

# **Handbook of Marine Macroalgae**

# **Handbook of Marine Macroalgae**

## **Biotechnology and Applied Phycology**

**Se-Kwon Kim**

*Pukyong National University*

 **WILEY-BLACKWELL**

A John Wiley & Sons, Ltd., Publication

This edition first published 2012  
© 2012 John Wiley & Sons, Ltd

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical and Medical business with Blackwell Publishing.

*Registered office:*

John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

*Other Editorial Offices:*

9600 Garsington Road, Oxford, OX4 2DQ, UK

111 River Street, Hoboken, NJ 07030-5774, USA

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at [www.wiley.com/wiley-blackwell](http://www.wiley.com/wiley-blackwell)

The right of the author to be identified as the author of this work has been asserted in accordance with the Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book. This publication is designed to provide accurate and authoritative information in regard to the subject matter covered. It is sold on the understanding that the publisher is not engaged in rendering professional services. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

The contents of this work are intended to further general scientific research, understanding, and discussion only and are not intended and should not be relied upon as recommending or promoting a specific method, diagnosis, or treatment by physicians for any particular patient. The publisher and the author make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation any implied warranties of fitness for a particular purpose. In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of medicines, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each medicine, equipment, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. Readers should consult with a specialist where appropriate. The fact that an organization or Website is referred to in this work as a citation and/or a potential source of further information does not mean that the author or the publisher endorses the information the organization or Website may provide or recommendations it may make. Further, readers should be aware that Internet Websites listed in this work may have changed or disappeared between when this work was written and when it is read. No warranty may be created or extended by any promotional statements for this work. Neither the publisher nor the author shall be liable for any damages arising herefrom.

*Library of Congress Cataloging-in-Publication Data*

Kim, Se-Kwon.

Handbook of marine microalgae : biotechnology and applied phyecology / Se-Kwon Kim.

p. cm.

Includes index.

ISBN 978-0-470-97918-1 (cloth)

1. Microalgae—Handbooks, manuals, etc.    2. Microalgae—Biotechnology—Handbooks, manuals, etc.
  3. Algology—Handbooks, manuals, etc.    4. Marine algae culture—Handbooks, manuals, etc.    I. Title.
- QK568.M52K56 2011  
579.8'1776—dc23

2011023327

A catalogue record for this book is available from the British Library.

This book is published in the following electronic formats: ePDF 9781119977094; Wiley Online Library 9781119977087; ePub 97811199776550; Mobi 9781119977667

Typeset in 9.75/11.75pt Minion by Aptara Inc., New Delhi, India

Printed in [Country] by [Printer]

First Impression 2012

# Contents

<b>List of Contributors</b>	<b>xvii</b>
<b>Preface</b>	<b>xxi</b>
<b>Editor</b>	<b>xxiii</b>

## **PART I Introduction to Algae and Their Importance**

<b>1 Biological Importance of Marine Algae</b>	<b>3</b>
<i>Ali A. El Gamal</i>	
1.1 Introduction	3
1.2 Interesting natural products and their biological activities from macroalgae (seaweeds)	4
1.2.1 Chlorophyta (green algae)	5
1.2.2 Phaeophyta (brown algae)	8
1.2.3 Rhodophyta (red algae)	17
Acknowledgment	27
References	27
<b>2 Seaweeds: The Wealth of Oceans</b>	<b>36</b>
<i>Upadhyayula Suryanarayana Murty and Amit Kumar Banerjee</i>	
2.1 Introduction	36
2.2 Need for marine resources	36
2.3 Various marine resources	36
2.4 Producers in the marine environment	37
2.5 Emergent plants	37
2.6 Seaweed diversity	37
2.7 Uses of seaweeds	37
2.8 Marine farming: global scenario	39
2.9 SEAPURA: an EU effort	39
2.10 Seaweed farming: an Indian scenario	40
2.11 Expanding the existing knowledge base: current research trends in exploring seaweeds	41
2.11.1 Metagenomics in understanding seaweeds	41
2.11.2 Role of bioinformatics	41
2.11.3 Data storage and retrieval	41
2.11.4 Different kind of information analysis	42
2.11.5 Phylogeographical and evolutionary analysis	42
2.12 Future prospects	42
2.13 Conclusion	43
References	43

<b>3 Eco-Biochemical Studies of Common Seaweeds in the Lower Gangetic Delta</b>	<b>45</b>
<i>Rajrupa Ghosh, Kakoli Banerjee and Abhijit Mitra</i>	
3.1 Seaweeds: an overview	45
3.2 Commercial uses of seaweeds	46
3.3 Indian scenario	46
3.4 Biochemical composition of seaweeds with special reference to Indian Sundarbans	51
References	55
 <b>4 Chemodiversity and Bioactivity within Red and Brown Macroalgae Along the French coasts, Metropole and Overseas Departements and Territories</b>	 <b>58</b>
<i>Nathalie Bourgougnon and Valerie Stiger-Pouvreau</i>	
4.1 Introduction	58
4.2 Exploitation of marine algal resources	60
4.2.1 International context	60
4.2.2 French and Breton context	60
4.3.3 French research network on marine bioactive compounds extracted from macroalgae	61
4.3 Why a focus on red and brown seaweeds?	64
4.4 Marine red seaweeds and biological activities	64
4.4.1 Polysaccharides	65
4.4.2 Phycoerythrin	67
4.5 Marine brown seaweeds and biological activities	68
4.5.1 Polysaccharides	68
4.5.2 Phenolic compounds (phloroglucinol and derived products)	69
4.5.3 Terpenes	72
4.6 The use of metabolites from marine red and brown algae for their chemical defense	73
4.6.1 Biotic interactions of marine red and brown algae (pathogens, grazing, etc.)	73
4.6.2 Biofouling	75
4.7 The use of metabolites as chemomarkers for taxonomy	81
4.8 Industrial uses of metabolites from marine red and brown algae	82
4.8.1 Algae for nutritional foods	82
4.8.2 Algae for health and cosmetics	85
4.8.3 Algae against microorganisms	88
4.10 Conclusion	89
Acknowledgments	89
References	90
 <b>5 Physiological Basis for the use of Seaweeds as Indicators of Anthropogenic Pressures: The Case of Green Tides</b>	 <b>106</b>
<i>Jesús M. Mercado</i>	
5.1 Introduction	106
5.2 Light absorption	107
5.3 Photosynthesis at sub- and saturating irradiance	108
5.4 Inorganic carbon acquisition	110
5.5 Does the high capacity for using bicarbonate favor the development of green tides?	111
5.6 Conclusions	111
Acknowledgments	112
References	112

<b>6 Significance of the Presence of Trace and Ultratrace Elements in Seaweeds</b>	<b>116</b>
<i>Antonio Moreda-Piñeiro, Elena Peña-Vázquez and Pilar Bermejo-Barrera</i>	
6.1 Introduction	116
6.2 Mineral content in seaweed	117
6.3 Trace and ultratrace elements in seaweeds	117
6.3.1 Legislation concerning seaweed consumption	117
6.3.2 Trace and ultratrace elements in seaweed: studies concerning seaweed edibility	147
6.3.3 Radionuclides in edible seaweed	148
6.4 Trace and ultratrace elements in seaweed: pollution biomonitoring	148
6.4.1 Seaweeds as bioindicators	148
6.4.2 Trace and ultratrace elements in seaweed: studies concerning environmental monitoring	150
6.4.3 Seaweeds as bioindicators of radioactive pollution	152
6.5 Chemical speciation	154
6.5.1 Importance of the chemical species of an element	154
6.5.2 Sources of organometallic species in the environment and foodstuffs	154
6.5.3 Organometallic compounds (elemental chemical species) in algae	154
6.5.4 Analytical chemistry of elemental speciation in algae	162
References	164

## PART II Isolation and Chemical Properties of Molecules Derived from Seaweeds

<b>7 Chemical Composition of Seaweeds</b>	<b>173</b>
<i>Ladislava Mišurcová</i>	
7.1 Introduction	173
7.2 Various components of seaweeds	174
7.2.1 Proteins and amino acids	174
7.2.2 Minerals	176
7.2.3 Vitamins	179
7.2.4 Lipids	181
7.2.5 Dietary fiber	182
7.3 Conclusion	186
References	186
<b>8 Structural Peculiarities of Sulfated Polysaccharides from Red Algae <i>Tichocarpus crinitus</i> (Tichocarpaceae) and <i>Chondrus pinnulatus</i> (Gigartinaceae) Collected at the Russian Pacific Coast</b>	<b>193</b>
<i>Anna O. Barabanova and Irina M. Yermak</i>	
8.1 Introduction	193
8.2 Carrageenan sources in the Russian Far East	196
8.3 The polysaccharide composition of algae in relation to the phase of its life cycle	197
8.3.1 The polysaccharides of <i>Chondrus pinnulatus</i> (Gigartinaceae)	197
8.3.2 The polysaccharides of <i>Tichocarpus crinitus</i> (Tichocarpaceae)	197
8.3.3 Influence of environmental conditions on polysaccharide composition of <i>T. crinitus</i>	199
8.4 The rheological and viscosity properties of carrageenan from <i>C. pinnulatus</i> and <i>T. crinitus</i>	200
References	201

<b>9 Extraction and Characterization of Seaweed Nanoparticles for Application on Cotton Fabric</b>	<b>205</b>
<i>Sivalingam Thambidurai</i>	
9.1 Introduction	205
9.2 Textile materials	205
9.2.1 Cotton fiber	205
9.2.2 Cotton yarn	206
9.2.3 Cotton fabric	207
9.2.4 Preparatory process	207
9.3 Antimicrobial agents	208
9.3.1 Organic chemicals	209
9.3.2 Inorganic nanoparticles	209
9.3.3 Oxygen bleach	209
9.3.4 Plant products	210
9.3.5 Chitin and chitosan	210
9.4 Seaweeds	211
9.4.1 Bioactive compounds from seaweed	211
9.5 Extraction and characterization	212
9.5.1 Crude extract	212
9.5.2 Nanoparticle extraction	212
9.5.3 Characterization of nanoparticles	212
9.6 Antibacterial finishing	216
9.6.1 Padding of extract	216
9.6.2 Antibacterial test	217
9.6.3 Antibacterial property	217
9.7 Permanent finish	217
Acknowledgments	217
References	218
<b>10 Enzyme-assisted Extraction and Recovery of Bioactive Components from Seaweeds</b>	<b>221</b>
<i>You-Jin Jeon, W.A.J.P Wijesinghe and Se-Kwon Kim</i>	
10.1 Introduction	221
10.2 Extraction of bioactive compounds from seaweeds	222
10.3 Role of cell wall degrading enzymes	222
10.4 Importance of enzyme treatment prior to extraction of bioactive compounds	222
10.5 Selection of the enzyme/s and the extraction conditions	222
10.6 Bioactive peptides from seaweeds	223
10.6.1 Polyphenols and brown algal phlorotannins	224
10.6.2 Carotenoids	225
10.6.3 Polysaccharides	225
10.7 Conclusions	226
References	226
<b>11 Structure and Use of Algal Sulfated Fucans and Galactans</b>	<b>229</b>
<i>Vitor H. Pomim</i>	
11.1 Introduction	229
11.2 Phylogenetic distribution	230
11.3 Common methods for extraction and structural analyses	230
11.3.1 Methods for isolation	230
11.3.2 Methods for detection, quantization, and purity control	231
11.3.3 Methods for molecular weight determination	233
11.3.4 Methods for structural characterization	233

CONTENTS		ix
11.4	General structural features related to phylogenetic occurrence	239
11.4.1	Phylogenetic implications: how has the 3-linked, $\beta$ -galactopyranose unit occurred in the marine environment throughout the course of evolution?	239
11.4.2	Restricted occurrence of SFs in brown algae	240
11.4.3	SGs in green algae	242
11.4.4	Red algal SGs occur usually in disaccharide repeating units within heterogeneous sulfation patterns: carrageenans and agarans	242
11.5	Industrial applications	242
11.5.1	SFs/fucoidans as food supplements and cosmetic hydrators	242
11.5.2	Carrageenans and agarans: the most industrially used SG molecules	244
11.6	Pharmacological properties	247
11.6.1	Antiviral actions	247
11.6.2	The use of SFs and SGs in therapy for preventing thrombosis and coagulation	249
11.6.3	Inhibiting inflammation	250
11.6.4	Pro- and antiangiogenic actions of SFs/fucoidans	251
11.6.5	Algal SPs helping the fight against tumor	253
11.6.6	Combating infection of parasites with algal SPs: a new avenue against parasitoses	254
11.6.7	Effects on cellular growth, migration and adhesion	254
11.7	Major conclusions	255
	Acknowledgments	255
	References	255
<b>12</b>	<b>Bioactive Metabolites from Seaweeds</b>	<b>262</b>
	<i>Jing Hu, Bin Yang, Xiuping Lin, Xue-Feng Zhou, Xian-Wen Yang, and Yonghong Liu</i>	
12.1	Introduction	262
12.2	Chemical constituents	263
12.2.1	Sesquiterpenes	263
12.2.2	Diterpenes	268
12.2.3	Other skeletons	271
12.2.4	Meroterpenoids	274
12.2.5	C <sub>15</sub> -acetogenins	275
12.2.6	Phlorotannins	277
12.2.7	Steroids	279
12.3	Conclusions	280
	References	281
<b>13</b>	<b>Seaweed Digestibility and Methods Used for Digestibility Determination</b>	<b>285</b>
	<i>Ladislava Mišurcová</i>	
13.1	Digestibility	285
13.1.1	Protein digestibility	285
13.2	Methods of seaweed digestibility assessment	287
13.2.1	<i>In vivo</i> methods of digestibility assessment	287
13.2.2	<i>In situ</i> methods of digestibility assessment	288
13.2.3	<i>In vitro</i> methods of digestibility assessment	289
13.3	Factors influencing digestibility of seaweed and seaweed products	291
13.3.1	Endogenous factors influencing seaweed digestibility	291
13.3.2	Exogenous factors influencing seaweed digestibility	292
13.4	Evaluation of seaweed digestibility	295
13.5	Contribution of seaweed to food and feed digestibility	296

13.6 Conclusion	297
References	297
<b>14 Metallation of Seaweed <i>Fucus vesiculosus</i> Metallothionein: As<sup>3+</sup> and Cd<sup>2+</sup> binding</b>	<b>302</b>
<i>Thanh T. Ngu and Martin J. Stillman</i>	
14.1 Introduction	302
14.2 Characterization of the rfMT	303
14.3 Equilibrium metallation studies of rfMT studied using ESI-MS and UV-visible absorption techniques	304
14.3.1 Equilibrium data for cadmium binding	304
14.3.2 Equilibrium data for arsenic binding	305
14.4 Dynamic metallation studies of rfMT studied using ESI-MS techniques	306
14.5 Conclusions	315
Acknowledgments	315
References	315
 <b>PART III Biological Properties of Molecules Derived from Seaweeds</b>	
<b>15 <i>In Vivo</i> and <i>in Vitro</i> Toxicity Studies of Fucoxanthin, a Marine Carotenoid</b>	<b>321</b>
<i>Yoshimi Niwano and Fumiaki Beppu</i>	
15.1 Introduction	321
15.2 <i>In vivo</i> oral toxicity study	321
15.3 <i>In vitro</i> and <i>in vivo</i> mutagenicity study	324
15.4 Conclusion	327
References	327
<b>16 Brown Seaweed Lipids as Potential Source of Omega-3 PUFA in Biological Systems</b>	<b>329</b>
<i>Kazuo Miyashita, Bhaskar Narayan, Takayuki Tsukui, Hiroyuki Kamogawa, Masayuki Abe, and Masashi Hosokawa</i>	
16.1 Introduction	329
16.2 Omega-3 and omega-6 PUFA	330
16.3 Importance of omega-3 PUFA on human health	331
16.4 Brown seaweed lipids	332
16.5 Bioconversion of LN to DHA	333
16.6 Hepatic DHA enhancement in mice by fucoxanthin	333
16.7 Conclusion	335
References	335
<b>17 Immune Regulatory Effects of Phlorotannins Derived From Marine Brown Algae (<i>Phaeophyta</i>)</b>	<b>340</b>
<i>Phuong Hong Nguyen, il-Whan Choi, Se-Kwon Kim and Won-Kyo Jung</i>	
17.1 Introduction	340
17.2 Anti-inflammatory effects of phlorotannins on RAW264.7 macrophage cells	343
17.3 Neuroprotective effects of phlorotannins on BV2 microglial cells	344
17.4 Anti-allergic effects of phlorotannins	344
17.4.1 Anti-asthma	344
17.4.2 Anti-rheumatoid arthritis (RA)	345
17.4.3 Other phlorotannins	345
17.5 Conclusion	346
Acknowledgments	346
References	346

<b>18</b>	<b><i>In Vivo</i> and <i>In Vitro</i> Studies of Seaweed Compounds</b>	<b>348</b>
	<i>Raquel Domínguez Gonzalez, Vanessa Romaris Hortas and Pilar Bermejo Barrera</i>	
18.1	Introduction	348
18.2	Methods to study compound bioaccessibility	349
18.2.1	<i>In vivo</i> methods	349
18.2.2	<i>In vitro</i> methods	349
18.3	<i>In vivo</i> versus <i>in vitro</i> methods	352
18.4	Methods with cell culture models	352
18.5	Conclusions	352
	References	352
<b>19</b>	<b>Brown Seaweed-Derived Phenolic Phytochemicals and Their Biological Activities for Functional Food Ingredients with Focus on <i>Ascophyllum nodosum</i></b>	<b>356</b>
	<i>Emmanouil Apostolidis and Chong M. Lee</i>	
19.1	Introduction: seaweed-derived functional food ingredients	356
19.2	Major commercial brown seaweeds	357
19.2.1	Ecology and characteristics	357
19.2.2	Health benefits	358
19.3	Brown seaweeds and phenolic phytochemicals	359
19.3.1	Brown seaweed phenolic phytochemicals and health benefits	359
19.3.2	<i>Ecklonia cava</i> health benefits	359
19.4	<i>Ascophyllum nodosum</i> : importance and health benefits	361
19.4.1	Health benefits	361
19.4.2	<i>Ascophyllum nodosum</i> phenolic phytochemical-mediated type 2 diabetes management	362
19.4.3	Future directions	364
19.5	Conclusions	365
	References	366
<b>20</b>	<b>Antiobesity and Antidiabetic Effects of Seaweeds</b>	<b>371</b>
	<i>Chang-Suk Kong and Se-Kwon Kim</i>	
20.1	Introduction	371
20.2	Antiobesity and antidiabetic effects of seaweed	372
20.2.1	Brown seaweed	372
20.2.2	Active components	373
20.3	Conclusions	375
	References	375
<b>21</b>	<b>Health Beneficial Aspects of Phloroglucinol Derivatives from Marine Brown Algae</b>	<b>378</b>
	<i>Noel Vinay Thomas and Se-Kwon Kim</i>	
21.1	Introduction	378
21.2	Phloroglucinol derivatives (phlorotannins) from marine brown algae	378
21.3	Health beneficial aspects of brown algal phlorotannins	381
21.3.1	Anti-inflammatory activity	381
21.3.2	Antioxidant activity	382
21.3.3	Anti-photoaging activity	382
21.3.4	Antitumor activity	383
21.3.5	MMP inhibition activity	384
21.3.6	Additional health beneficial aspects of phlorotannins	384
21.4	Conclusions and future prospects	385
	References	385

<b>22 Biological Effects of Proteins Extracted from Marine Algae</b>	<b>387</b>
<i>Taek-Jeong Nam</i>	
22.1 Introduction	387
22.2 Stimulatory effect of a glycoprotein from <i>LAMINARIA Japonica</i> on cell proliferation	387
22.3 Chemoprotective effect of marine algae extracts against acetaminophen toxicity	389
22.3.1 Effect of a glycoprotein from <i>Hizikia fusiformis</i> on acetaminophen-induced liver injury	390
22.3.2 Chemoprotective effects of a protein from the red algae <i>Porphyra yezoensis</i> in drug-induced liver injury	395
References	396
<b>23 Functional Ingredients from Marine Algae as Potential Antioxidants in the Food Industry</b>	<b>398</b>
<i>Isuru Wijesekara, Mahinda Senevirathne, Yong-Xin Li and Se-Kwon Kim</i>	
23.1 Introduction	398
23.2 Marine algae-derived functional ingredients and their antioxidant effect	399
23.2.1 Phlorotannins	399
23.2.2 Sulfated polysaccharides	399
23.2.3 Carotenoids	400
23.3 Conclusion	401
References	401
<b>24 Algal Carotenoids as Potent Antioxidants</b>	<b>403</b>
<i>Kazuo Miyashita, M. Airanthi K. Widjaja-Adhi, Masayuki Abe, and Masashi Hosokawa</i>	
24.1 Introduction	403
24.2 Algal carotenoids	404
24.3 Carotenoids as dietary antioxidants	405
24.4 Brown seaweeds as rich source of antioxidants	406
24.5 Antioxidant activity of algal carotenoids	408
24.6 Antiobesity and antidiabetic effect of fucoxanthin	409
24.7 Conclusion	410
References	410
<b>PART IV Biotechnology of Seaweeds</b>	
<b>25 Anti-HIV Activities of Marine Macroalgae</b>	<b>417</b>
<i>Thanh-Sang Vo, Dai-Hung Ngo and Se-Kwon Kim</i>	
25.1 Introduction	417
25.2 Potential anti-HIV agents from marine macroalgae	417
25.2.1 Sulfated polysaccharides	417
25.2.2 Phlorotannins	419
25.2.3 Diterpenes	420
25.2.4 Lectins	420
25.2.5 Bioactive peptides	421
25.3 Conclusion	421
References	421
<b>26 Biotechnology of Seaweeds: Facing the Coming Decade</b>	<b>424</b>
<i>Lin Hanzhi, Qin Song and Jiang Peng</i>	
26.1 Introduction	424
26.2 Biotechnology of seaweeds in 'blue farming'	424

26.3	Biotechnology of seaweeds in the chemical industry and pharmacy	425
26.4	Biotechnology of seaweeds in a changing world: their role in bioremediation and bioenergy	426
	Acknowledgment	427
	References	427
<b>27</b>	<b>Current Trends and Future Prospects of Biotechnological Interventions Through Plant Tissue Culture in Seaweeds</b>	<b>431</b>
	<i>Abdul Bakrudeen Ali Ahmed and Rosna Mat Taha</i>	
27.1	Introduction	431
27.2	Explants, sterilization and methods used in seaweed production	432
27.2.1	Active chemicals and mechanism in seaweed production	433
27.2.2	Polyamines as growth promoters in seaweed production	433
27.2.3	Plant growth regulators' role in seaweed production	434
27.3	Micropropagation of seaweeds	434
27.4	Callus and cell suspension culture in seaweed production	435
27.5	Bioprocess technology and cell culture in seaweed production	436
27.6	Remarks and conclusion	438
	References	438
<b>28</b>	<b>Detoxification Mechanisms of Heavy Metals by Algal–Bacteria Consortia</b>	<b>441</b>
	<i>Enrique J. Peña-Salamanca, Ana Lucia Rengifo-Gallego and Neyla Benitez-Campo</i>	
28.1	Introduction	441
28.2	Mechanisms used by algae in heavy metals tolerance and removal	442
28.2.1	Production of extracellular binding-polypeptides	442
28.2.2	Exclusion mechanism	443
28.2.3	Internal detoxification	443
28.2.4	Metal transformation	443
28.3	Algal–bacterial mechanisms involved in heavy metal detoxification	444
28.3.1	Biosorption	444
28.3.2	Bioaccumulation	445
28.3.3	Biotransformation and biomineralization	445
28.4	Algal–bacteria consortia in the red alga <i>Bostrychia calliptera</i> (Rhodomelaceae)	445
28.5	Biological treatment of heavy metals	446
28.6	Biotechnological applications	447
28.7	Conclusions and future remarks	448
	References	448
<b>PART V Natural Resource Management and Industrial Applications of Seaweeds</b>		
<b>29</b>	<b>Manufacturing Technology of Bioenergy Using Algae</b>	<b>453</b>
	<i>Gyung-Soo Kim</i>	
29.1	Introduction	453
29.2	Bioethanol types and characteristics	453
29.3	Foreign and domestic bioethanol industries and technologies	454
29.4	Algal biomass characteristics	455
29.5	Red algae bioethanol production technology	455
29.5.1	Overview	455
29.5.2	Saccharification process	456
29.5.3	Fermentation process	457
29.5.4	Separation and distillation process	459

29.6	Future technology outlook	459
	Acknowledgments	459
	References	459
<b>30</b>	<b>Seaweed as an Adsorbent to Treat Cr(VI)-Contaminated Wastewater</b>	<b>461</b>
	<i>Saroj Sundar Baral</i>	
30.1	Importance of chromium	461
30.2	Harmful effects of Cr(VI)	461
30.3	Different methods of treatment	462
30.3.1	Adsorption method	462
30.4	Case study on adsorptive removal of Cr(VI) from aqueous solution using seaweed <i>Hydrilla verticillata</i>	465
30.4.1	Materials and method	465
30.4.2	Results and discussion	465
	References	475
<b>31</b>	<b>Using the Biomass of Seaweeds in the Production of Components of Feed and Fertilizers</b>	<b>478</b>
	<i>Katarzyna Chojnacka</i>	
31.1	Introduction	478
31.2	Seaweeds in fertilizers	478
31.2.1	General aspects of using seaweeds and their extracts as fertilizers	478
31.2.2	Seaweed extracts as fertilizers	479
31.2.3	Plant biostimulants from seaweeds	479
31.2.4	Commercial seaweed fertilizers	479
31.2.5	Studies on cultivation of plants on seaweed derived fertilizers	479
31.2.6	Seaweed fertilizer as value-added product from manure	480
31.3	Seaweeds in feeds for animals	481
31.3.1	General aspects of using seaweeds and their extracts in animal diet	481
31.3.2	Seaweeds in feeds – historical aspects	481
31.3.3	Nutritional properties of seaweeds	482
31.3.4	Seaweed nutraceuticals	482
31.3.5	Studies on animal breeding using seaweed meals	482
31.3.6	Studies on animal breeding using seaweed extracts	483
31.3.7	Integrated processes – aquaculture	484
31.4	Using the biomass of seaweeds enriched with microelements by biosorption in nutrition of plants and animals	484
31.4.1	Microelement hunger	485
31.4.2	Biofortification of food	485
31.4.3	Using biosorption to increase bioavailability of microelements	485
31.4.4	Seaweeds as biosorbents – carriers of microelements in nutrition of plants and animals – to produce biofortified food	486
31.5	Conclusions	486
	Acknowledgments	487
	References	487
<b>32</b>	<b>Applications of Seaweed in Meat-Based Functional Foods</b>	<b>491</b>
	<i>Susana Cofrades, Inés López-López and Francisco Jiménez-Colmenero</i>	
32.1	Introduction	491
32.2	Meat-based functional foods	491
32.3	Seaweed as a functional food ingredient in meat products	492
32.3.1	Application of specific seaweed components in meat products	492
32.3.2	Incorporation of seaweeds into meat products	494

32.4	Conclusions	495
	Acknowledgment	496
	References	496
<b>33</b>	<b>Industrial Applications of Macroalgae</b>	<b>500</b>
	<i>A. Malshani Samaraweera, Janak K. Vidanarachchi and Maheshika S. Kurukulasuriya</i>	
33.1	Introduction	500
33.2	Composition of seaweeds	500
33.2.1	Seaweed polysaccharides	501
33.2.2	Polyphenols	502
33.2.3	Mycosporine-like amino acids (MAAs)	502
33.3	Seaweeds as vegetables: their nutritive value	503
33.3.1	Fatty acids	503
33.3.2	Amino acids	504
33.3.3	Minerals	504
33.3.4	Antinutrients and toxic factors	504
33.4	Applications as functional foods	505
33.4.1	Dietary fiber as prebiotics	505
33.4.2	Microencapsulation of bacteria as probiotics	505
33.5	Application of seaweeds as antioxidants in the food industry	506
33.6	Industrial applications of phycocolloids	508
33.6.1	Extraction of seaweed phycocolloids	508
33.6.2	Phycocolloids in food preparation	509
33.6.3	Edible food coatings	510
33.6.4	Other applications of phycocolloids	510
33.7	Biomedical applications	510
33.7.1	Antioxidant activity	510
33.7.2	Antitumor and immunomodulatory activity	511
33.7.3	Anti-inflammatory activity	512
33.7.4	Anticoagulant activity	512
33.7.5	Applications in tissue engineering	512
33.8	Macroalgal-derived cosmeceuticals	513
33.9	Applications in agriculture	514
33.10	Applications in pollution detection and control	515
33.11	Utilization of macroalgae for energy production	515
33.12	Conclusions	516
	References	516
<b>34</b>	<b>Application of Seaweeds in the Food Industry</b>	<b>522</b>
	<i>Cristina García Sartal, María Carmen Barciela Alonso and Pilar Bermejo Barrera</i>	
34.1	Introduction	522
34.2	Compounds extracted from algae of interest to the human nutrition industry	522
34.2.1	Macroalgae-extracted compounds	522
34.2.2	Microalgae-extracted compounds	524
34.3	Animal feeding	527
34.3.1	Terrestrial animal feed	527
34.3.2	Poultry	528
34.3.3	Aquaculture	528
34.4	Fertilizers	528
34.5	Conclusion	529
	References	529

<b>35 A Dimensional Investigation on Seaweeds: Their Biomedical and Industrial Applications</b>	<b>532</b>
<i>Sudha Narayanan Parapurath, Hebsibah Elsie Bernard, Dhanarajan Malli Subramaniamc and Ramya Ramamurthy</i>	
35.1 Introduction	532
35.1.1 Introduction to algae	532
35.1.2 Types of seaweeds	532
35.1.3 Components of algae	533
35.1.4 Nutritive value of seaweeds	534
35.2 Biomedical applications of seaweeds	534
35.2.1 Biomedical importance of seaweeds	534
35.2.2 Antioxidant properties of seaweeds	535
35.2.3 Antibacterial effects of seaweeds	535
35.2.4 Antiviral properties of seaweeds	535
35.2.5 Heme-agglutinating properties of seaweeds	536
35.2.6 Hepatoprotective and anticancer properties	536
35.2.7 Seaweed consumption and weight loss	536
35.3 Industrial applications of seaweeds	537
35.3.1 Seaweeds as a fertilizer	537
35.3.2 Seaweeds for cosmetics and agar production	537
35.3.3 Seaweeds used for wastewater treatment	537
35.3.4 Seaweed as a fuel	538
35.4 Conclusion	538
Acknowledgment	538
References	538
<b>36 Seaweed Polysaccharides – Food Applications</b>	<b>541</b>
<i>Vazhiyil Venugopal Menon</i>	
36.1 Introduction	541
36.2 Major functions of polysaccharides in a food system	541
36.2.1 Water-binding capacity	541
36.2.2 Gelation	541
36.2.3 Emulsions and foams	542
36.3 Interactions of polysaccharides with food components	542
36.4 Major food applications of polysaccharides	542
36.4.1 Seaweed polysaccharides	543
36.5 Regulatory and commercial aspects	551
References	552
<b>Index</b>	<b>557</b>

# Contributors

**Masayuki Abe**

Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan  
and  
Kaneka Co., 3-2-4, Nakanoshima, Kita-ku, Osaka 530-8288, Japan

**Abdul Bakrudeen Ali Ahmed**

Institute of Biological Sciences, Faculty of Science,  
University of Malaya, Kuala Lumpur 50603, Malaysia

**María Carmen Barciela Alonso**

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

**Emmanouil Apostolidis**

University of Rhode Island, 6 Rhodey Ram Way, Kingston, RI 02881, USA

**Amit Kumar Banerjee**

Bioinformatics Group, Biology Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500607, Andhra Pradesh, India

**Kakoli Banerjee**

Department of Marine Science, University of Calcutta, 35 B.C. Road, Kolkata-700019, India

**Anna O. Barabanova**

Pacific Institute of Bioorganic Chemistry Far-East Branch of Russian Academy of Sciences, pr. 100-letya Vladivostoka 159, Vladivostok-690022, Russia

**Saroj Sundar Baral**

Department of Chemical Engineering, Birla Institute of Technology & Science, Pilani- K. K. Birla Goa Campus, Goa 403-726, India

**Pilar Bermejo-Barrera**

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

**Hebsibah Elsie Bernard**

Department of Biochemistry, DKM College, Thiruvalluvar University, Vellore – 632 001, Tamil Nadu, India

**Neyla Benitez-Campo**

Applied Plant Biology Research Group, Department of Biology, Universidad del Valle, A.A. 25360 Cali Colombia

**Fumiaki Beppu**

Faculty of Fisheries, Hokkaido University, 3-1-1 Minato, Hakodate-0418611, Japan

**Nathalie Bourgougnon**

College Doctoral International de l'Université, Euripenne de Bretagne (UEB), Directrice du College Doctoral de l'Université de Bretagne –Sud (UBS), Laboratoire de Biotechnologie et Chimie Marines, France

**Katarzyna Chojnacka**

Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology, Poland

**Susana Cofrades**

Instituto de Ciencia y Tecnología de Alimentos y Nutrición-ICTAN (Formerly Instituto del Frío) (CSIC). Ciudad Universitaria, 28040-Madrid, Spain

**Ali A. El Gamal**

Department of Pharmacognosy, College of Pharmacy, Mansoura University, Mansoura, Egypt

**Rajrupa Ghosh**

Department of Marine Science, University of Calcutta, 35 B.C. Road, Kolkata 700019, India

**Raquel Domínguez Gonzalez**

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

**Lin Hanzhi**

Key Laboratory of Experimental Marine Biology, Chinese Academy of Sciences at Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

**Vanessa Romaris Hortas**

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

**Masashi Hosokawa**

Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan

**Jing Hu**

Key Laboratory of Marine Bio-resources Sustainable Utilization/Guangdong Key Laboratory of Marine Material Medica/Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

**You-Jin Jeon**

School of Marine Biomedical Sciences, Jeju National University, Jeju 690-756, Republic of Korea

**Francisco Jiménez-Colmenero**

Instituto de Ciencia y Tecnología de Alimentos y Nutrición-ICTAN (Formerly Instituto del Frío) (CSIC). Ciudad Universitaria, 28040-Madrid, Spain

**Won-Kyo Jung**

Department of Marine Life Science, and Marine Life Research & Education Center, Chosun University, Gwangju-501759, Republic of Korea

**Hiroyuki Kamogawa**

Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan

**Gyung-Soo Kim**

Biolsystems Corporation, JoongPyung B/D 6F 64-1, Umyeon-dong, Seocho-gu, Seoul 137-900, Republic of Korea

**Se-Kwon Kim**

Department of Chemistry, Marine Bioprocess Research Center, Pukyong National University, Busan 608-737, Republic of Korea

**Chang-Suk Kong**

Department of Food and Nutrition, College of Medical and Life Science, Silla University, Busan 617-736, Republic of Korea

**Maheshika S. Kurukulasuria**

Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya-20400, Sri Lanka

**Chong M. Lee**

University of Rhode Island, 6 Rhodey Ram Way, Kingston, RI 02881, USA

**Yong-Xin Li**

Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan 608-737, Republic of Korea

**Xiuping Lin**

Key Laboratory of Marine Bio-resources Sustainable Utilization/Guangdong Key Laboratory of Marine Material Medica/Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

**Yonghong Liu**

Key Laboratory of Marine Bio-resources Sustainable Utilization/Guangdong Key Laboratory of Marine Material Medica/Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

**Ines López-López**

Instituto de Ciencia y Tecnología de Alimentos y Nutrición-ICTAN (Formerly Instituto del Frío) (CSIC). Ciudad Universitaria, 28040-Madrid, Spain

**Vazhiyil Venugopal Menon**

Seafood Technology Section, Food Technology Division, Bhabha Atomic Research Center, Mumbai 400085, India

**Jesús M. Mercado**

Centro Oceanográfico de Málaga. Instituto Español de Oceanografía. Puerto Pesquero s/n. Apdo. 285, Fuengirola-29640, Spain

**Ladislava Mišurcová**

Tomas Bata University in Zlín, Faculty of Technology, Department of Food Technology and Microbiology, Czech Republic

**Abhijit Mitra**

Department of Marine Science, University of Calcutta, 35 B.C. Road, Kolkata-700019, India

**Kazuo Miyashita**

Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan

**Antonio Moreda-Piñeiro**

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

**Taek-Jeong Nam**

College of Fisheries Science, Pukyong National University, Busan 608-737, Republic of Korea

**Bhaskar Narayan**

Department of Meat, Fish & Poultry Technology, CFTRI, Mysore 570 020, India

**Dai-Hung Ngo**

Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan, Republic of Korea

**Thanh T. Ngu**

Department of Chemistry, The University of Toronto, Toronto, Ontario, Canada

**Phuong Hong Nguyen**

Department of Marine Life Science, and Marine Life Research & Education Center, Chosun University, Gwangju-501759, Republic of Korea

**Yoshimi Niwano**

New Industry Creation Hatchery Center, Tohoku University, 6-6-10 Aoba, Aramaki, Aoba-ku, Sendai, Miyagi-9808579, Japan

**Sudha Narayanan Parapurath**

Department of Chemistry, DKM College, Thiruvalluvar University, Vellore - 632 001, Tamil Nadu, India

**Enrique J. Peña-Salamanca**

Applied Plant Biology Research Group, Department of Biology, Universidad del Valle, A.A. 25360 Cali, Colombia

**Elena Peña-Vázquez**

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

**Jiang Peng**

Key Laboratory of Experimental Marine Biology, Chinese Academy of Sciences at Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

**Mahinda Senevirathne**

Marine Bioprocess Research Center, Pukyong National University, Busan 608-737, Republic of Korea

**Valerie Stiger-Pouvreau**

College Doctoral International de l'Université, Euripenne de Bretagne (UEB), Directrice du College Doctoral de l'Université de Bretagne - Sud (UBS), Laboratoire de Biotechnologie et Chimie Marines, France

**Upadhyayula Suryanarayana Murty**

Bioinformatics Group, Biology Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500607, Andhra Pradesh, India

**Vitor H. Pomin**

Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, GA 30602, USA and

Federal University of Rio de Janeiro, Medical Biochemistry Institute, Rio de Janeiro, RJ, Brazil

**Ramya Ramamurthy**

Research Scholar, Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

**Ana Lucia Rengifo**

Applied Plant Biology Research Group, Department of Biology, Universidad del Valle, A.A. 25360 Cali, Colombia

**A. Malshani Samaraweera**

Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya-20400, Sri Lanka

**Cristina García Sartal**

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

**Qin Song**

Key Laboratory of Experimental Marine Biology, Chinese Academy of Sciences at Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

and

Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

**Martin J. Stillman**

Department of Chemistry, University of Western Ontario, London, Ontario, Canada

**Dhanarajan Malli Subramaniam**

Jaya College of Arts and Science, Thirunindravur, University of Madras, Tamil Nadu, India

**Rosna Mat Taha**

Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia

**Sivalingam Thambidurai**

Department of Industrial Chemistry, School of Chemistry, Alagappa University, Karaikudi-630003, Tamil Nadu, India

**Noel Vinay Thomas**

Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan 608-737, Republic of Korea

**Takayuki Tsukui**

Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan

**Janak K. Vidanarachchi**

Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya-20400, Sri Lanka

**Thang-Sang Vo**

Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan, Republic of Korea

**M. Airanthi K. Widjaja-Adhi**

Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido-0418611, Japan

**Isuru Wijesekara**

Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan 608-737, Republic of Korea

**Wijesinghe W.A.J.P**

School of Marine Biomedical Sciences, Jeju National University, Jeju 690-756, Republic of Korea

**Bin Yang**

Key Laboratory of Marine Bio-resources Sustainable Utilization/Guangdong Key Laboratory of Marine Material Medica/Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

**Xian-Wen Yang**

Key Laboratory of Marine Bio-resources Sustainable Utilization/Guangdong Key Laboratory of Marine Material Medica/Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

**Irina M. Yermak**

Pacific Institute of Bioorganic Chemistry Far-East Branch of Russian Academy of Sciences, pr. 100-letya Vladivostoka 159, Vladivostok-690022, Russia

**Xue-Feng Zhou**

Key Laboratory of Marine Bio-resources Sustainable Utilization/Guangdong Key Laboratory of Marine Material Medica/Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

# Preface

Marine environment becoming the most explored habitat because of its chemical and biological diversity. Recently, marine floral and faunal exploration and exploitation becoming a great deal of interest which is the key to combat various diseases. Among the marine sources, algae or seaweeds are the more valuable sources of structurally diverse bioactive compounds. Even though, seaweed salads have been supplied as a regular diet, much information is not available whether the algal food has any significance on human health. For example, the beneficial effects of seaweeds and their bioactive substances like phlorotannins, sulphated polysaccharides, peptides and carotenoid pigments extend their applications from eco-biotechnological to the industrial standpoint. Hence, the utilization of marine macroalgal substances as potential biological and industrial products should be well established worldwide to gain various health and medical benefits. Although Asians consume seaweeds because of the known importance in their daily lives, many of the westerners might not think of the 'seaweed' as a nutritional or a daily supplement in their food. It is because of the term 'weed', which generally represents the unwanted plants in any ecosystem. Hence, I would like to introduce a more appropriate term "sea-vegetables" in this book, which could bring a positive notion in human beings to think 'algae' or 'seaweed' as consumable vegetables from sea.

The present book "*Handbook of Marine Macroalgae: Biotechnology and Applied Phycology*", describes the characteristic feature of marine macroalgal substances, source species, types, production and applications (biological, biotechnological, industrial). There are four discriminating parts present in the present book: **Part-I** deals with an overview of introduction and prospects of marine macroalgal introduction, their eco-physiological and biochemical importance along with various aspects of macroalgal biodiversity; **Part-II** provides a general and complex aspects of

isolation, extraction and physicochemical properties of various marine macroalgal compounds; **Part-III** discusses various biological and biomedical applications; **Part-IV** deals an over view on the *in vitro* cultivation other biotechnological prospects of marine macroalgae; and **Part-V** provides the information on the industrial utilization of marine macroalgae with their resource management strategies. Each part is a collection of comprehensive information on the past and present research of marine macroalgae, compiled of proficient scientists worldwide. Although significant activities and applications of marine macroalgal derived substances have been shared by various chapters, specific and unique biological, biomedical and industrial applications have been covered individually. Functional foods I personally intended to mention that the present findings and the recent information in this book will be helpful to the upcoming researchers to establish a phenomenal research from wide range of research areas.

I express my sincere thanks to all the authors, who have contributed in this book and their relentless effort was the result of scientific attitude and immense perseverance descended from their present and past experiences. I am grateful to the experts, who have provided state-of-the-art contributions that are included in this book. I also thank the personnel of Wiley-Blackwell publishers for their continual support, which is essential for the successful completion of the present task.

I hope that the fundamental as well as applied contributions in this book might serve as a potential research and development leads for the benefit of humankind. Altogether, algal biotechnology will be the hottest field in future towards the enrichment of targeted algal species, which further establishes a sustainable oceanic environment. The present book would be a reference book for the emerging students in the academic and industrial research.

Se-Kwon Kim

# Editor

**Se-Kwon Kim**, PhD, is currently working as a professor of marine biochemistry in the Department of Chemistry, Pukyong National University (PKNU), Busan, South Korea.

Dr. Kim received his MSc and PhD degrees from PKNU and joined as a faculty member in the same university. He conducted his postdoctoral research at the Bioprocess laboratory, Department of Food Science and Technology, University of Illinois, Urbana-Champaign, Illinois USA (1988–1989). He became a visiting scientist at the Memorial University of Newfoundland in Canada (1999–2000).

In the year 2004, Dr. Kim became the Director for ‘Marine Bioprocess Research Center (MBPRC)’ at Pukyong National University. He served as president for the ‘Korean Society of Chitin and Chitosan’ (1986–1990), and the ‘Korean Society of Marine Biotechnology’ (2006–2007). Dr. Kim was also the Chairman for 7<sup>th</sup> Asia-Pacific Chitin and Chitosan Symposium, which was held in South Korea in 2006. He is one of the board members of ‘International Society of Marine Biotechnology (IMB)’ and ‘International Society for Nutraceuticals and Functional Foods (ISNFF)’.

He was the editor-in-chief of the Korean Journal of Life Sciences (1995–1997), the Korean Journal of Fisheries Science and Technology (2006–2007) and the Korean Journal of Marine Bioscience and Biotechnology (2006–till date). To the credit for his research, he won the best paper awards from the American Oil Chemists’ Society (AOCS) and the Korean Society of Fisheries Science and Technology (KS-FST) in 2002.

His major research interests are investigation and development of bioactive substances derived from marine organisms and their application in oriental medicine, cosmetics and nutraceuticals via marine bioprocessing and mass-production technologies. Furthermore, he expanded his research fields especially in the field of dietary supplements from sea vegetables for the development of anti-diabetic, anti-arthritic, anti-hypertensive, anti-cancer, anti-aging substances towards the health promotion of senior citizens.

To date, he has authored over 450 research papers and holds 72 patents. In addition, he has written or edited more than 30 books.

# **PART I**

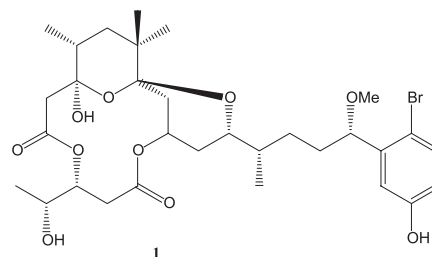
## **Introduction to Algae and Their Importance**

# Biological Importance of Marine Algae

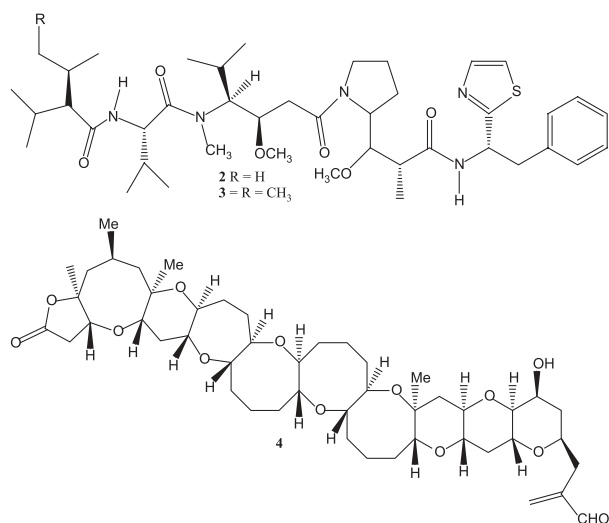
Department of Pharmacognosy, College of Pharmacy, King Saud University, KSA

Algae are a heterogeneous group of plants with a long fossil history. Two major types of algae can be identified: the macroalgae (seaweeds) occupy the littoral zone, which included green algae, brown algae, and red algae, and the microalgae are found in both benthic and littoral habitats and also throughout the ocean waters as phytoplankton (Garson, 1989). Phytoplankton comprise organisms

The true origins of compounds found in marine invertebrates have been a subject of discussion. They may vary from compound to another, but there are strong hints that dietary or symbiotic algae are one of the participants in the production of these metabolites. For example, as early as 1977, the blue-green algae, *Lyngbya majuscula* was recognized as the source of aplysiatoxin 1 found in the sea hares *Aplysia* that feed on this alga (Mynderse *et al.*, 1997). Similarly, a series of highly active antitumor compounds, dolastatin 2 and 3, isolated from sea slugs are considered to be of blue-green algal origin (Shimizu, 2000). Also, eukaryotic algae and various dinoflagellate metabolites are found in shellfish and other invertebrates as toxins (Shimizu, 2000). Brevetoxins 4, ciguatoxins 5, and dinophysistoxins-1&2 and 6 and 7 are well known examples of paralytic shellfish toxins (Hall and Strichartz, 1990).



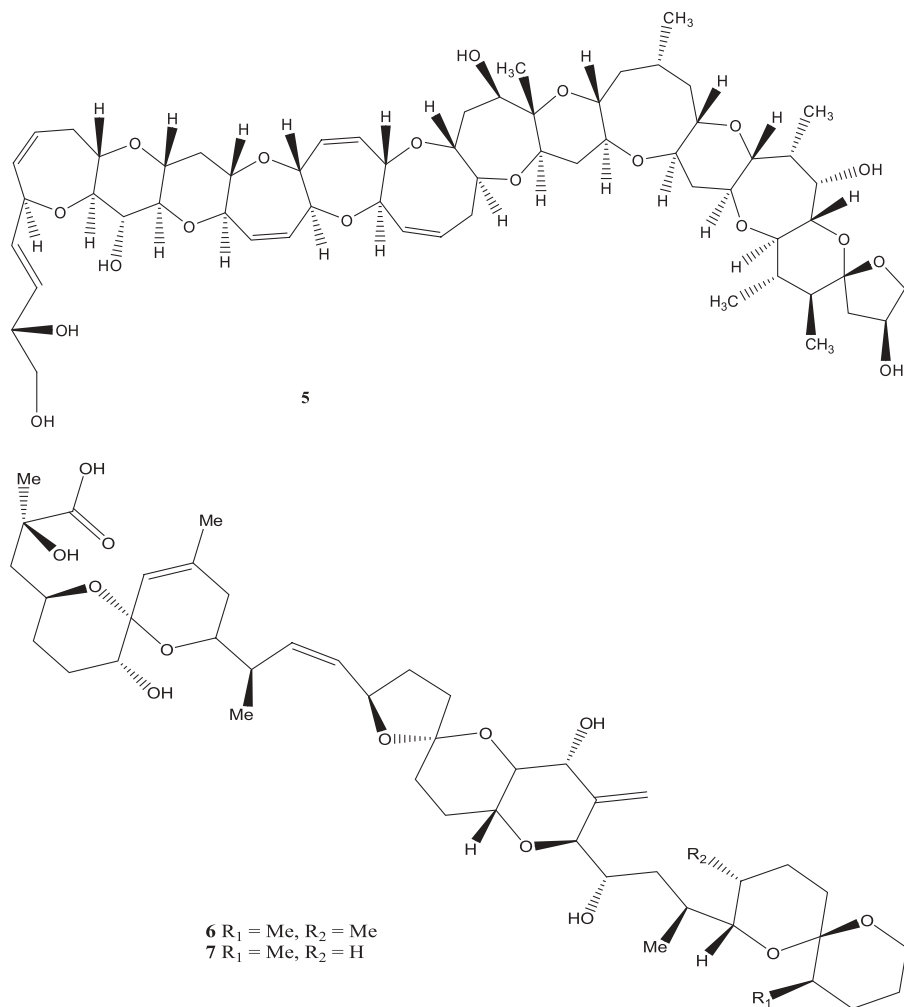
*Handbook of Marine Macroalgae: Biotechnology and Applied Phycology*, First Edition. Edited by Se-Kwon Kim.  
© 2012 John Wiley & Sons, Ltd. Published 2012 by John Wiley & Sons, Ltd.



## 1.2 Interesting natural products and their biological activities from macroalgae (seaweeds)

Marine macroalgae or seaweeds have been used as foods especially in China and Japan and crude drugs for treatment of many diseases such as iodine deficiency (goiter, Basedow's disease and hyperthyroidism). Some seaweeds have also been used as a source of additional vitamins, treatment of various intestinal disorders, as vermifuges, and as hypocholesterolemic and hypoglycemic agents. Seaweeds have been employed as dressings, ointments and in gynecology (Trease and Evanes, 1996).

Macroalgae can be classified into three classes: green algae (Chlorophyta), brown algae (Phaeophyta) and red algae (Rhodophyta) (Garson, 1989).

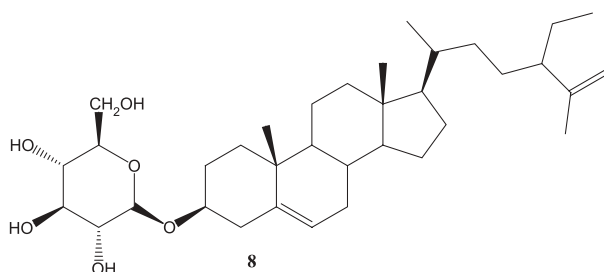


### 1.2.1 Chlorophyta (green algae)

The characteristic green color of green algae is mainly due to the presence of chlorophyll *a* and *b* in the same proportion like higher plants (Bold and Wynne, 1985). There are few reports of novel secondary metabolites among the Chlorophyta than the other algal division; the following are the most important biologically active natural products isolated from these algae.

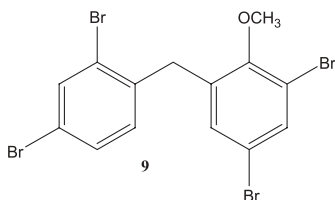
#### Anti-inflammatory substances

An anti-inflammatory, 3- $\beta$ -D-glucopyranosylstigmasta-5,25-diene **8** have been isolated by Awad in 2000 (Awad, 2000) from the green alga *Ulva lactuca*.



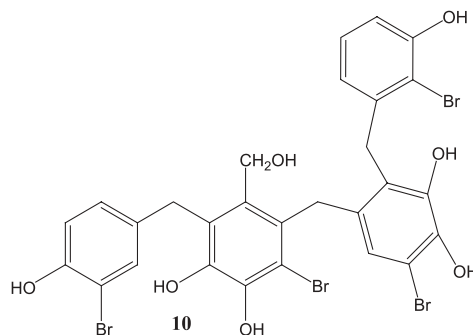
Habu is a deadly snake found in Okinawa where 200–300 people are bitten by the snake every year. A patient must be given immediate medical treatment with the serum prepared from a horse-developed antibody by injection of snake toxin. However, about 20% of the patients are allergic to the serum.

In order to develop an alternative drug, Okinawa Prefectural Institute of Public Health has been conducting screening strategies to find a compound with anti-inflammatory activity, which can be measured by the suppression of inflammation caused by the injection of toxin into a mouse limb. A diphenyl ether **9** isolated from an alga was found to be effective in this assay (Higa, 1989). The extract of the green alga *Cladophora fascicularis* was separated by different chromatographic methods to produce 2-(2',4'-dibromophenoxy)-4,6-dibromoanisole (Kuniyoshi, Yamada and Higa, 1985), the first example of diphenyl ether from green algae. It was also active in inhibiting the growth of *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Kuniyoshi, Yamada and Higa, 1985).

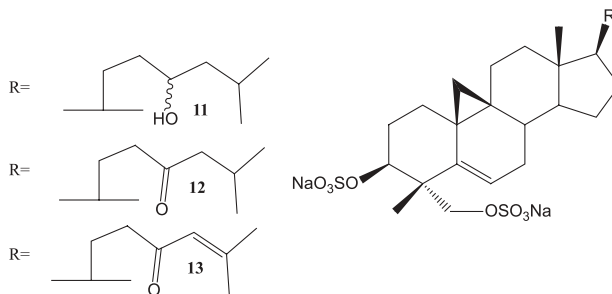


#### Cytotoxic and immunosuppressive activities

Bioassay-guided fractionation utilizing inhibitory activity against inosine 5'-monophosphate dehydrogenase inhibitor (IMPDH) leads to the isolation of a new brominated diphenylmethane derivative. Isorawsonol **10** was isolated from the tropical green alga *Arrainvillia rawsonii* by Chen and colleagues in 1994 (Chen *et al.*, 1994). The activity of IMPDH has been linked with cellular proliferation and inhibition of that enzyme has been demonstrated to have anticancer and immunosuppressive effects (Chen *et al.*, 1994).



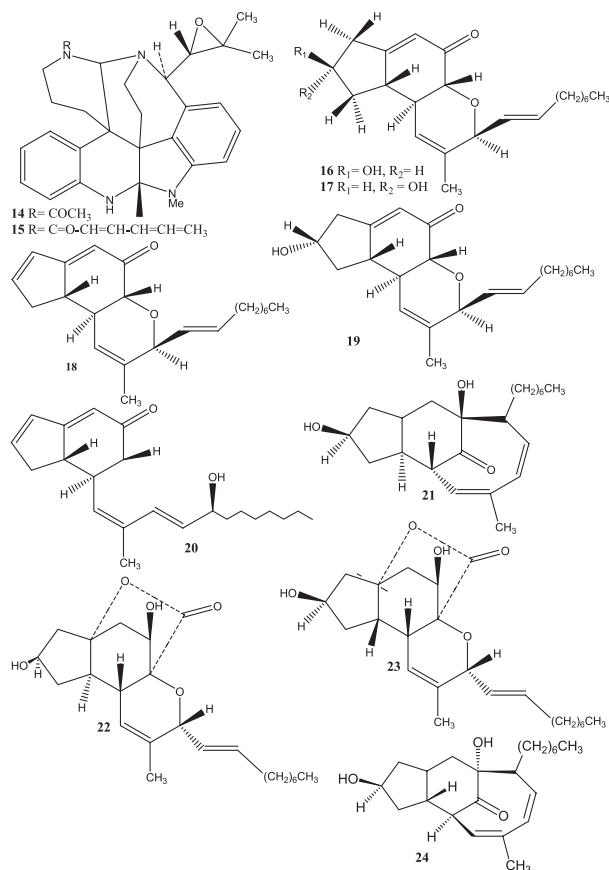
Bioactivity-directed fractionation of the extract of the green alga *Tydemania expeditionis* using the protein tyrosine kinase pp60<sup>v-src</sup> led to the isolation of three new cycloartenol disulfates **11–13**; they showed modest inhibition of this enzyme (Govindan *et al.*, 1994).



Communesins A **14** and B **15**, exhibiting cytotoxic activity against cultured P-388 lymphocytic leukemia cells, were isolated from the mycelium of a strain of *Penicillium* species stuck on the marine alga *Enteromorpha intestinalis* (Numata *et al.*, 1993).

Penostatins A **16**, B **17**, C **18**, D **19** (Takahashi *et al.*, 1996) and E **20** (Iwamoto *et al.*, 1999) have been isolated from a strain of *Penicillium* species originally separated from the marine alga *Enteromorpha intestinalis* (L.) Link (Ulvaaceae). The compounds A–C and E exhibited significant cytotoxicity against the cultured P388 cell line (Iwamoto *et al.*, 1999; Takahashi *et al.*, 1996). Penostatins F, G, H **21–23** and I **24**

were isolated from a strain of *Penicillium* originally separated from the marine alga *Enteromorpha intestinalis* (L.) Link (Ulvaceae). All the compounds exhibit significant cytotoxicity against cultured P388 cells (Iwamoto *et al.*, 1998).

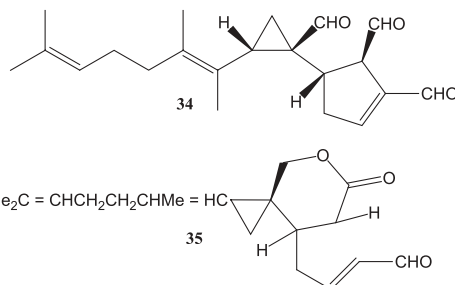
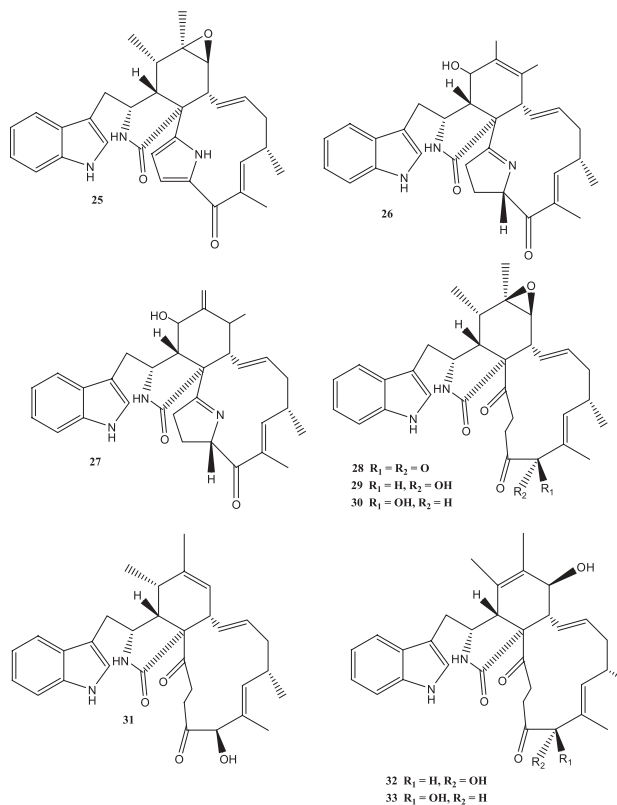


The novel compounds cytochalasins, penochalasins A–C 25–27 (Numata *et al.*, 1996), D–H 28–32, and chaetoglobosin O 33 (Iwamoto *et al.*, 2001) were isolated from a strain of *Penicillium* species originally separated from the marine alga *Enteromorpha intestinalis*. All these compounds exhibited potent cytotoxic activity against cultured P388 cells.

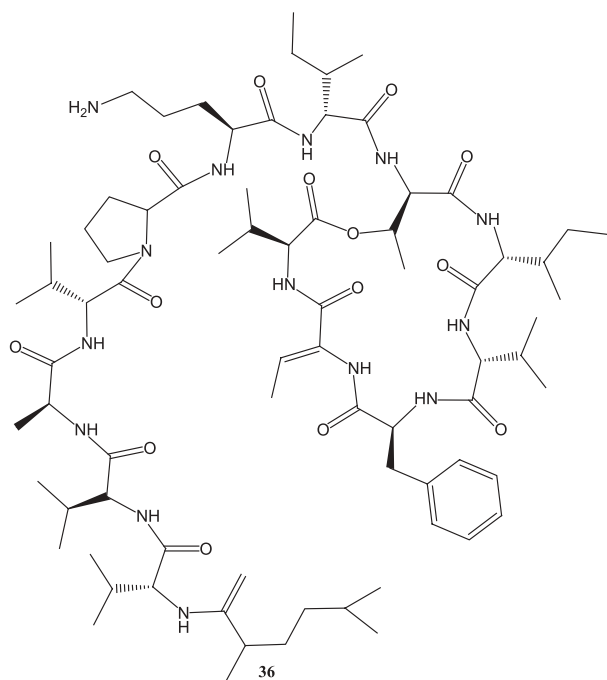
Four new diterpenoid metabolites were isolated from several species of the green algae *Halimeda* (Udoteaceae). These new compounds show potent antimicrobial and cytotoxic properties in bioassays. Among these four compounds were halimediatrial 34 and halimedalactone 35 (Paul and Fenical, 1983). Halimediatrial 34 is a diterpene trialdehyde that was extracted from *Halmida lamouroux* (Chlorophyta, Udoteaceae) species. This compound was found to be toxic

towards reef fishes, and significantly reduces feeding in herbivorous fishes (Paul and Fenical, 1983).

The cyclic depsipeptide kahalalide F 36 was originally isolated from both the mollusc *Elysia rufescens* and from the dietary source, the green alga *Bryopsis* sp. (Hamann and Scheuer, 1993) was introduced into Phase I trials by Pharma Mar as a lead compound against prostate cancer.



The green alga *Bryopsis* sp. was the source of the cyclic depsipeptides kahalalide P 37 and Q 38, with moderate inhibition of the HL-60 cell lines (Dmitrenok *et al.*, 2006).

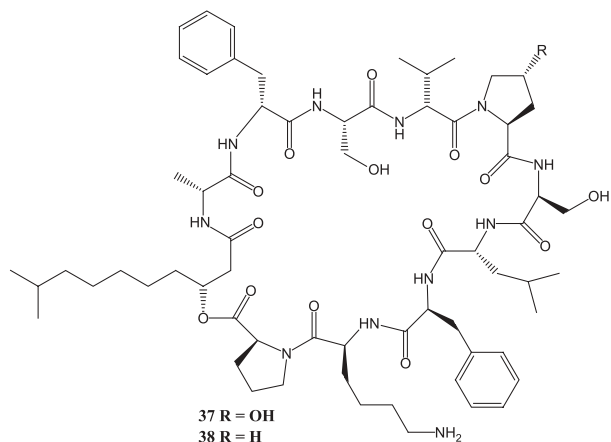


### Antibacterial activity

Cycloeudesmol **39** is an antibiotic cyclopropane containing sesquiterpene; it was isolated from the marine alga *Chondria oppositoclada* Dawson (Fenical and Sims, 1974). Cycloeudesmol was found to be a potent antibiotic against *Staphylococcus aureus* and *Candida albicans*.

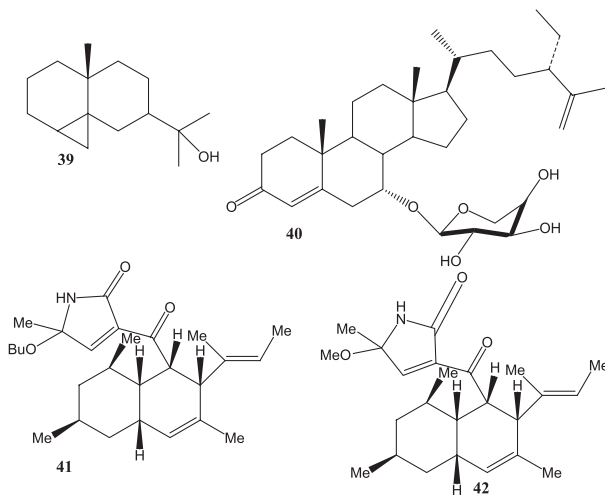
Lyengaroside A **40** was isolated from the green alga *Codium iyengarii* and displayed a moderate antibacterial activity (Ali *et al.*, 2002).

Green algae extract of *Caulerpa prolifera* exhibited moderate to significant activity against unidentified strains of marine bacteria (Smyrniotopoulos *et al.*, 2003).



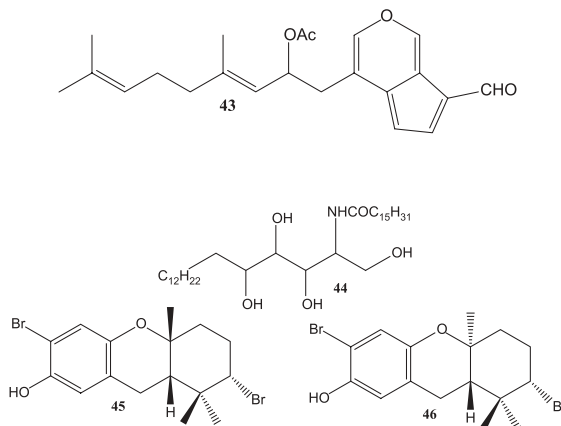
### Antiplasmodial activity

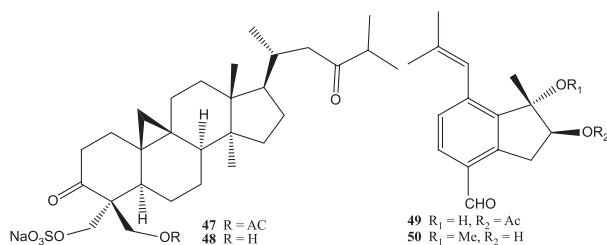
The endophytic and obligate marine fungus *Ascochyta salicorniae* was isolated from the green alga *Ulva* spp.. *Ascochyta salicorniae* was found to produce the unprecedented and structurally unusual tetrameric acid contiguous metabolites ascosalipyrrolidinones A **41** and B **42**. Ascosalipyrrolidinone A **41** has antiplasmodial activity toward *Plasmodium falciparum* strains K1 and NF-54, as well as showing antimicrobial activity and inhibiting tyrosine kinase p56lck (Osterhage *et al.*, 2000).



### Antiviral activity

Halitunal **43** is a novel diterpene aldehyde possessing a unique cyclopentadieno [c] pyran ring system; it has been isolated from the marine alga *Halimeda tuna*. Halitunal shows antiviral against murine coronavirus A59 *in vitro* (Koehn *et al.*, 1991).





In 1992 Garg and coworkers (Garg *et al.*, 1992) isolated the antiviral derivative, sphingosine, *N*-palmitoyl-2-amino 1,3,4,5-tetrahydroxyoctadecane **44**, which demonstrated antiviral activity and *in vivo* protection against Semliki forest virus (SFV). This compound was isolated from the Indian green alga *Ulva fasciata*.

#### Antimutagenic activity

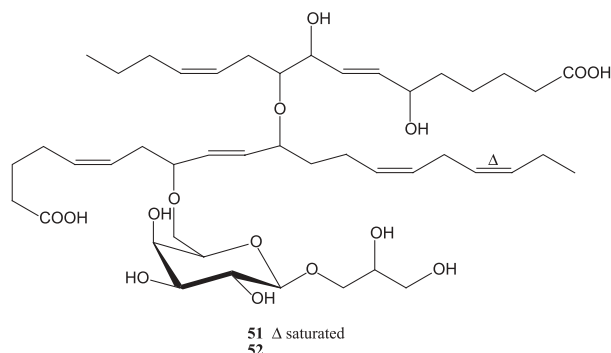
Two new compounds, cymobarbatol **45** and 4-isocymobarbatol **46** were isolated from the marine green alga *Cymopolia barbat*. Both compounds were found to be non-toxic over a broad concentration range against *Salmonella typhimurium* strains T-98 and T-100. Both compounds exhibited strong inhibition of the mutagenicity of 2-aminoanthracene and ethylmethanesulfonate towards, respectively, the T-98 strains plus a metabolic activator and T-100 (Wall *et al.*, 1989).

#### Antifungal activity

Capisterones A **47** and B **48** are triterpene sulfate esters isolated from the green alga *Penicillus capitatus*. Both compounds exhibited potent antifungal activity against the marine algal pathogen *Lindra thallasiae* (Puglisi *et al.*, 2004).

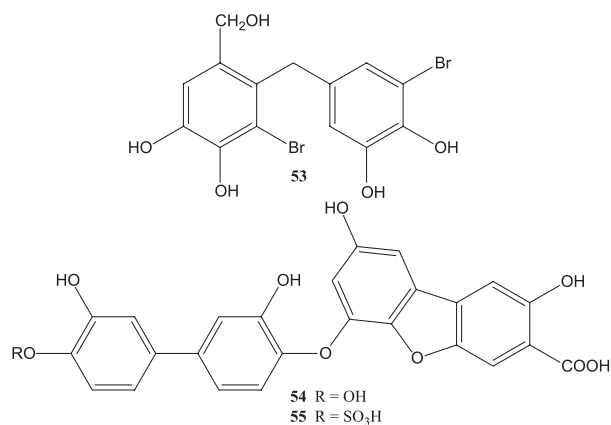
Two sesquiterpenes, caulerpals A **49** and B **50** were isolated from green alga *Caulerpa taxifolia* in addition to the known caulerpin (Aguilar-Santos, 1970); they were shown to be potent inhibitors of human protein tyrosine phosphatase 1 B (hPTP I B) (Mao, Guo and Shen, 2006). Capisterones A **47** and B **48**, originally isolated from *Penicillus capitatus* (Garg *et al.*, 1992), were re-isolated and absolute stereochemistry assigned using electronic CD. In addition, the capisterones have been shown to significantly enhance fluconazole activity in *Saccharomyces cerevisiae* (Li *et al.*, 2006).

A new class of ether-linked glycolipids, nigricanosides A **51** and B **52** were isolated as methyl esters from the green alga *Avrainvillea nigrans*. Nigricanoside A dimethyl ester was found to be a potent antimetabolic agent, acting by stimulating the polymerization of tubulin and inhibiting the proliferation of both MCF-7 and HCT-116 cells (Williams *et al.*, 2007).



#### Protein tyrosine phosphatase 1B inhibitors (PTP1B)

Hydroxyisoavrainvilleol **53** was originally isolated from the tropical green alga *Avrainvillea nigrans* (Colon *et al.*, 1987) but has now been isolated from red alga *Polysiphonia urceolata* as a protein tyrosine phosphatase 1B inhibitor (PTP1B) (Liu *et al.*, 2008). A vanillic acid biphenyl derivative **54** and the sulfate adduct **55** were isolated from the Australian green alga *Cladophora socialis* as a protein tyrosine phosphatase 1B (PTPa1B) inhibitor (Feng *et al.*, 2007).



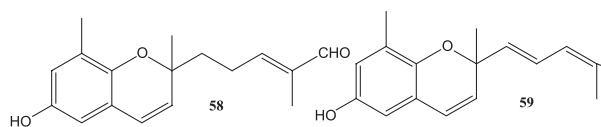
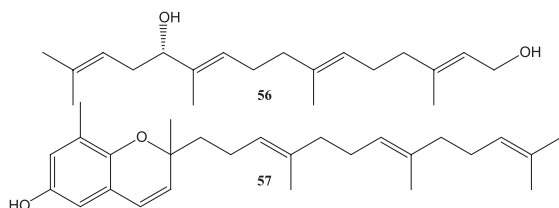
### 1.2.2 Phaeophyta (brown algae)

The brown color of these algae results from the dominance of the xanthophyll pigments and fucoxanthin; this masks the other pigments, chlorophyll *a* and *c*,  $\beta$  carotenes, and other xanthophylls (Bold and Wynne, 1985). Food reserves of brown algae are typically complex polysaccharides and higher alcohols. The principal carbohydrate reserve is laminaran. The cell walls are made of cellulose and alginic acid. Many bioactive metabolites have been isolated from brown algae with different pharmacological activities as shown below:

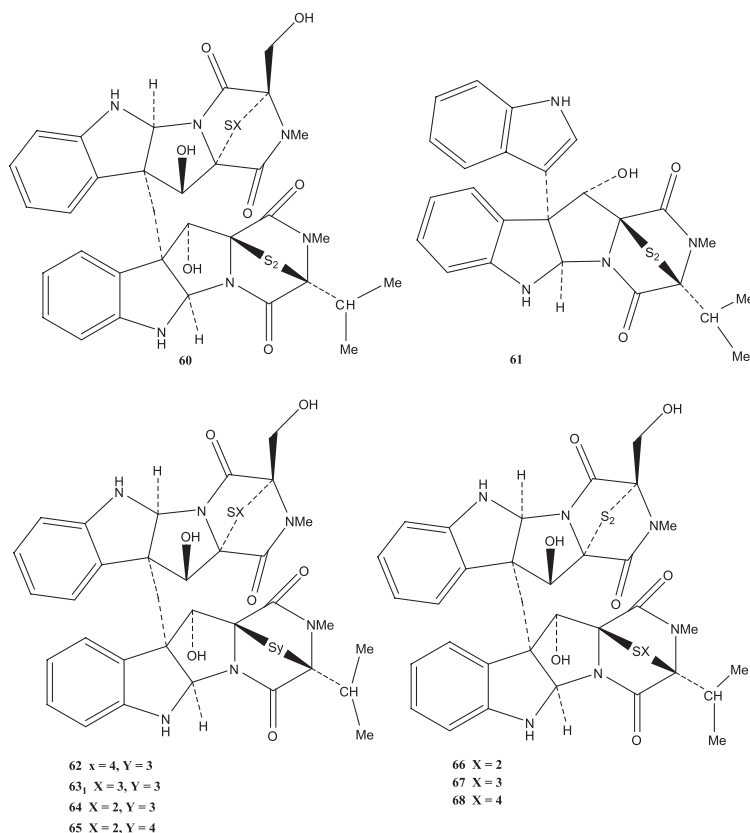
### Cytotoxic and antitumor activity

A linear cytotoxic diterpene bifurcadiol **56** was isolated from the brown alga *Bifurcaria bifurcata* by Guardia and colleagues in 1999 (Guardia *et al.*, 1999), which exhibits cytotoxicity against cultured human tumor cell lines (A549, SK-OV-3, SKL-2, XF 498, and HCT).

Meroterpenoids, sargol, sargol-I and sargol-II **57–59** were isolated from the brown alga *Sargassum tortile* and showed cytotoxic activity (Numata *et al.*, 1991).



Leptosins A, B, C (I, X = 4,3,2 **60**), D, E and F (II, X = 2,3,4 **61**), belonging to a series of epipolythiodioxopiperazine derivatives, have been isolated from the mycelia of a strain of *Leptosphaeria* species attached to marine alga *Sargassum tortile*. All these compounds showed potent cytotoxicity against cultured P388 cells, except leptosins A and C, which exhibited significant antitumor activity against sarcoma 180 ascites (Takahashi *et al.*, 1994). Further investigation of the secondary metabolites of this fungus has led to the isolation of four additional cytotoxic compounds, named leptosins G, G1, G2 **62–64** and H **65** (Takahashi *et al.*, 1995a). Leptosins K, K1 **66–67** and K<sub>2</sub> **68** were also isolated and showed a potent cytotoxic activity against P388 cell line (Takahashi *et al.*, 1995b).



Leptosins I **69** and J **70** have been also isolated from the mycelia of a strain of *Leptosphaeria* species OUPS-4 attached to the marine alga *Sargassum tortile*. These compounds exhibited significant cytotoxic activity against cultured P388 cells (Takahashi *et al.*, 1994a,b).

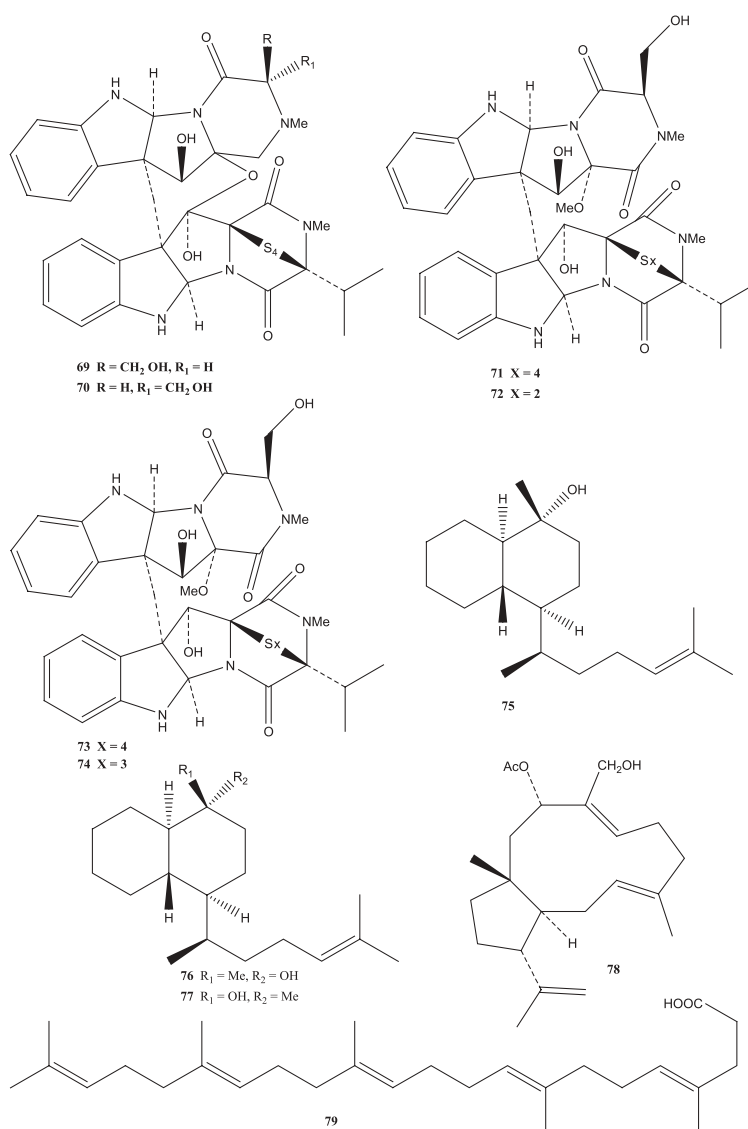
Leptosins M, MI, N and N1 **71–74** that have been isolated from a strain of *Leptosphaeria* species were originally separated from the marine alga *Sargassum tortile*. All these compounds exhibited significant cytotoxicity against cultured P388 cells. In addition, leptosin M proved to exhibit significant cytotoxicity against human cancer cell lines, and to

inhibit specifically two protein kinases, PTK and CaMKII, and human topoisomerase II (Yamada *et al.*, 2002).

Three cytotoxic diterpenes dictyotins A, B and C **75–77** were isolated from the brown alga *Dictyota dichotoma* by Wu and coworkers in 1990 (Wu, Li and Li, 1990).

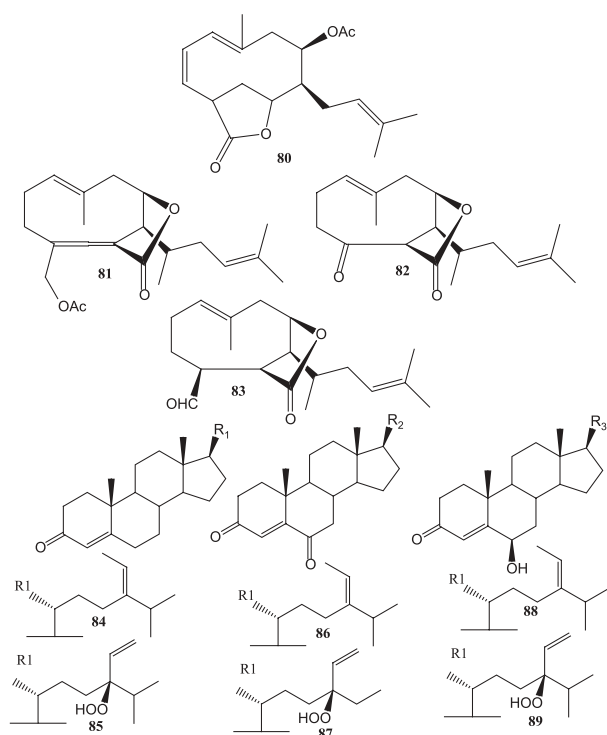
Dolabellane, a type of diterpene **78**, has been isolated from unidentified species of *Dictyota* and exhibits significant cytotoxicity. (Tringali, Prattellia and Nicols, 1984).

A cytotoxic compound named as turbinaric acid **79** was isolated from *Turbinaria ornate* (Asari, Kusumi and Kaki-sawa, 1989).



Four diterpenes with xenicane and norxenicane **80–83** have been isolated from another species of *Dityota dichotoma* from Okinawa Island. In addition, they showed antitumor activity.

24-Ethylcholesta-4,24(28)-diene 3-one **84**, 24-ethylcholesta-4,28(29)-diene-3-one **85**, 24-ethylcholesta-4,24(28)-diene-3,6-dione **86**, 24 $\beta$ -hydroperoxy-24-ethylcholesta-4,28(29)-diene-3, 6-dione **87**, 60-hydroxy-24-ethylcholesta-4,24(28)-diene-3-one **88**, 24-hydroperoxy-6 $\beta$ -hydroxy-24-ethylcholesta-4,28(29)-diene-3-one **89** were isolated from the brown alga *Turbinaria conoides*. These oxygenated fucosterols exhibited cytotoxicity against various cancer cell lines (Sheu *et al.*, 1999) including P-388, KB, A-549 and HT-29.

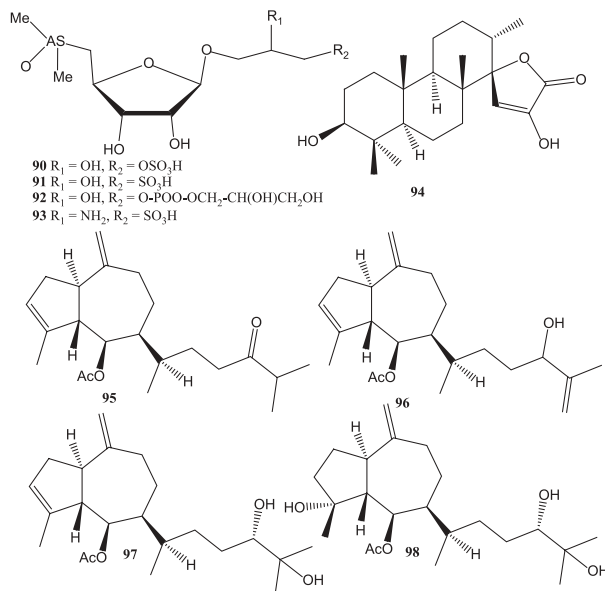


Four arsenic-containing ribofuranosides **90–93** together with inorganic arsenic have been isolated from the brown alga *Hizikia fusiforme*, which is eaten in Japan under the name hijiki (Edmonds, Morita and Shibata, 1987).

Stypolactone **94**, a diterpenoid of mixed biogenesis, has been isolated from the brown algae *Stypopodium zonale* and showed weak cytotoxic activity *in vitro* against the A-549 and H-116 cell lines (Dorta *et al.*, 2002).

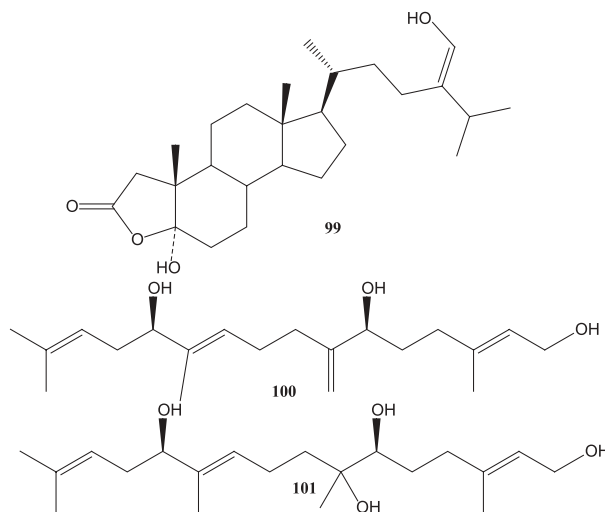
Four hydroazulene diterpenes, dictyone acetate **95**, dictyol F monoacetate **96**, isodictyol monoacetate **97**, and cystoseirol monoacetate **98** were isolated from the brown alga *Cystoseira myrica* collected in the Gulf of Suez showed

a moderate cytotoxicity against the murine cancer cell line KA3IT, but reduced cytotoxicity against normal NIH3T3 (Ayyad *et al.*, 2003).

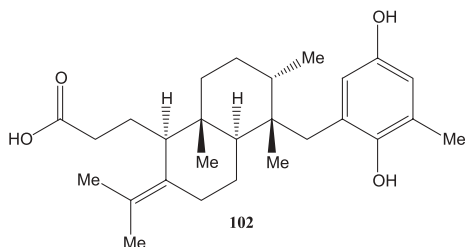


Sterols **99** isolated from *Stypopodium carpophyllum* exhibited cytotoxic activity against several cultured cancer cell lines (Tang *et al.*, 2002).

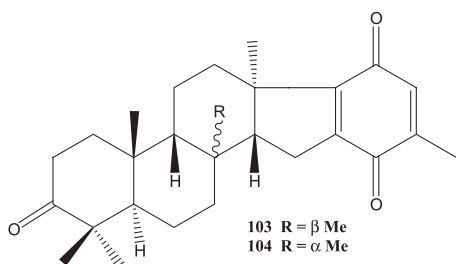
Two cytotoxic trihydroxylated diterpenes based on 12-hydroxygeranylgeraniol **100** and **101** were isolated from the brown alga *Bifurcaria bifurcata* (Gulioli *et al.*, 2004).



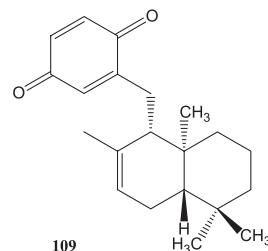
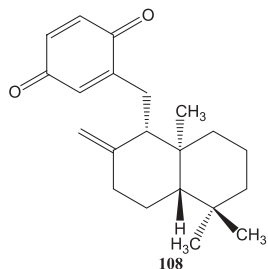
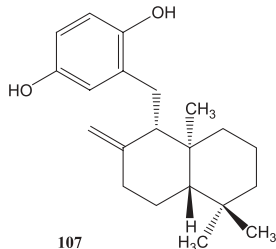
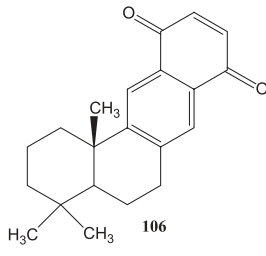
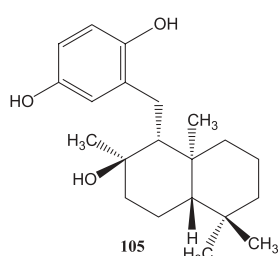
The tropical brown alga *Stypopodium zonale* collected from the coast of Tenerife was the source of terpenoid **C 102**; the methyl ester of C exhibited *in vitro* cytotoxic activity against HT-29, H-116 and A-549 (Dorta *et al.*, 2002).



The brown alga *Taonia atomaria* was a source of meroditerpenes atomarianones A **103** and B **104**, cytotoxic agents against the NSCLC-N6 and A-549 cell lines (Abatis *et al.*, 2005).



(+)-Yahazunol **105** (Ochi *et al.*, 1979) and cyclozonarone **106** (Kurata, Tanguchi and Suzuki, 1996) were showed cytotoxic activity against several human tumor cell lines, while zonarol **107**, zonarone **108** and isozonarol **109** (Fenical *et al.*, 1973) isolated from brown algae also displayed cytotoxicity against various human tumor cell lines (Laube, Beil and Seifert, 2005).



The brown alga *Perithalia capillaris* yielded new bis-prenylated quinones **110**, **111**, both are inhibitors of superoxide production in human neutrophils *in vitro* and of proliferation of HL-60 cells (Blackman, Dragar and Wells, 1979).

Two diterpenes, 4,18-dihydroxydictyolactone **112** and 8 $\alpha$ ,11 dihydroxypachydictyol A **113**, were isolated from a *Dictyota* sp. (Jongaramruong and Kongkam, 2007). In bioassays, 4,18-dihydroxydictyolactone was strongly cytotoxic (NCI-H187) (Jongaramruong and Kongkam, 2007).

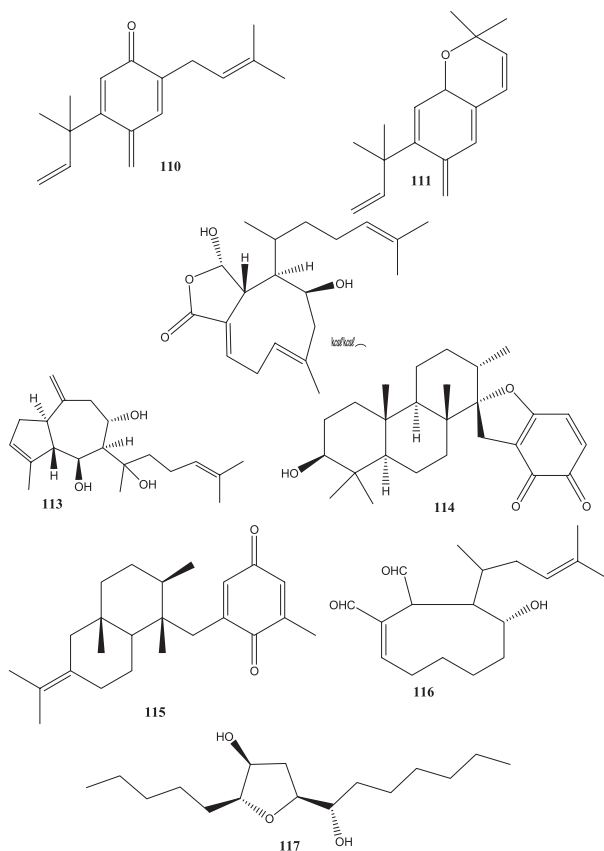
#### *Ichthyotoxins and feeding-deterrent substances from brown algae*

Stypoldione **114** was isolated from the brown alga *Stypodium zonale*, which showed an ichthyotoxic effect. When fresh *S. zonale* is placed in an aquarium, water soon turns to a rust color and is rendered extremely toxic to the reef-dwelling herbivorous dam shellfish *Eupomacentrus leucostictus*. The fish immediately senses the toxins and attempts to jump out of the aquarium. This behavior is followed by erratic response to external stimuli, apparent difficulty in obtaining oxygen, loss of equilibrium, narcosis and eventually death. The toxic symptoms were then proved to be due to stypoldione isolated from *S. zonale* (Gerwick *et al.*, 1979). Styloquinonic acid **115** was isolated from the lipophilic extract of the same alga (Wessels, Konig and Wright, 1999) and showed inhibition of tyrosine kinase p56<sup>lck</sup> enzyme. Tyrosine kinase inhibitory activity was determined by enzyme-linked immunosorbent assay using a commercial test kit (Wessels, Konig and Wright, 1999).

The brown alga *Dictyota spinulosa* appeared not to be eaten by herbivores so that its constituents were examined by Tanaka and Higa in 1984 (Tanaka and Higa, 1984) and they isolated a new diterpene, hydroxydictyodial **116** as a major component among several other related compounds. Hydroxydictyodial has also been isolated from *Dictyota crenulata* (Kirkup and Moore, 1983).

#### *Nematocidal activity*

Chemical analysis of the brown alga *Notheia anomala* collected from the rock platforms along the southern coast of



Australia yielded *cis*-dihydroxytetrahydrofuran **117** derivatives. Tetrahydrofuran from *Notheia anomala* are reported for the first time as potent and selective inhibitors of the larval development of the parasitic nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis* (Capon *et al.*, 1998).

### Antifungal activity

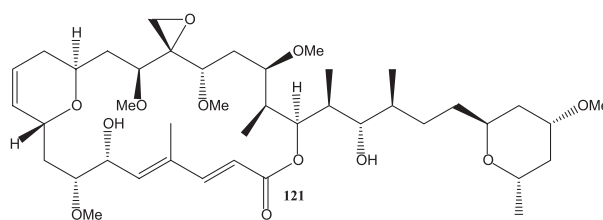
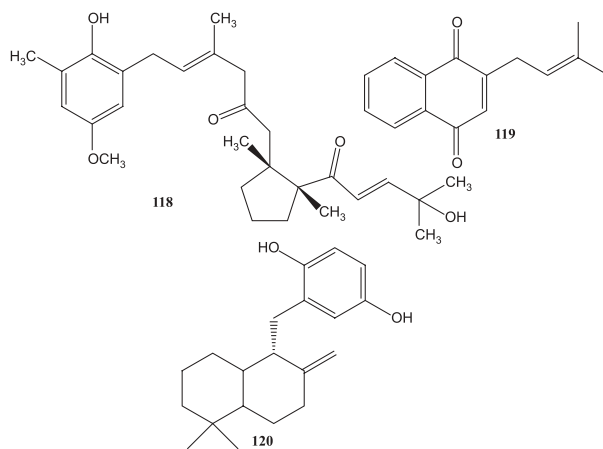
A meroditerpenoid has been isolated from the brown alga *Cystoseira tamariscifolia* and characterized as methoxybifurcarenone **118**. It possesses antifungal activity against three

tomato pathogenic fungi and antibacterial activity against *Agrobacterium tumefaciens* and *Escherichia coli* (Bennamara *et al.*, 1999).

A 1,4-naphthaquinone derivative (deoxylapachol) **119**, from a New Zealand brown alga *Landsburgia quercifolia* was isolated by the bioactivity-directed isolation method. It showed activity against P388 leukemic cells (IC<sub>50</sub> 0.6 µg/ml) and was also antifungal (Perry, Bluent and Munro, 1991).

An antifungal compound named as (+)-zonarol **120** was isolated from the brown alga *Dictyopteris zonaroides* by Fenical *et al.*, (1973).

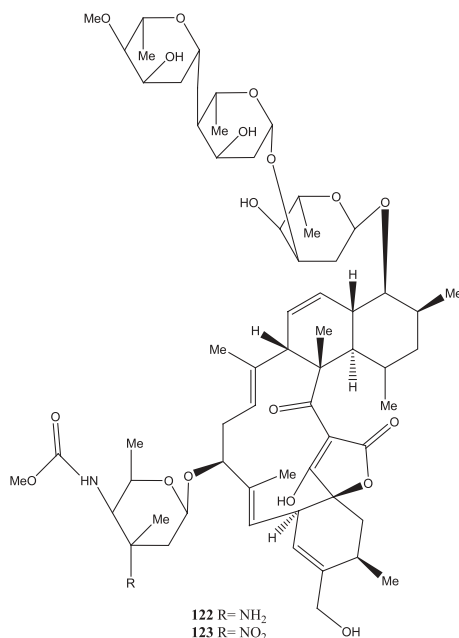
Lobophorolide **121** was isolated from the common brown alga *Lobophora variegata* and displayed a potent and highly specific activity against the marine filamentous fungi *Dendrophiella salina* and *Lindra thalassiae* and a potent activity against *C. albicans* and was also antineoplastic (Kubaneck *et al.*, 2003).



### Anti-inflammatory activity

Two new anti-inflammatory macrolides, lopophorins A **122** and B **123** have been isolated from the fermented broths of a marine bacterium isolated from the surface of the Caribbean brown alga *Lobophora variegata* (Dictyotales). The new compounds are distantly related to antibiotics of the Kijanimicin class and are potent inhibitors of tropical

PMA-induced edema in the mouse ear assay when administered either topically or intraperitoneally (Jiang, Jensen and Fenical, 1999).



(*Z*)-Sargaquinone **124**, the more saturated analog **125**, and the known sargaquinone (Ishitsuka *et al.*, 1979) were isolated from the brown alga *Taonia atomaria* and were anti-inflammatory agents by inhibition of leukotriene biosynthesis (Tziveleka *et al.*, 2005).

### Algicidal activity

A chlorine-containing perhydroazulene diterpene, dictyol J **126**, was isolated from the brown alga *Dictyota dichotoma* along with two known diterpenes, dictyolactone (Finer *et al.*, 1979) and sanadaol (Ishitsuka, Kusumi and Kakisawa, 1982). All three metabolites were algicidal to the bloom-forming species *Heterosigma akashiwo* and *Karenia mikimotoi*. Dictyolactone also displayed a moderate activity against the dinoflagellate *Alexandrium catanella*.

### Hepatoprotective activity

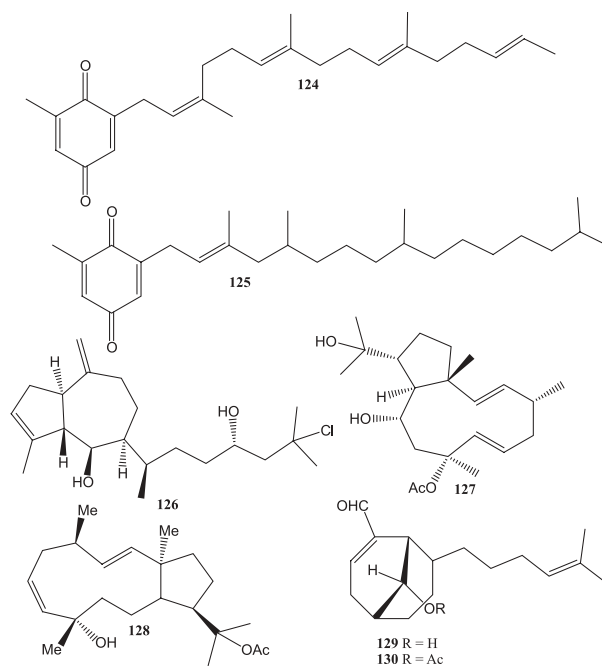
Phloroglucinol (Cross, Bevan and Briggs, 1907) and phloroglucinol derivatives eckstolonol (Kang *et al.*, 2003), eckol, phlorofucofuroeckol A (Fukuyama *et al.*, 1990) and

dieckol (Fukuyama *et al.*, 1983) were isolated from the brown alga *Ecklonia stolonifera* as hepatoprotective agents (Kim *et al.*, 2005).

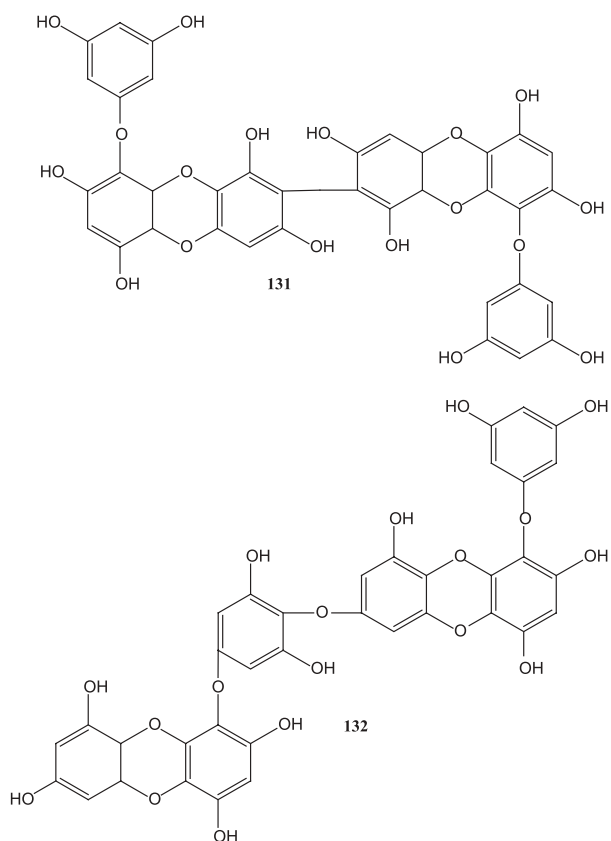
### Antiviral activity

A new dollabelladiene derivative **127** and the previously isolated 10,18-diacetoxy-8-hydroxy 2,6-dollabelladiene **128** (Ireland and Faulkner, 1977) were characterized from the brown alga *Dictyota pfaffi* (Barbosa *et al.*, 2004). Both compounds showed strong anti-human syncytial virus (HSV)-1 activity *in vitro* but little inhibition of human immunodeficiency virus (HIV)-1 reverse transcriptase.

The diterpenes (6*R*)-6-hydroxy dichototomo-3,14-diene-1,17-dial **129**, and the 6-acetate derivative **130**, from the brown alga *D. menstrualis* (Pereira *et al.*, 2004) exhibited antiretroviral activity *in vitro*.

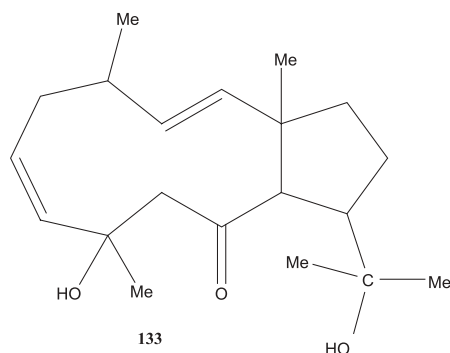


The phlorotannin derivatives 8,8'-bieckol **131** (Fukuyama *et al.*, 1989) and 8,4''-bieckol **132** from the brown alga *Ecklonia cava*, are inhibitors of HIV-1 reverse transcriptase (RT) and protease. Both compounds inhibited the RT more potently than the protease and the inhibitory activity of 8,8'-bieckol against HIV-I was comparable to that of a reference compound nevirapine.



### Protection against herbivorous animals

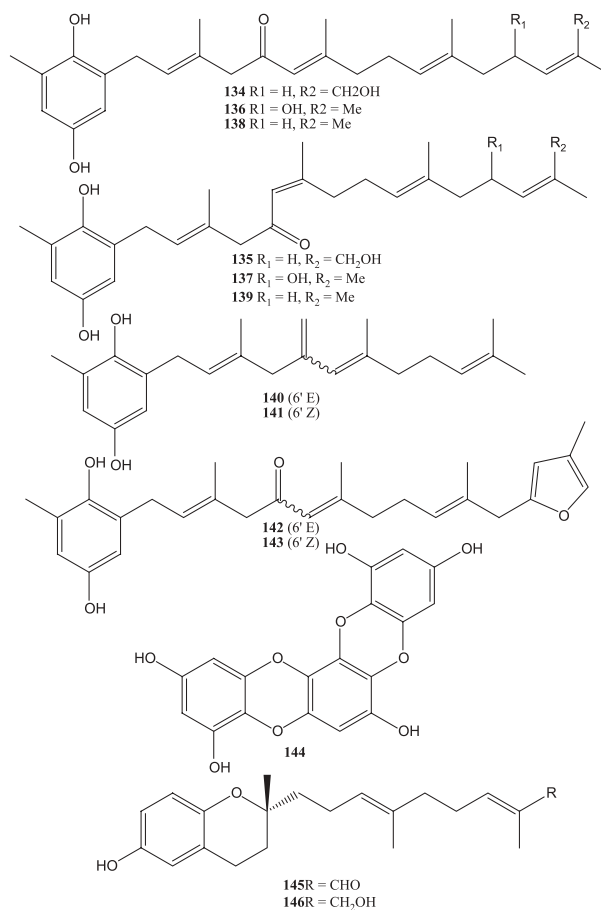
Dolabellane 1 **133**, originally isolated from the opisthobranch mollusk *Dolabella californica* (Ireland and Faulkner, 1977) has been characterized as the major secondary metabolite and active chemical defense against herbivores (sea urchins and fish) in the brown alga *Dictyota pfaffi* (Barbosa *et al.*, 2003).



### Free radical scavenger and antioxidant activities

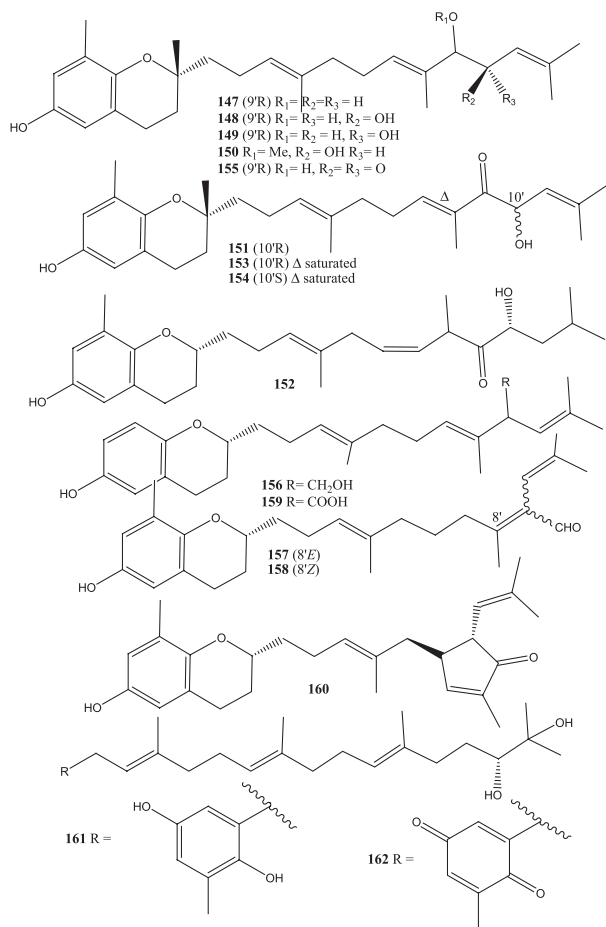
Several prenyl toluquinones were isolated from the brown alga *Cystoseira crinita*. Compounds **134–141** exhibited potent radical-scavenging effects while **142** and **143** were less active (Fisch *et al.*, 2003).

The brown alga *Ecklonia stolonifera* collected from South Korea yielded a new phlorotannin, eckstolonol **144**, which possessed a potent 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Kang *et al.*, 2003).



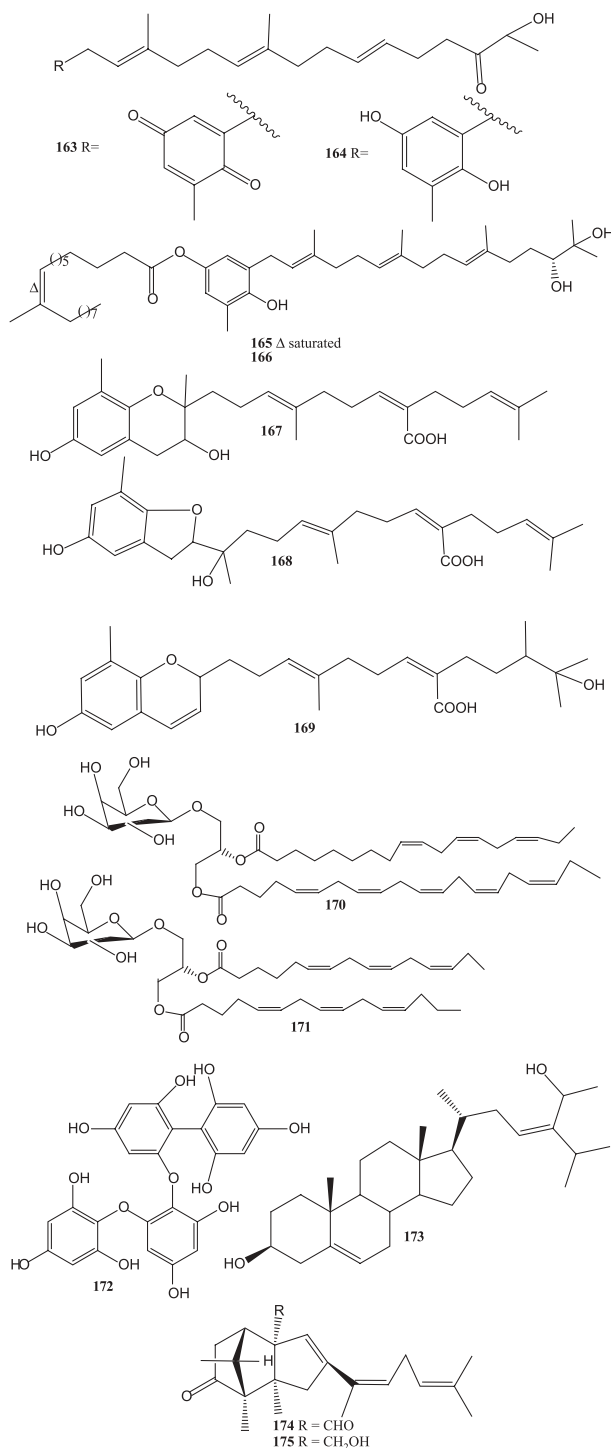
The sargachromanols A–P (compounds **145–160**, meroterpenoids of the chromene class, were isolated from the brown alga *Sargassum siliquastrum*. All the isolated compounds exhibited significant activity in the DPPH assay while compounds **151** and **159** were also inhibitors of butyl choline esterase (Jang *et al.*, 2005). The known plastoquinones (**161** and **162**) were isolated from the brown alga *S. micracanthum*. Compound **161** displayed significant antioxidant activity, while in contrast **162** was potentially

active against human cytomegalovirus (HCMV) *in vitro* (Iwashima *et al.*, 2005). *Sargassum micracanthum* (brown alga) was the source of strongly antioxidant plastoquinones **163–166**, while compounds **164–166** showed antiproliferative effects against 26-L5 cells (Mori *et al.*, 2005).



The tetraprenyltoluquinols, thunbergols **167** and B **168**, were isolated from the brown alga *Sargassum thunbergii* and were scavengers of the DPPH radical and of ONOO from morpholinolysynonimine (SIN-I) (Seo *et al.*, 2006).

Brown alga *Sargassum thunbergii* afforded a novel chromene, sargothunbergol A **169**, as a free radical scavenger (DPPH assay) (Seo, Park and Nam Bull, 2007). Two monogalactosyl diacylglycerols **170** and **171** were isolated from *S. thunbergii* (Kim *et al.*, 2007). Fucodiphlorethol G **172**, a tetrameric phlorotannin, was isolated from *Ecklonia cava*, and was a strong radical scavenger (DPPH assay) (Ham *et al.*, 2007).



The known compounds taondiol (Gonzalez, Darias and Martin, 1971) isoeptaondiol (Roviroso *et al.*, 1992) stypodiol, (Gerwick and Fenical, 1981), stypoldione (Gerwick *et al.*, 1979) and sargaol (Numata *et al.*, 1992), isolated

from the brown alga *Taonia atomaria* exhibited free radical-scavenging activity (DPPH and chemiluminescence tests) (Nahas *et al.*, 2007).

### Antidiabetic activity

*In vivo* testing of fucosterol, which was isolated from the brown alga *Pelvetia siliquosa*, demonstrated that it is the main antidiabetic principle from *Pelvetia siliquosa* (Lee *et al.*, 2004).

### Antihypertensive activity

Some known phlorotannins isolated from the brown alga *Ecklonia stolonifera*, namely eckol (Fukuyama *et al.*, 1983), phlorofucufuroeckol A (Fukuyama *et al.*, 1990) and dieckol (Fukuyama *et al.*, 1983) were shown to have marked inhibitory activity against angiotensin-converting enzyme (ACE) (Jung *et al.*, 2006).

### Morphological abnormality in a plant pathogen

*Stytopodium carpophyllum* from South China Sea was the source of two new bioactive sterols A **173** and B **99**. These sterols induced morphological abnormality in the plant pathogenic fungus *Pyricularia oryzae* (Tang *et al.*, 2002a).

### Antifeedent activity

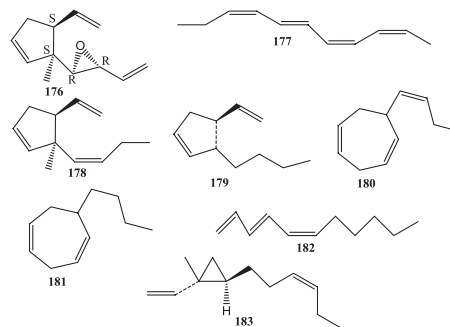
Two diterpenoids with a novel skeleton, diterpenoids A **174** and B **175**, were isolated from the brown alga *Dilophus okamurae* and displayed antifeedent activity against young abalone (Suzuki, Yamada and Kurata, 2002). 10,18-diacetoxy-8-hydroxy 2,6-dollabeladiene **128** (Ireland and Faulkner, 1977) was the antifeedent compound of brown alga *D. pfaffi* against the sea urchin *Lytechinus variegatus* and generalist fishes (Barbosa *et al.*, 2004).

### Gamete-releasing, gamete-attracting and sperm-attractants pheromone from brown algae

Most algae form some sort of spore, which is a cell that is often motile and serves to reproduce the organism. Algae also have sex, often a very simple kind of sex where the algae themselves act as gametes, but sometimes very complicated with egg and sperm-like cells.

(+)-Caudoxirene **176** is a new gamete-releasing and gamete-attracting pheromone isolated from brown alga *Perithalia cudata* (Muller *et al.*, 1988). Giffordene **177** is another gamete-attractant of brown algae *Giffordia* (*Hinksia mitchellae*) (Boland *et al.*, 1987). The female gametes of *Chorda tomentosa* secrete a mixture of multifidene **178**, 3-butyl 4-vinylcyclopentene **179**, ectocarpene **180** and (–)-dictyopterene C **181** that trigger an explosive

discharge of spermatozoid from ripe antheridia prior to chemotaxis (Maier *et al.*, 1984). Two sperm-attractants of *Cystophora siliquosa* and *Hormosira hanksii* were identified as cystophorene **182** and hormosirene **183** (Muller *et al.*, 1985).



## 1.2.3 Rhodophyta (red algae)

The red color of these algae results from the dominance of the pigments phycoerythrin and phycocyanin; these mask the other pigments, chlorophyll *a* (no chlorophyll *b*),  $\beta$ -carotene, and a number of unique xanthophylls (Bold and Wynne, 1985). The walls are made of cellulose, agars and carrageenans. Several red algae are eaten; amongst these is dulse (*Palmaria palmata*) and carrageen moss (*Chondrus crispus* and *Mastocarpus stellatus*). However, “Nori” popularized by the Japanese is the single most valuable marine crop grown by aquaculture with a value in excess of 1 US billion \$.

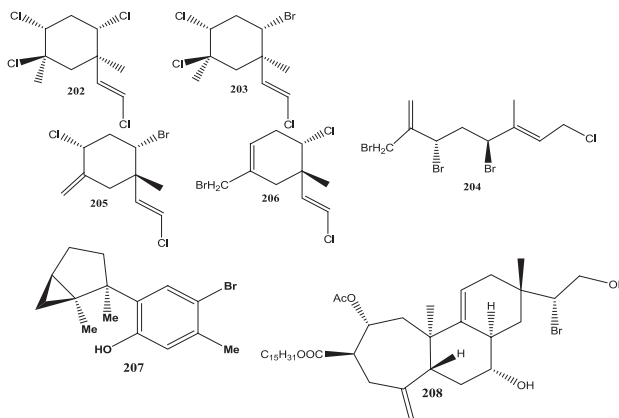
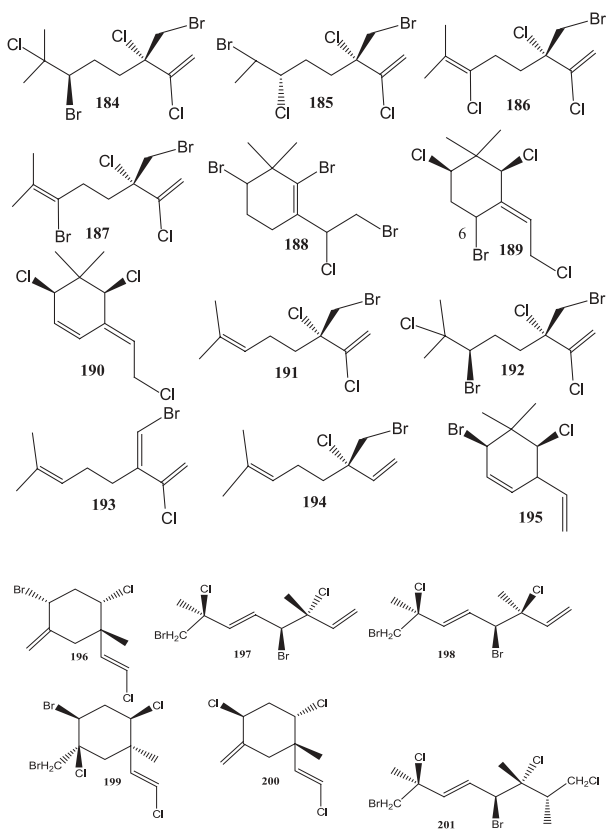
The red algae *Kappaphycus* and *Betaphycus* are now the most important sources of carrageenan, a commonly used ingredient in food, particularly yogurt, chocolate milk, and prepared puddings. *Gracilaria*, *Gelidium*, *Pterocladia*, and other red algae are used in manufacture of the all-important agar, used widely as a growth medium for microorganisms and biotechnological applications.

There are about 8000 species of red algae, most of which are marine. These are found in the intertidal and subtidal zones to depths of up to 40, or occasionally, 250 m. Red algae are considered as the most important source of many biologically active metabolites in comparison to the other algal class.

### Cytotoxic activity

Halmon **184** is a polyhalogenated monoterpene isolated from the red alga *Portieria hornemanii* and is considered as a novel *in vitro* antitumor agent by the National Cancer Institute (NCI). The NCI Decision Network Committee selected halmon as a preclinical drug for development

(Fuller *et al.*, 1992, 1994). Ten halogenated monoterpenes **185–194**, related to the novel antitumor compound halomon **184** or to the carbocyclic analog (Fuller *et al.*, 1992), have been isolated from different geographic collections of the red alga. These compounds were comparatively evaluated alongside compounds **184** and **190** in the US National Cancer Institute's *in vitro* human cancer cell line screening panel. The results provide insights into structure/activity relationships in this series as follows. Compounds **184–187** exhibited similar cytotoxicity to that reported earlier for **184** (Fuller *et al.*, 1992). These results suggested that halogen at C<sub>7</sub> was not essential to the activity. In contrast, compound **191** was relatively weakly cytotoxic and the minimally differential activity showed no significant correlation to that of **184**, indicating that a halogen at C<sub>6</sub> was essential for the characteristic activity of **184–187**. The halogen at C<sub>2</sub> was required for halomone-like activity. Carbocyclic compounds such as **188** and **195** were considerably less cytotoxic than **204–207**. Compound **189** was more comparable to the overall (panel-averaged) potency to halomon. However, there was little differential response of the cell lines, and consequently no significant correlation to the profile of **184**.

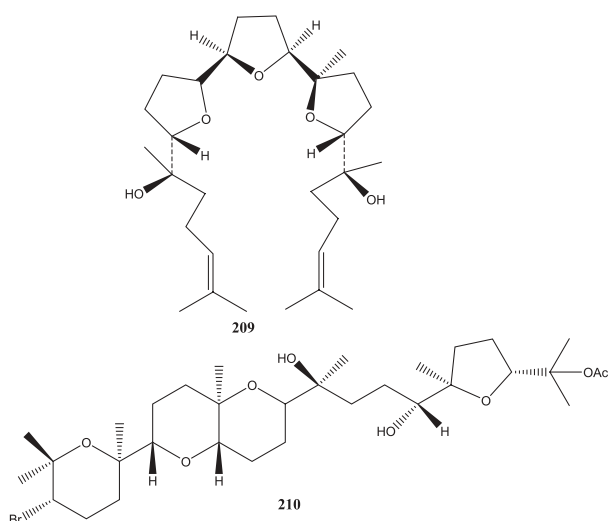


The polyhalogenated monoterpene content of six samples of the tropical marine red alga *Plocamium hamatum* **196–206**, collected from the southern, central and northern regions of the Great Barrier Reef, Australia was assessed. The biological activities of compounds **197–203** and **206** were assessed and indicated that compounds **199** and **201** have moderate cytotoxic activity. (Koing, Wright and Linden, 1999).

The invention of laurinterol (LOEL) **207**, which was isolated from *Laurencia okamurai* is considered as invention for the prevention and inhibition of melanoma (Moon-Moo, Sang-Hoon and Se-Kwon, 2009). LOEL can effectively inhibit the growth of melanoma cells by inducing apoptosis therein without adverse effect as in synthetic medicines. Thus, LOEL exhibited a dose-dependent inhibitory effect on the growth of melanoma cells as it was observed that cells are treated with LOEL at 10 µg/ml and the growth of melanoma cells by was inhibited 50%. Addition of 1 µg/ml of LEOL exerted 30% inhibition on the growth of melanoma cells in the presence of fetal bovine serum (FBS) (Moon-Moo, Sang-Hoon and Se-Kwon, 2009).

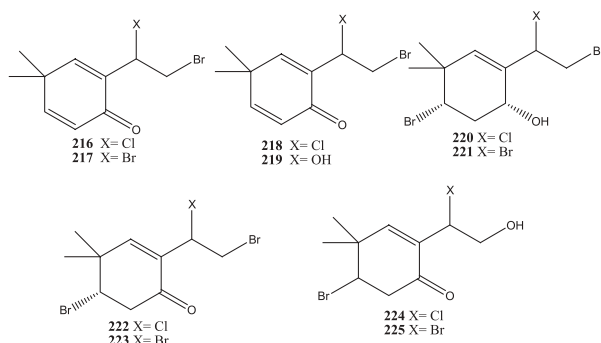
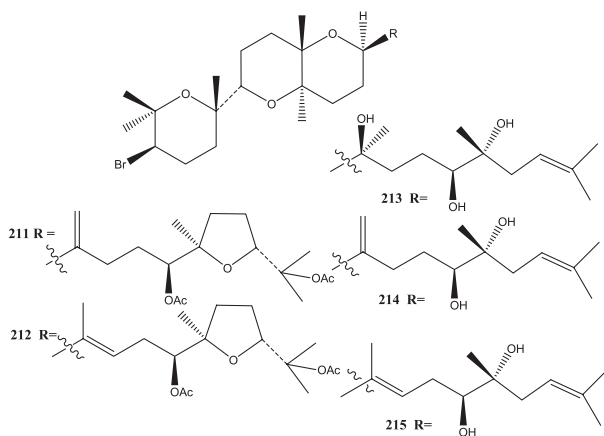
2-Acetoxy-15-bromo-6,17-dihydroxy-3-palmitoyl-neoparguera-4(19), 9(11)-diene **208**, a novel secoparguerane skeleton has been isolated from the red alga *Laurencia obtuse* from Okinawa and showed a cytotoxic activity (Cortes *et al.*, 1990).

Two new cyclic ethers consisting of squalene carbon skeleton, teurilene **209** and thysiferyl 23-acetate **210**, have been isolated from the red alga *Laurencia obtuse* (Suzuki *et al.*, 1985). Thysiferyl 23-acetate **210** (bromo ether) showed remarkable cytotoxic property (ED<sub>50</sub> of 0.3 µg/ml) against P388 *in vitro* cell line



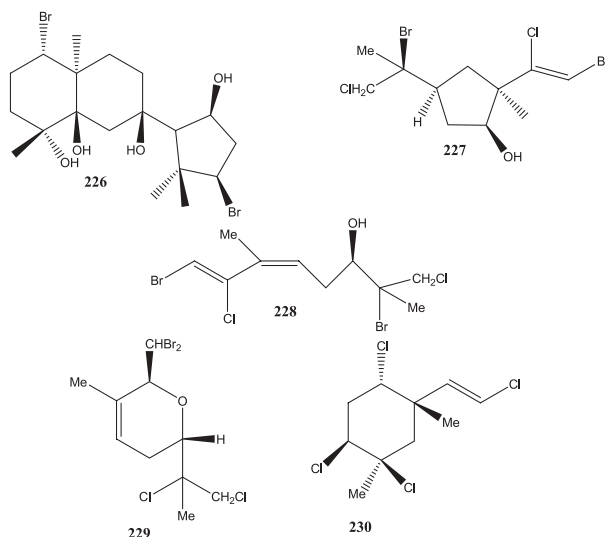
Five new cytotoxic triterpenes: triterpenoids 28-anhydrothysiferyl diacetate [15,28-didehydro-15-deoxythysiferyl] diacetate **211**, 15-anhydrothysiferyl diacetate [15,16-didehydro-15-deoxy-thysiferyl] diacetate **212**, magireol-A **213**, magireol B **214** and magireol C **215** were isolated from Japanese red alga *Laurencia obtuse* (Suzuki *et al.*, 1987).

Several cyclic monoterpenes **217–225** have been isolated from the Japanese red alga *Desmia hornemanni*, and some chemical modification has been done on these compounds to find the most active one for cytotoxic activity (Higa, 1985). Compound **216** exhibited relatively high activity against P388, A549 lung carcinoma, and HCT-8 human colon adenocarcinoma.



Okianwa red alga *Laurencia yonaguniensis* was the source of neoirietetraol **226**, a brominated diterpene based on the rare neoirieane skeleton; it was toxic to brine shrimp and was also active against marine bacteria *Alcaligenes aquamarinus* and *E. coli* (Takahashi *et al.*, 2002).

Europlacamioide C **227**, perfuroplacamioide **228**, pirene **229** and tetrachlorinated cyclohexane **230** from the red alga *Plocium carttilagineum* (Argandona *et al.*, 2002) exhibited selective cytotoxicity against human tumor cell lines with pirene showing a specific and irreversible effect on SW480 cells (de Ines *et al.*, 2004).

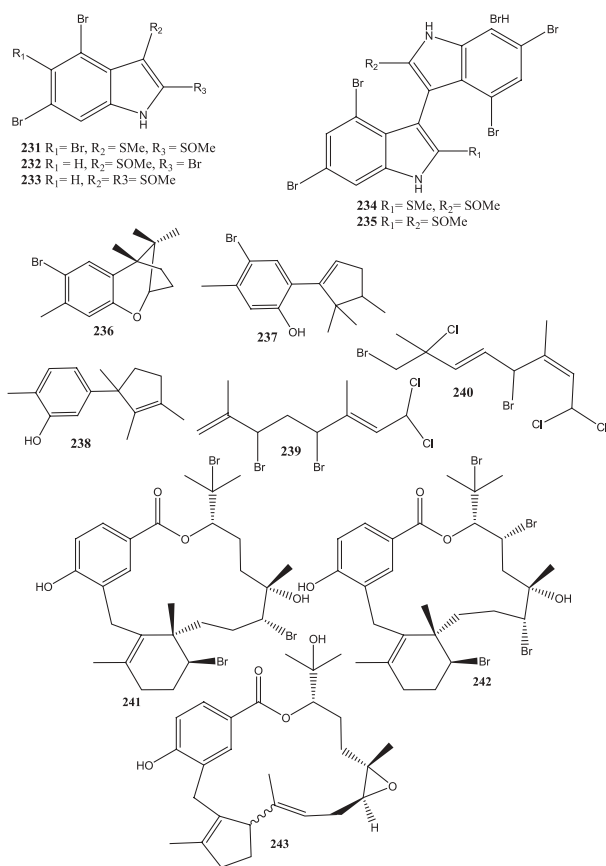


Five sulfur-containing polybromindoles **231–235** were isolated from the red alga *Laurencia brongniartii*, of which **234** and **235** were active against P388 cells and **234** against HT-29 cells (El Gamal, Wang and Duh, 2005). The cuparene

sesquiterpenes **236–238**, isolated from the red alga *L. microcladia* were cytotoxic against the NSCLC-N6 and A549 cancer cell lines. (Kladi *et al.*, 2005).

Plocaralides B **239** and C **240** isolated from *Plocamium* species (Steirle, Wing and Sims, 1979; Higgs, Vanderah and Faulkner, 1977) and *Aplysia californica* (Ireland, 1976) displayed moderate activity against the human esophageal cancer cell line WHCOI (Knott *et al.*, 2005).

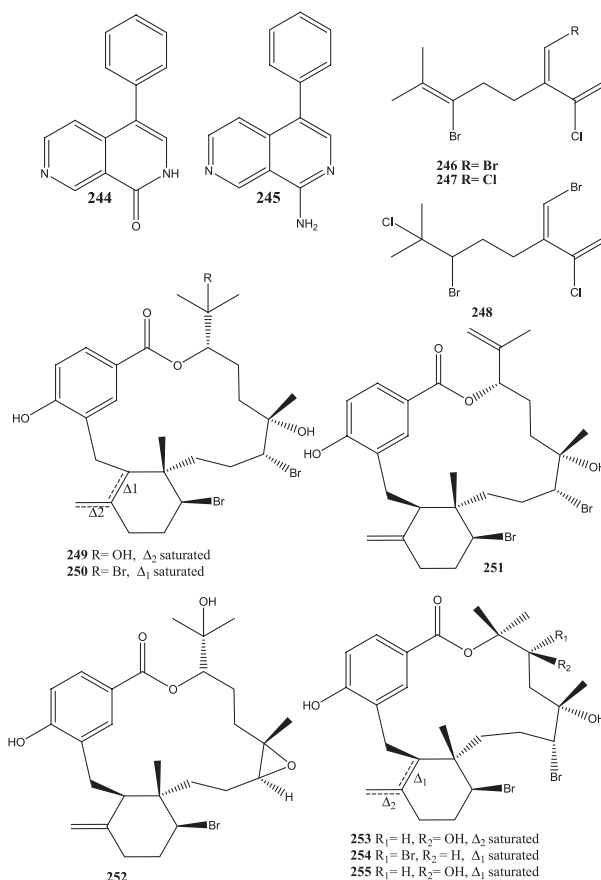
The red alga *Callophycus serratus* was the source of three antibacterial and antifungal diterpene-benzoate compounds, bromophycolides A **241** and B **242**, and a non-halogenated compound **243**. Bromophycolide A **241** was cytotoxic against several human tumor cell lines by specific induction of apoptosis (Kubaneck *et al.*, 2005).



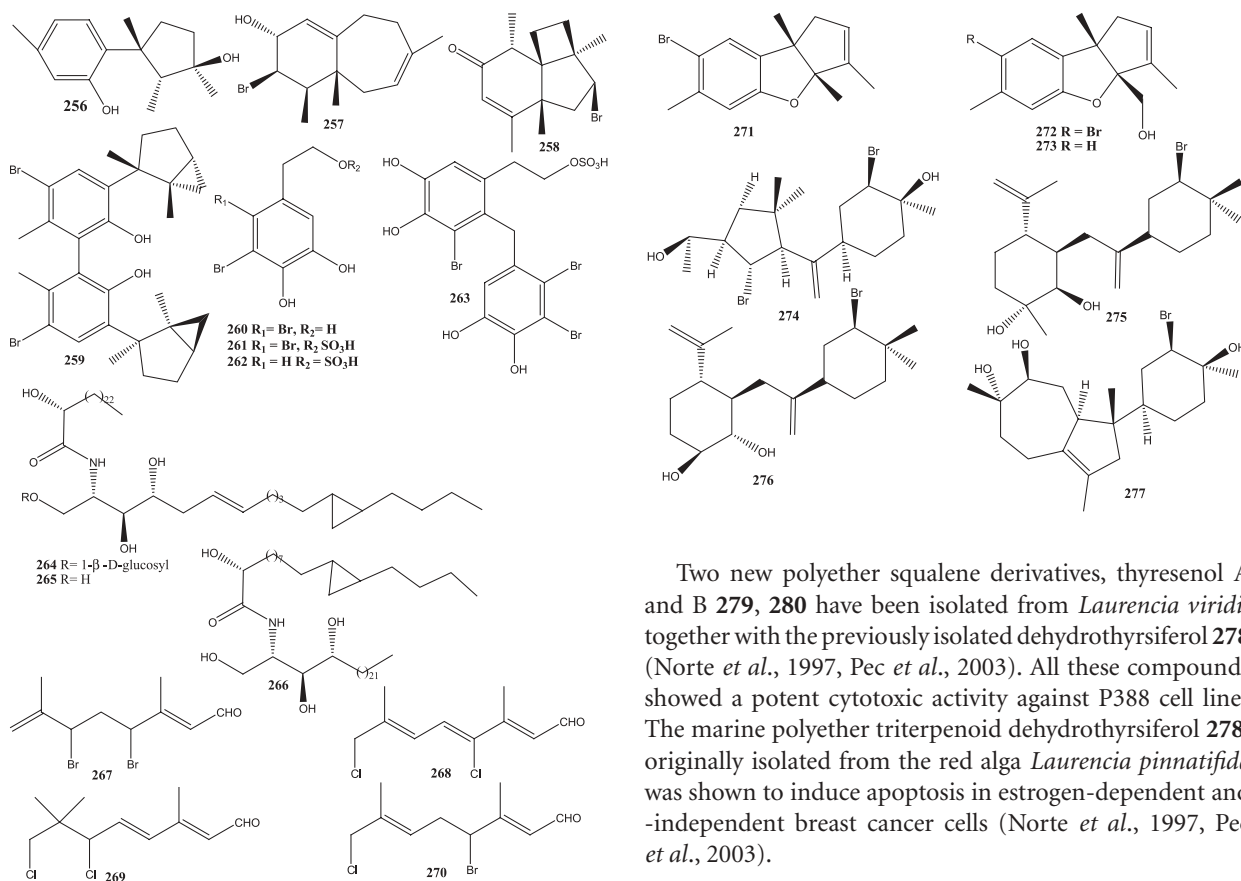
The alkaloids 2,7-naphthyridine lophocladines A **244** and B **245** were isolated from the red alga *Lophocladia* sp. Lophocladine A displayed affinity to *N*-methyl-D-aspartate (NMDA) receptors and was also a  $\delta$ -opioid receptor antagonist, while lophocladine B **245** was moderately active against NCI-H460 human lung tumor and MDA-MB-435 breast cancer cell lines and shown to be an inhibitor of microtubules (Gross *et al.*, 2006).

Three halogenated monoterpenes **246–248** were isolated from the red alga *Portiera hornemannii* along with the known compound halomon (Fuller *et al.*, 1992). Both halomon **184** and **248** were moderate inhibitors of DNA methyl transferase-1 (Andrianasolo *et al.*, 2006).

Bromophycolides C-I **249–255** are diterpene-benzoate macrolides isolated from the red alga *Callophycus serratus* with modest activity against a range of human tumor cell lines. (Kubaneck *et al.*, 2006).



The red alga *Laurencia obtusa* was a source of sesquiterpenes 3,7-dihydroxydihydrolaurene **256**, perforenol B **257** and **258**, while *L. microcladia* yielded a dimeric sesquiterpene **259**. Compounds **256–258** were tested against five human tumor cell lines and the Chinese hamster ovary (CHO) cell line. Perforenol B **257** exhibited strong activity while sesquiterpenes **256** and **258** exhibited weak activity. The sesquiterpene **259** was moderately cytotoxic against NSCLC-N6 and A549 lung cancer cell lines (Kladi *et al.*, 2006). The red alga *Rhodomela confervoides* was the source of four bromophenols **260–263**. They exhibited moderate cytotoxicity against several human cancer cell lines (Ma *et al.*, 2006).



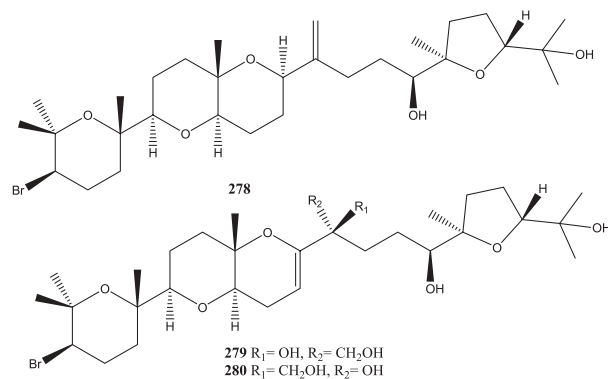
The red alga *Gracilaria asiatica* was the source of three cyclopropyl derivatives, the cerebroside gracilarioside **264** and the ceramides gracilamides A **265** and B **266**, which were mildly cytotoxic to the human A375-S2 melanoma cell line (Sun *et al.*, 2006).

Four somewhat air-unstable halogenated monoterpene aldehydes **267–270** were characterized from the red alga *Plocamium corallorhiza*, of which **287** was significantly cytotoxic against an esophageal cell line (Mann *et al.*, 2007).

Three sesquiterpenes, aplysin-9-ene **271**, epiaplysinol **272** and debromoepiaplysinol **273**, were isolated from the red alga *Laurencia tristicha*. Debromoepiaplysinol **273** displayed selective cytotoxicity to the HeLa cell line (Sun *et al.*, 2007).

Diterpenes neorogioldiol B **274** and prevezol B **275** isolated from the red alga *Laurencia obtusa* displayed significant cytotoxicity against the human tumour cell lines MCF7, PC3, HeLa, A431, and K562, while prevezol C **276** exhibited significant cytotoxicity against HeLa and A431 cell lines. Prevezol D **277** was moderately active against all cell lines (Ilopoulou *et al.*, 2003).

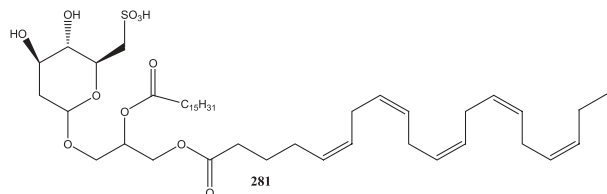
Two new polyether squalene derivatives, thyresenol A and B **279**, **280** have been isolated from *Laurencia viridis* together with the previously isolated dehydrothysiferol **278** (Norte *et al.*, 1997, Pec *et al.*, 2003). All these compounds showed a potent cytotoxic activity against P388 cell lines. The marine polyether triterpenoid dehydrothysiferol **278**, originally isolated from the red alga *Laurencia pinnatifida* was shown to induce apoptosis in estrogen-dependent and -independent breast cancer cells (Norte *et al.*, 1997, Pec *et al.*, 2003).



### Antiviral activity

Sulquinovosyldiacylglycerol, KM043 **281**, a new sulfolipid KM043, which belongs to the 6-sulf-α-D-quinovopyranosyl-(1→3')-1',2'-diacylglycerol (SQDG) class of compounds has been isolated from the marine red alga *Gigartina tenella* (Ohata *et al.*, 1998) as a potent inhibitor of eukaryotic DNA and HIV-1 reverse transcriptase type 1. The inhibition was dose dependent, and complete (more than 90%) inhibition of DNA polymerase α (pol.α),

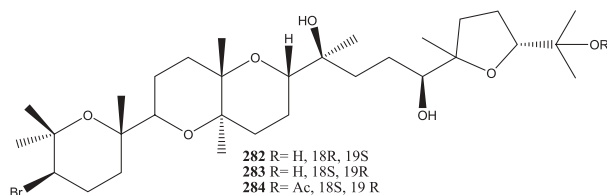
DNA polymerase  $\beta$  (pol. $\beta$ ) and HIV-reverse transcriptase type 1 (HIV-RT) was observed at concentrations 5, 10 and 30  $\mu$ M, respectively.



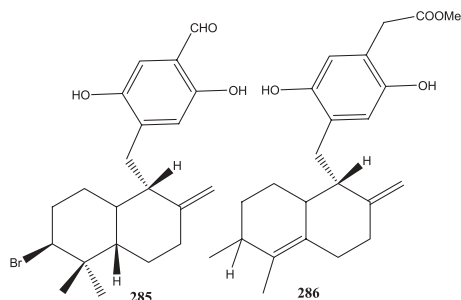
2,3,6-Tribromo4,5-dihydroxybenzyl methyl ether (Park *et al.*, 1999) isolated from the red alga *Symphyclocladia latiuscula* was active against wild type HSV-I, as well as APr HSV-I and TK-HSV-I and significantly delayed the appearance of lesions in infected mice without toxicity (Park *et al.*, 2005).

The invasive species *Caulerpa racemosa* was the source of the known compound sulfoquinovosyldiaclyglycerol, previously isolated from a terrestrial plant (Amarquaye *et al.*, 1994) and from the marine brown alga *Ishige okamurai* (Tang *et al.*, 2002b), and displayed selective antiviral activity against Herpes simplex virus 2 (HSV-2) (Wang *et al.*, 2007).

Venustatriol **282**, thysiferol **283** and thysiferyl 23-acetate **284** were isolated from the red alga *Laurencia venusta* and all displayed significant antiviral activity against vesicular stomatitis virus (VSV) and HSV-I (Sakemi *et al.*, 1986).

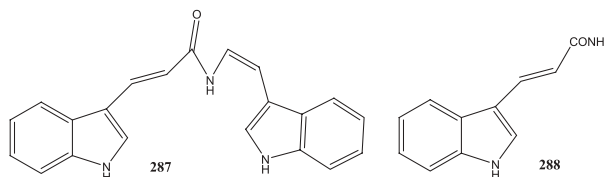


During a survey of marine organisms for anti-HIV RT activities, two new sesquiterpene hydroquinones, peyssonol A **285** and B **286** have been isolated from the active anti-HIV RT extracts of the Red Sea alga *Peyssonnelia* spp. (Talpir *et al.*, 1994).



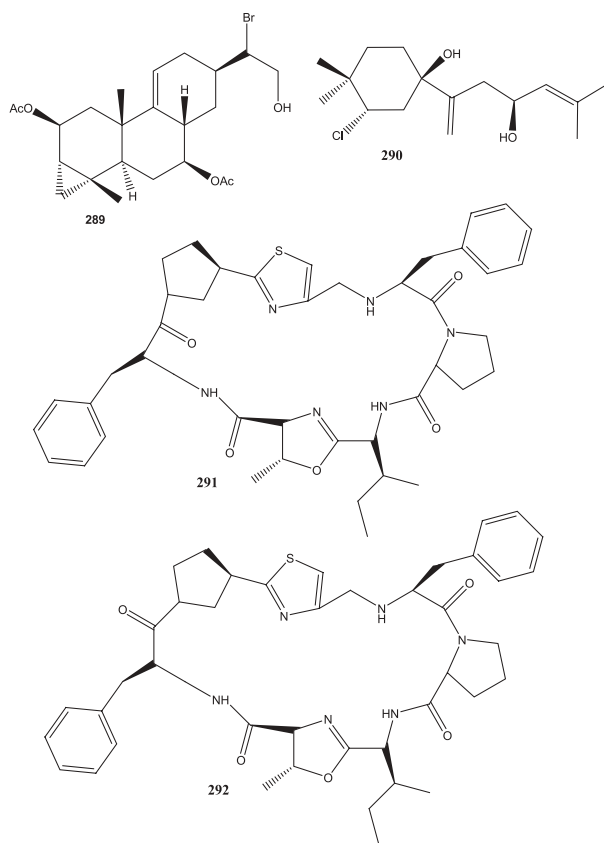
### Anthelmintic activity

Chondriamide C **307**, a new bis(indole) amide and 3-indolacrylamide **308** have been isolated from the red alga *Chondria atropurpurea* and showed anthelmintic activity against *Nippostrongylus brasiliensis* (Davty *et al.*, 1998).



Brominated diterpenes of the parguerene and isoparguerene series were isolated from the red alga *Jania rubens* including the novel deoxyparguerol-7-acetate **309**. All the isolated diterpenes had anthelmintic activity (Awad, 2004).

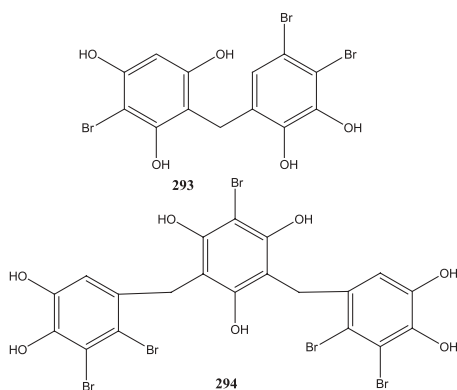
The red alga *Laurencia scoparia* was a source of halogenated  $\beta$ -bisabolene sesquiterpenes **310** (Awad, 2004; Davty *et al.*, 2006). It showed weak *in vitro* anthelmintic activity against *Nippostrongylus brasiliensis* (Davty *et al.*, 2006).



### Anti-inflammatory activity

Chemical investigation of the marine red alga *Ceratodictyon spongiosum* containing the symbiotic sponge *Sigmadia symbiotica* collected from Indonesia, afforded two isomers of a new bioactive thiazole-containing cyclic heptapeptide: *cis,cis*-ceratospongamide **291** and *trans,trans*-ceratospongamide **292** (Tan *et al.*, 2000). Isolation of these peptides was assisted by bioassay-guided fractionation using a brine shrimp toxicity assay. *trans,trans*-ceratospongamide exhibits potent inhibition to sPLA2 expression in a cell-based model for anti-inflammation ( $ED_{50}$  32 nM), whereas the *cis,cis* isomer is inactive. *trans,trans*-Ceratospongamide was also shown to inhibit the expression of a human-sPLA2 (secreted phospholipase A2) promoter-based reporter by 90%. The degree of anti-inflammatory activity of compounds **291** and **292** was measured as the inhibition of secreted phospholipase A2 by hepatocellular carcinoma cells stimulated with 1L-1 $\beta$ . The *trans,trans* form is a potent inhibitor of sPLA2 expression with  $ED_{50}$  32  $\mu$ M. Both compounds showed only moderate potency in the brine shrimp toxicity assay.

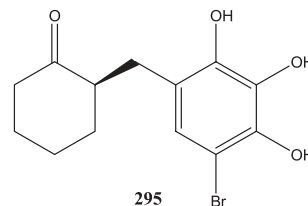
The anti-inflammatory bromophenolic metabolites named vidalols A **293** and B **294** were isolated from the Caribbean red alga *Vidalia obtusiloba* that acts through the inhibition of phospholipase enzyme (Wiemer, Idler and Fenical, 1991). The new compounds were discovered as part of an organized effort to isolate new naturally occurring anti-inflammatory agents with a focus upon those that may function through inhibition of phospholipase A2.



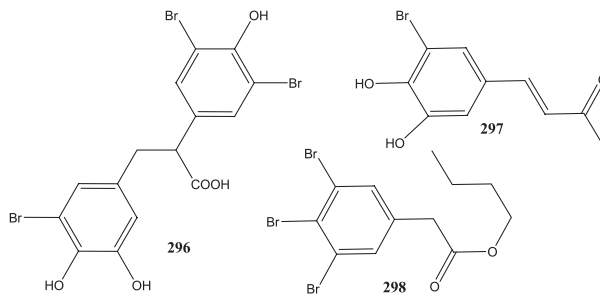
### Free radical scavenger activity

(2R)-2-(2,3,6-tribromo-4,5-dihydroxybenzyl) cyclohexanone **295** was isolated from the red alga *Symphycladia latiussula*, which has a free radical scavenger activity. The antioxidant activity was expressed and calculated in terms of  $IC_{50}$  [ $\mu$ g/ml or  $\mu$ M required to inhibit 1,1-diphenyl-2-

picrylhydrazyl radical, (DPPH), formation by 50%] (Choi *et al.*, 2000).



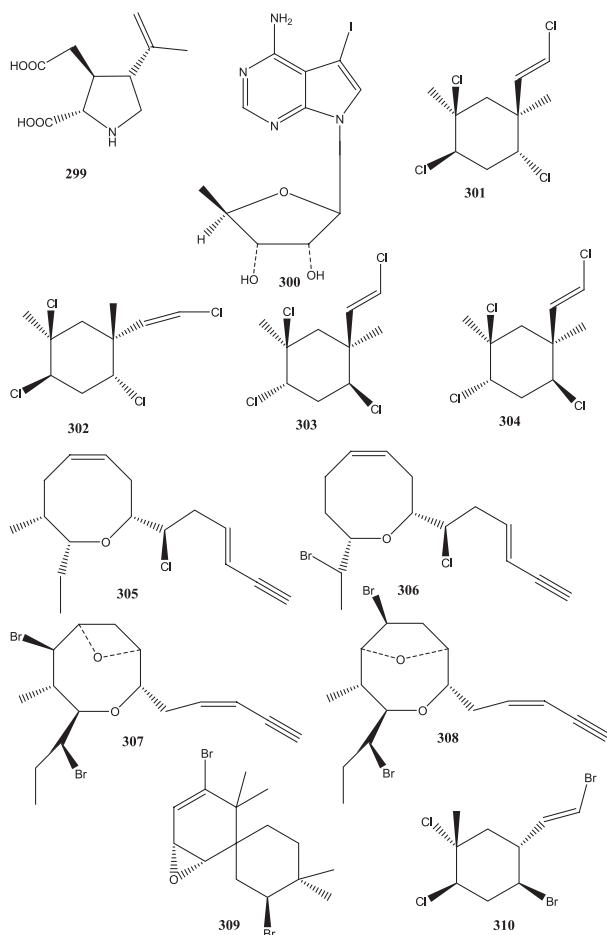
Three bromophenols **296–298** and the previously reported 1,2-bis(3-bromo-4,5-dihydroxyphenyl) ethane (Kurata, Amiya and Nakano, 1976) were isolated from the red alga *Polysiphonia urceolata* All compounds were potent DPPH radical scavengers (Li *et al.*, 2007).



Five known bromophenols, bis (2,3,6-tribromo-4,5-dihydroxyphenyl) methane (Wang *et al.*, 2005), bis (2,3,6-tribromo-4,5-dihydroxybenzyl) ether (Kurata and Amiya, 1980), 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (Kim *et al.*, 2002), 2,3,6-tribromo-4,5-dihydroxymethylbenzene (Li *et al.*, 2007) and 2,3,6-tribromo-4,5-dihydroxybenzaldehyde (Kurata and Amiya, 1980) were co-isolated and were also potent free radical scavengers (Duan, Li and Wang, 2007).

### Neurophysiological activity

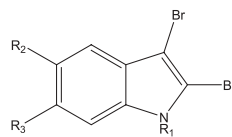
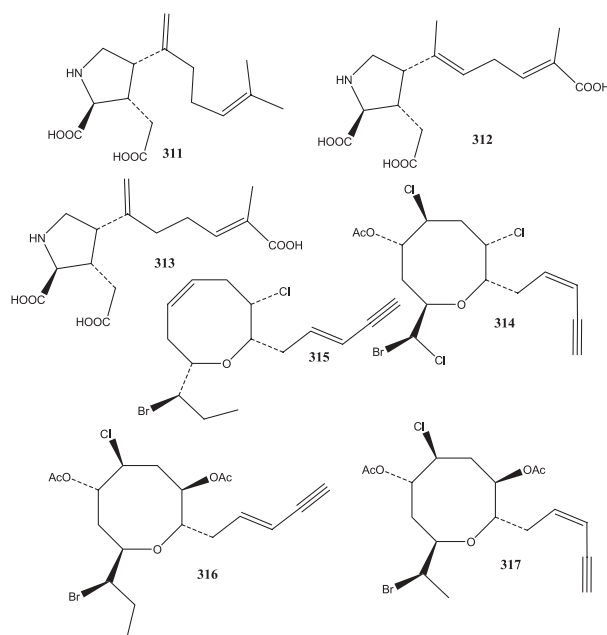
The amino acid ( $\alpha$ -alkokainic acid **299** isolated from the red alga *Digenea simplex* showed a potent neurophysiological activity in mammals (Biscoe *et al.*, 1975; Ferkany and Coyle, 1983). 5-Iodo-5'-deoxy-tubercidin **300** was isolated from the red alga *Hypnea valendiae*, which causes pronounced relaxation of muscles and hypothermia in mice and it blocks polysynaptic and monosynaptic reflexes. This compound is one of the most interesting algal metabolites which were discovered by using a bioassay-directed isolation procedure (Kazlauskas *et al.*, 1983).



### Insecticidal activity

The insecticidal and acaricidal polyhalogenated monoterpenes **301–304** have been isolated from Chilean specimens of the red alga *Plocamium cartilagineum*. The insecticidal activity of these compounds proved to be effective against the Aster leafhopper (San-Martin, Negrete and Roviroa, 1991). Laurepinacine **305** and islaurepinnacin **306** are acetylinic sesquiterpene ethers isolated from the red alga *Laurancia pinnata* that demonstrated insecticidal activity (Fukuzawa and Masamune, 1981). (*Z*)-Laureatin **307**, (*Z*)-isolaureatin **308** and deoxyprepacifenol **309** are other related compounds from the red alga *Laurencia nipponica* Yamada. They show strong insecticidal activity against the mosquito larvae *Culex pipens pallens* (Watanabe, Umeda and Miyakado, 1989; El Sayed *et al.*, 1997). Telfairine **310** is another related monoterpene reported from the red alga *Plocamium telfairia*, with strong insecticidal activity against the mosquito larva *Culex pipens pallens* (Watanabe *et al.*, 1988).

The new insecticidal amino acids, namely isodomic acid A **311**, isodomic acid B **312** and isodomic acid C **313**, were isolated from the red alga *Chondria arnata*. They show significant insecticidal activity when they are injected subcutaneously into the abdomen of American cockroach (Maeda *et al.*, 1986). *Laurencia obtusa*, collected from off Symi Island in the Greece, Aegean Sea was the source of C<sub>15</sub> aceto-genins 13-epilaurencienyne (*3Z*) **314**, 13-epinnatifidenyne (*3E*) **315** and two diacetoxypentadec-3-en-1-yne derivatives (**316**, **317**). Compounds **314** and **315** exhibited strong toxicity against ants with considerable knockdown effect from the first day, while compounds **315** and **316** exhibited gradual toxicity that was escalated at the fourth day with >70% mortality (Ilopulou *et al.*, 2002).

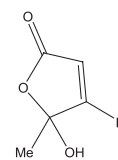


**318** R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = H, R<sub>3</sub> = Br

**319** R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = Br, R<sub>3</sub> = H

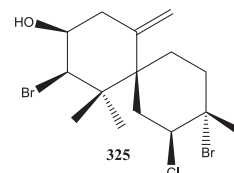
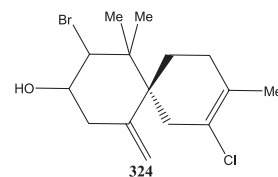
**320** R<sub>1</sub> = H, R<sub>2</sub> = R<sub>3</sub> = Br

**321** R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = R<sub>3</sub> = Br



**322** R = Br

**323** R = Cl



### Antimicrobial activity

The antimicrobial activity of the red alga *Laurencia brongniarti* against *Bacillus subtilis* (a Gram-positive bacterium) and *Saccharomyces cerevisiae* (yeast) has been traced to the four polybrominated indoles **318–321** (Carter *et al.*, 1978).

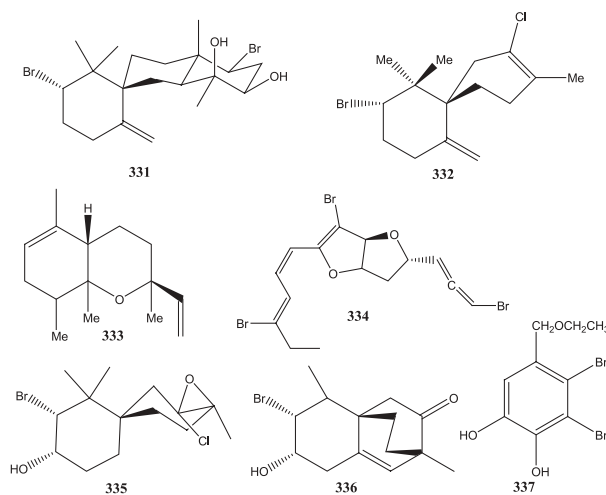
From the air-dried red alga *Beckerella subcostatum*, bromobeckerelide **322** epimer (the major fraction) and chlorobeckerelide **323** epimers (the minor fraction) were isolated. In laboratory tests, both compounds showed activity against *Bacillus subtilis* (Ohta, 1977).

From the MeOH extract of *Marginisporum aberans*, showing antimicrobial activity against *Bacillus subtilis*, *p*-hydroxybenzaldehyde, dichloroacetamide, and 3,5-dinitriguaiacol were obtained. All these compounds showed activity against *Bacillus subtilis* (Ohta and Takagi, 1977).

Elatol **324**, a halogenated sesquiterpene alcohol from the red alga *L. elata* (Sims, Lin and Wing, 1974), inhibited six species of human pathogenic bacteria with significant antibacterial activities against *Staphylococcus epidermidis*, *Klebsiella pneumonia* and *Salmonella* sp. (Vairappan, 2003). Iso-obtusol **325** from the red alga *L. obtusa* (Gonzalez *et al.*, 1976, 1979) exhibited antibacterial activity against four bacterial species with significant activity against *K. pneumonia* and *Salmonella* sp.

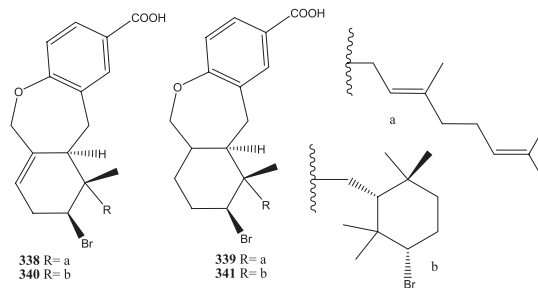
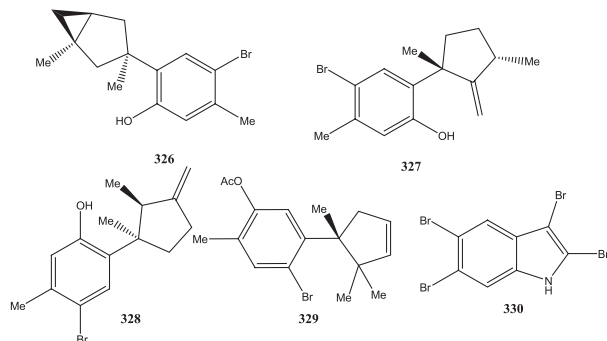
Halogenated metabolites from the red alga *Laurencia* species were tested for antibacterial activity against 22 strains of human pathogenic bacteria, including seven strains of antibiotic-resistant bacteria. Laurinterol **326** (Irie *et al.*, 1966), isolaurinterol **327** (Irie *et al.*, 1970), *allo*-laurinterol **328** (Kazlauskas *et al.*, 1976), cupalaurinol **329** (Ichiba and Higa, 1986) and 2,3,5,6-tetra-bromoindol **330** (Carter *et al.*, 1978) displayed a wide spectrum of antibacterial activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae* and vancomycin-resistant *Enterococcus faecalis* and *E. faecium*. Laurinterol and *allo*-laurinterol were particularly effective (Vairappan *et al.*, 2004).

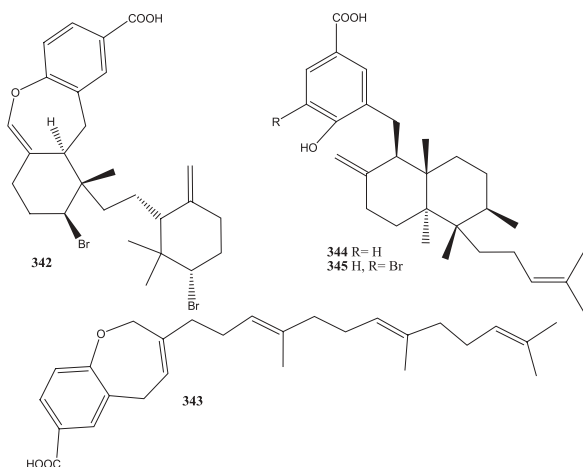
The red alga *Laurencia mariannensis* afforded a number of new metabolites: the brominated diterpene, 10-hydroxykahukuene B **331**, two sesquiterpenes, 9-deoxyelatal **332** and isodactyloxene A **333**, one brominated C15-acetogenin, laurenmariallene **334**, and two new naturally occurring halogenated sesquiterpenes **335** and **336** that were obtained previously as intermediates in a biomimetic synthetic study of rhodolaureol and rhodolauradiol (Gonzalez *et al.*, 1982). Both 10-hydroxykahukuene B **331** and laurenmariallene **334** had modest antibacterial activity.



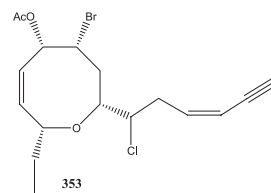
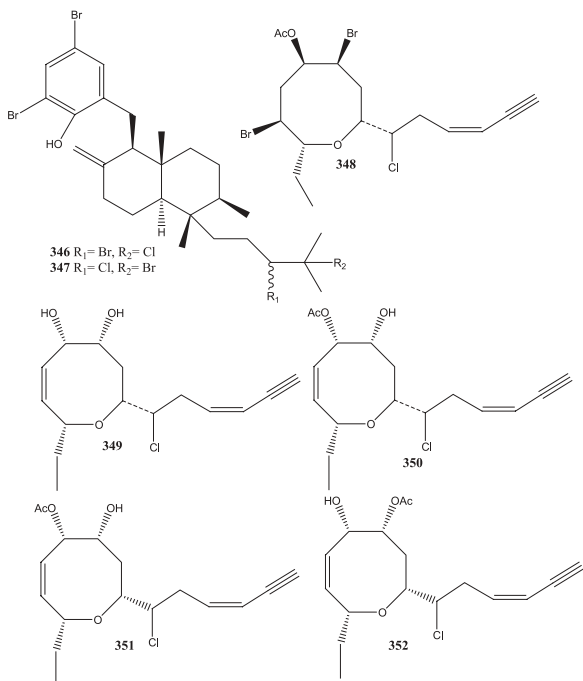
Lanosol enol ether **357**, originally isolated from the brown alga *Fucus vesiculosus* has been shown to be an antibacterial and antifungal component of the brown alga *Osmundaria serrata* (Barreto and Meyer, 2006).

Eight novel diterpenebenzoic acids, callophycoic acids A–H **338–345**, and two halogenated diterpene-phenols, callophycols A **346** and B **347**, were isolated from red alga *Callophycus serratus*, some of which displayed moderate antibacterial, antimalarial, antitumor, and antifungal activity (Lane *et al.*, 2007).





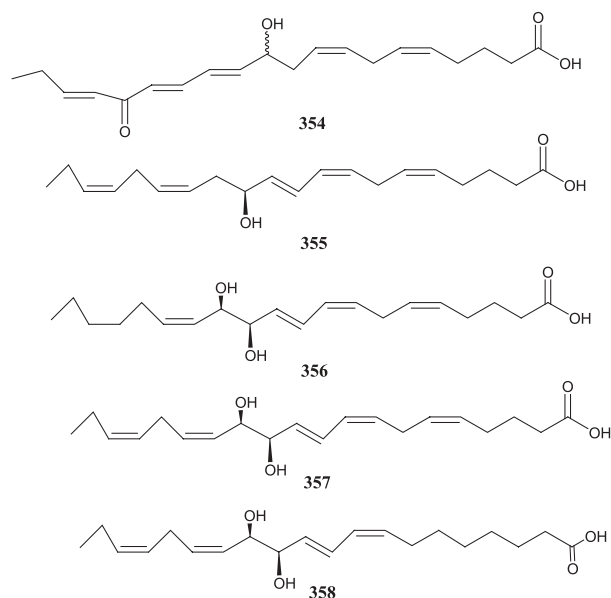
Five new  $C_{15}$  eight-membered cyclic ethers (**348**, **350–353**) (Kladi *et al.*, 2008) with a characteristic terminal *cis*-ene-yne moiety in addition to the previously reported acetylenic chlorodiols **349** (Blunt *et al.*, 1981) were isolated from the red alga *Laurencia glandulifera*. All these metabolites were tested for their antistaphylococcal activity and the minimum inhibitory concentration (MICs) of **349–352** were in the range of 8–256  $\mu\text{g/ml}$ .



### Lipoxygenase inhibitor

The eicosanoids are biologically active arachidonic acid derivatives frequently found in marine organisms. Ptilodene **354** (new fatty acid) is an eicosanoid from the red alga *Ptilotaflificina* sp. that showed inhibitory activity to human 5-lipoxygenase, dog kidney  $\text{Na}^+/\text{K}^+$ -ATPase and the growth of several pathogenic Gram-positive and -negative bacteria (Lopez and Gerwick, 1988). Another eicosanoid derivative, which is a potent inhibitor of platelet aggregation, is 12-(*S*)-hydroxyeicosapentaenoic acid **355** isolated from the red alga *Murrayella pericladus* (Bernari *et al.*, 1994).

Three biologically active eicosanoids, (12*R*,13*R*)-dihydroxy-eicosa-5(*Z*),8(*Z*),10(*E*), 14(*Z*)tetraenoic acid **356**, (12*R*,13*R*)-dihydroxyeicosa-5(*Z*),8(*Z*),10(*E*),14(*Z*),17(*Z*)-pentaenoic acid **357** and (10*R*,11*R*)-dihydroxyoctadeca-6(*Z*),8(*E*),12(*Z*)-trienoic acid **358** were isolated from the red alga *Farlowia mollis* (Solem, Jiang and Gerwick, 1989).



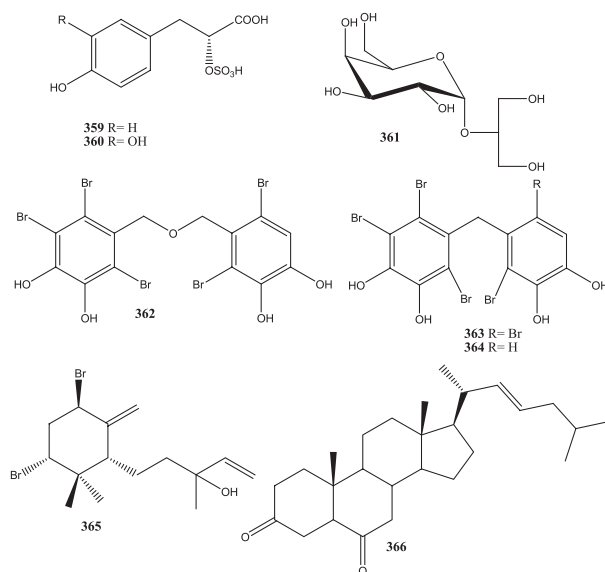
### Antifeedent activity

Two phenylpropanoic acid derivatives, tichocarpols A **359** and B **360** were isolated from the red alga *Tichocarpus crinitus*. These two compounds along with floridoside **361** (Roh *et al.*, 1994) which is also isolated from the alga, exhibited

antifeedant activity against the sea urchin *Strongylocentrotus intermedius* (Ishii *et al.*, 2004).

#### Aldose reductase inhibitor activity

The new bromophenols **362–364** and two bromophenols known previously only as synthetic compounds (Diers *et al.*, 2004; Nishizawa and Satoh, 1975; Lightowler and Ryland, 1964) isolated from the red alga *Symphyclocladia latusecula* have significant aldose reductase inhibitors (Wang *et al.*, 2005).



#### Antimalarial activity

Snyderol sesquiterpene **365** derivative isolated from the red alga *Laurencia obtusa* was active against D6 and W2 clones of the malarial parasite *Plasmodium falciparum* (Topeu *et al.*, 2003).

#### Anti-elastase activity against porcine pancreas elastase

3,6-Diketo steroid **366** was isolated from the red alga *Hypnea musciformis* collected on the Atlantic coast of Morocco exhibited anti-elastase activity against porcine pancreas elastase (Gosavi *et al.*, 1995).

#### Inhibition of isocitrate lyase enzyme

A number of bromophenols isolated from the red alga *Odonthalia corymbifera* exhibited potent inhibitory activity against isocitrate lyase, an important enzyme in the rice fungal pathogen, *Magnaporthe grisea*.

The compounds 3,5-dibromo-4-hydroxyphenylethylamine (Diers *et al.*, 2004) 2,20,3,30-tetrabromo-

4,40,5,50-tetrahydroxydiphenylmethane (Craigie and Gruenig, 1967), 2,3-dibromo-4,5-dihydroxybenzyl alcohol (Hodgkin, Craigie, and McInnes, 1966), 2,3-dibromo-4,5-dihydroxybenzyl methyl ether (Katsui *et al.*, 1967), 2,20,3-tribromo-30,4,40,5-tetrahydroxy-60-hydroxymethyldiphenylmethane (Kurata and Amiya, 1977) and 3-bromo-4-(2,3-dibromo-4,5-dihydroxybenzyl)-5-methoxymethylpyrocatechol also protected rice plants from infection by *Magnaporthe grisea* (Lee *et al.*, 2007). This was the first report of 3,5-dibromo-4-hydroxyphenylethylamine as a natural product (Lee *et al.*, 2007).

## Acknowledgment

The author is indebted to Elsevier for publication of the major part of this work in *Saudi Pharmaceutical Journal*, **18**, 1–25, 2010.

## References

- Abatis, D., Vigias, C., Galanakis, D., *et al.* (2005) Atomarianones A and B: two cytotoxic meroditerpenes from the brown alga *Taonia atomaria*. *Tetrahedron Lett.*, **46**, 8525–8529.
- Aguilar-Santos, G. (1970) Caulerpin, a new red pigment from green algae of the genus *Caulerpa*. *J. Chem. Soc., C*, 842–843.
- Ali, M.S., Saleem, M., Yammdagni, R. and Ali, M.A. (2002) Steroid and antibacterial glycosides from marine green alga *Codium iyengarii* Borgesen. *Nat. Prod. Lett.*, **16**, 407–413.
- Amarquaye, A., Che, C.T., Bejar, E., Malone, M.H. and Fong, H.H. (1994) A new glycolipid from *Byrsonima crassifolia*. *Planta Med.*, **60**, 85–86.
- Andrianasolo, E.H., France, D., Cornell-Kennon, S. and Gerwick, W.H. (2006) DNA methyl transferase inhibiting halogenated monoterpenes from the Madagascar red marine alga *Portieria hornemannii*. *J. Nat. Prod.*, **69**, 576–579.
- Argandona, V.H., Roviroso, J., San-Martin, A., *et al.* (2002) Antifeedant effects of marine halogenated monoterpenes. *J. Agric. Food. Chem.*, **50**, 7029–7033.
- Asari, F., Kusumi, T. and Kakisawa, H. (1989) Turbinaric acid, a cytotoxic secosqualene carboxylic acid from the brown alga *Turbinaria ornate*. *J. Nat. Prod.*, **52**, 1167–1169.
- Awad, N.E. (2000) Biologically active steroid from the green alga *Ulva lactuca*. *Phytother. Res.*, **14**, 641–643.
- Awad, N.E. (2004) Bioactive brominated diterpenes from the marine red alga *Jania rubens* (L.) Lamx. *Phytother. Res.*, **18**, 275–279.

- Ayyad, S.-E. N., Abdel-Halim, O.B., Shier, W.T. and Hoye, T.R. (2003) Cytotoxic hydroazulene diterpenes from the brown alga *Cystoseira myrica*. *Z. Naturforsch., C., Biosci.*, **58**, 33–38.
- Barbosa, J.P., Pereira, R.C., Abrantes, J.L., *et al.* (2004) *In vitro* antiviral diterpenes from the Brazilian brown alga *Dictyota paffii*. *Planta Med.*, **70**, 856–860.
- Barbosa J.P., Teixeira, V.L., Villca, R., Pereira, R.C., Abrantes, J.L. and da Paixao, Frugulhetti, I.C.P. (2003) A dolabelane diterpene from the Brazilian brown alga *Dictyota paffii*. *J. Biochem. Syst. Ecol.*, **31** 1451–1453.
- Barreto, M. and Meyer, J.J.M. (2006) Isolation and antimicrobial activity of a lanosol derivative from *Osmundaria serrata* (Rhodophyta) and a visual exploration of its biofilm covering. *S. Afr. J. Bot.*, **72**, 521–528.
- Bennamara, A., Abourrichi, A., Berrada, M., *et al.* (1999) Methoxybifurcarenone: an antifungal and antibacterial meroditerpenoid from the brown alga *Cystoseira tamariscifolia*. *Phytochemistry*, **52**, 37–40.
- Bernari, M.W. and Gerwick, W.H. (1994) Eicosanoids from the tropical red alga *Murrayella pericladus*. *Phytochemistry*, **36**, 1233–1240.
- Biscoe, T.J., Evans, R.H., Headley, P.M., Martin, M. and Watkins, J.C. (1975) Domic and quisqualic acids as potent amino acids excitants of frog and rat spinal neurons. *Nature*, **255**, 166–167.
- Blackman, A.J., Dragar, C. and Wells, R.J. (1979) A new phenol from the brown alga *Perithalia caudata* containing a “reverse” isoprene unit at the 4-position. *J. Aust. J. Chem.*, **32**, 2783–2786.
- Blunt, J.W., Lake, R.J., Munro, M.H.G. and Yorke, S.C. (1981) A new vinyl acetylene from the red alga *laurencia thysifera*. *Aust. J. Chem.*, **34**, 2393–2400.
- Bold, H.C. and Wynne, M.J. (1985) *Introduction to the Algae: Structure and Reproduction*, 2nd edn. Prentice-Hall Inc., Englewood Cliffs, NJ, pp. 1–33.
- Boland, W., Jaenicke, L., Muller, D.G. and Gassmann, G. (1987) Giffordene, 2Z,4Z,6E, 8Z-undecatetraene, is the odoriferous principle of the marine brown alga *Giffordia mitchellae*. *Experientia*, **43**, 466–468.
- Capon, R.I., Barrow, R.A., Rochfort, S., *et al.* (1998) Marine nematodes: tetrahydrofuran from a southern Australian brown alga, *Notheia anomala*. *Tetrahedron*, **54**, 2227–2242.
- Carter, D.C., Moore, R.E., Mynderse, J.S., Niemczura, W.P. and Todd, J.S. (1984) Structure of majusculamide C a cyclic depsipeptide from *Lyngbya majuscula*. *J. Org Chem.*, **49**, 236–241.
- Carter, G.T., Rinehart, J.r., Li, L.H. and Kuentzel, S.L. (1978) Brominated indoles from *Laurencia Brongniartii*. *Tetrahedron Lett.*, **19**, 4479–4482.
- Chen, I.L., Gerwick, W.H., Schatzman, R. and Laney, M. (1994) Isorawsonol and related IMO dehydrogenase inhibitors from the tropical alga *Avrainvillea rawsoni*. *J. Nat. Prod.*, **57**, 947–952.
- Choi, J.S., Park, H.J., Jung, H.A., Chung, H.Y., Jung, J.H. and Choi, W.C. (2000) A cyclohexanonyl bromophenol from the red alga *Symphyclocladia latiuscula*. *J. Nat. Prod.*, **63**, 1705–1706.
- Craigie, J.S. and Gruenig, D.E. (1967) Bromophenols from red algae. *Science*, **157**, 1058–1059.
- Cross, C. F., Bevan, E.J. and Briggs, J.F. (1907) Lignonephloroglucid formation without a color reaction *Chem. Ztg.*, **31**, 725–727.
- Colon, M., Guevara, P., Gerwick, W.H. and Ballantine, D. (1987) 5'-Hydroxyisoavrainvilleol, a new diphenylmethane derivative from the tropical green alga *Avrainvillea nigricans*. *J. Nat. Prod.*, **50**, 368–374.
- Cortes, D., Yolanda, T.M., D'Ocon, M.P., *et al.* (1990) Norstephalagine et atherospermidine, deux aporphines d'artabotrys maingayi relaxantes du muscle lisse. *J. Nat. Prod.*, **53**, 503–508.
- Davyt, D., Entz, W., Fernandez, R., *et al.* (1998) A new indol derivative from the red alga *Chondra atropurpurea*. Isolation, structure determination, and anthelmintic activity. *J. Nat. Prod.*, **61**, 1560–1563.
- Davyt, D., Fernandez, R., Suescun, L., *et al.* (2006) Bisabolanes from the red alga *Laurencia scoparia*. *J. Nat. Prod.*, **69**, 1113–1116.
- de Ines, C., Argandona, V.H., Riviroso, J., *et al.* (2004) Cytotoxic activity of halogenated monoterpenes from *Plocamium cartilagineum*. *Z. Naturforsch., C. Biosci.*, **59**, 339–344.
- Diers, J.A., Pennaka, H.K., Peng, J., Bowling, J.J., Duke, S.O. and Hamann, M.T. (2004) Structural activity relationship studies of zebra mussel antifouling and antimicrobial agents from verongid sponges *J. Nat. Prod.*, **67**, 2117–2021.
- Dorta, E., Cueto, M., Bito, I. and Darias, J. (2002) New terpenoids from the brown alga *Stypopodium zonale*. *J. Nat. Prod.*, **65**, 1727–1730.
- Dorta, E., Cueto, M., Diaz-Marrero, A.R. and Darias, J. (2002) Stypolactone, an interesting diterpenoid from the brown alga *Stypopodium zonale*. *Tetrahedron Lett.*, **65**, 9043–9046.
- Dmitrenok, A., Iwashita, T., Nakajima, T., Sakamoto, B., Namikoshi, M. and Nagai, H. (2006) New cyclic desipeptides from the green alga species; application of a carboxypeptidase hydrolysis reaction to the structure determination. *Tetrahedron*, **62**, 1301–1308.
- Duan, X-J., Li, X-M. and Wang, B-G. (2007) Highly brominated mono- and bis-phenols from the marine red alga *Symphyclocladia latiuscula* with radical-scavenging activity. *J. Nat. Prod.*, **70**, 1210–1213.

- Edmonds, S.L., Morita, M. and Shibata, Y. (1987) Isolation and identification of arsenic- containing ribfurnao-side and inorganic arsenic from Japanese edible seaweed *Hizikia fusiforme*. *J. Chem. Soc. Perkin. Trans.*, **1**, 577–580.
- El Gamal, A.A., Wang, W.-L. and Duh, C.-Y. (2005) Sulfur-containing polybromoindoles from the Formosan red alga *Laurencia brongniartii*. *J. Nat. Prod.*, **68**, 815–817.
- El Sayed, K.A., Dunbar, D.C., Perry, T.L., Wilkins, S.P. and Hamann, M.T. (1997) Marine natural products as prototype insecticidal agents. *J. Agric. Food Chem.*, **45**, 2735–2739.
- Faulkner, D.J. (1984) Marine natural products. *Nat. Prod. Rep.*, **1**, 251–280.
- Faulkner, D.J. (1984) Marine natural products. *Nat. Prod. Rep.*, **1**, 551–598.
- Faulkner, D.J. (1986) Marine natural products. *Nat. Prod. Rep.*, **3**, 1–33.
- Faulkner, D.J. (1987) Marine natural products. *Nat. Prod. Rep.*, **4**, 539–576.
- Faulkner, D.J. (1988) Marine natural products. *Nat. Prod. Rep.*, **5**, 613–663.
- Faulkner, D.J. (1990) Marine natural products. *Nat. Prod. Rep.*, **7**, 269–309.
- Faulkner, D.J. (1991) Marine natural products. *Nat. Prod. Rep.*, **8**, 97–147.
- Faulkner, D.J. (1992) Marine natural products. *Nat. Prod. Rep.*, **9**, 323–364.
- Faulkner, D.J. (1993) Marine natural products. *Nat. Prod. Rep.*, **10**, 497–539.
- Faulkner, D.J. (1994) Marine natural products. *Nat. Prod. Rep.*, **11**, 355–394.
- Faulkner, D.J. (1995) Marine natural products. *Nat. Prod. Rep.*, **12**, 223–269.
- Faulkner, D.J. (1996) Marine natural products. *Nat. Prod. Rep.*, **13**, 75–125.
- Faulkner, D.J. (1997) Marine natural products. *Nat. Prod. Rep.*, **14**, 259–302.
- Faulkner, D.J. (1998) Marine natural products. *Nat. Prod. Rep.*, **15**, 113–158.
- Faulkner, D.J. (1999) Marine natural products. *Nat. Prod. Rep.*, **16**, 33–43.
- Faulkner, D.J. (2000) Marine natural products. *Nat. Prod. Rep.*, **17**, 7–55.
- Faulkner, D.J. (2001) Marine natural products. *Nat. Prod. Rep.*, **18**, 1–49.
- Faulkner, D.J. (2002) Marine natural products. *Nat. Prod. Rep.*, **19**, 1–48.
- Feng, Y., Carroll, A.R., Addepalli, R., Fechner, G.A., Avery, V.M. and Quinn, R.J. (2007) Vanillic acid derivatives from the green algae *Cladophora socialis* as potent protein tyrosine phosphatase 1B inhibitors. *J. Nat. Prod.*, **70**, 1790–1792.
- Fenical, W. and Sims, J.J. (1974) Cycloeudesmol, an antibiotic cyclopropane conatinnin sesquiterpene from the marine alga, *Chondria oppositoclada* Dawson. *Tetrahedron Lett.*, **13**, 1137–1140.
- Fenical, W., Sims, J.J., Squatrito, D., Wing, R.M. and Radlick, P. (1973) Marine natural products, VII. Zonarol and isozonarol, fungitoxic hydroquinones from the brown seaweeds *Dictyopteris zonarioides*. *J. Org. Chem.*, **38**, 2383–2386.
- Ferkany, J.W. and Coyle, J.T. (1983) Kainic acid selectively stimulates the release of endogenous excitatory acidic amino acids. *J. Pharmacol. Exp. Ther.*, **225**, 399–406.
- Finer, I., Clardy, I., Fenical, W., et al. (1979) Structures of dictyodial and dictyolactone, unusual marine diterpenoids. *J. Org. Chem.*, **44**, 2044–2047.
- Fisch, K.M., Bohm, V., Wrightand, A.D. and Konig, G.M. (2003) Antioxidative meroterpenoids from the brown alga *Cystoseira crinita*. *J. Nat. Prod.*, **66**, 968–975.
- Fukuzawa, A. and Masamune, T. (1981) Laurepinnacin and isolaurepinnacin: new acetylenic cyclic ethers from the marine alga *Laurencia pinnata* Yamada. *Tetrahedron Lett.*, **22**, 4081–4084.
- Fukuyama, Y., Kodama, M., Miura, I., et al. (1990) Antiplasmin inhibitor. VI. Structure of phlorofucofuroeckol A, a novel phlorotannin with both dibenzo-1,4-dioxin and dibenzofuran elements, from *Ecklonia kurome* Okamura. *Chem. Pharm. Bull.*, **38**, 133–135.
- Fukuyama, Y., Miura, I., Kinzyo, Z., et al. (1983) Antiplasmin inhibitors, polyhydroxydibenzo-*P*-dioxins isolated from *Ecklonia kurome* Okamura. *Yuki Kogobutsu Tonnokai Koen Yoshishu*, **26**, 126–133.
- Fukuyama, Y., Kodaama, M., Miura, I., et al. (1989) Antiplasmin inhibitor. V. Structures of novel dimeric eckols isolated from the brown alga *Ecklonia kurome* Okamura. *Chem. Pharm. Bull.*, **37**, 2438–2440.
- Fuller, R.W., Cardellina, J.H., Kato, Y., et al. (1992) A pentahalogenated monoterpene from the red alga *Portieria hornemannii* produced a novel cytotoxicity profile against a diverse panel of human tumor cell lines. *J. Med. Chem.*, **35**, 3007–3011.
- Fuller, R.W., Cardellina, J.H., Jurek, J., et al. (1994) Isolation and structure/activity features of halomon-related anti-tumor monoterpenes from the red alga *Portieria hornemannii*. *J. Med. Chem.*, **37**, 4407–4411.
- Garg, H.S., Sharma, M., Bhakuni, D.S., Pramanik, B.N. and Bose, A.K. (1992) An antiviral sphingosine derivative from the green alga *Ulva fasciata*. *Tetrahedron Lett.*, **33**, 1641–1644.
- Garson, J. (1989) Marine natural products. *Nat. Prod. Rep.*, **6**, 143–170.
- Gerwick, W.H. and Fenical, W. (1981) Ichthyotoxic and cytotoxic metabolites of the tropical brown alga

- Stypopodium zonale* (Lamouroux) Papenfuss. *J. Org. Chem.*, **46**, 22–27.
- Gerwick, W.H., Fenical, W., Fritsch, N. and Clardy, J. (1979) Stypotriol and stypoldione; ichthyotoxins of mixed biogenesis from the marine alga *Stypopodium zonale*. *Tetrahedron Lett.*, **20**, 145–148.
- Gonzalez, A.G., Darias, J. and Martin, J.D. (1971) Taondiol, a new component from *Taonia atomaria*. *Tetrahedron Lett.*, **12**, 2729–2732.
- Gonzalez, A.G., Darias, J., Diaz, A., Fournero, J.D., Martin, J.D. and Perez, C. (1976) Evidence for the biogenesis of halogenated chamigrenes from the red alga *Laurencia obtuse*. *Tetrahedron Lett.*, **17**, 3051–3054.
- Gonzalez, A.G., Delgado, M.J., Martin V.S., Martinez-Ripoll, M. and Fayos, J. (1979) X-ray study of sesquiterpene constituents of the alga *L. obtusa* leads to structure revision. *Tetrahedron Lett.*, **29**, 2717–2718.
- Gonzalez, A.G., Martin, J.D., Martin, V.S., Norte, M. and Perez, R. (1982) Biomimetic approach to the synthesis of rhodolaureol and rhodolauradiol. *Tetrahedron Lett.*, **23**, 2395–2398.
- Gosavi, K., Babu, J. M., Mathur, H.H. and Bhadbhade, M. (1995) Isolation and x-ray structure of a new 3,6-diketo steroid from red alga *Hypnea musciformis*. *Chem. Lett.*, **7**, 519–520.
- Govindan, M., Abbas, S.A., Schmitz, E.I., Lee, R.H., Papkoff, I.S. and Slate, D.L. (1994) New cycloartanol sulfates from the alga *Tydemania expeditionis*: inhibitor of the protein tyrosin kinase pp60. *J. Nat. Prod.*, **57**, 74–78.
- Gross, H., Goeger, D.E., Hills, P., et al. (2006) Lophocladines, bioactive alkaloids from the red alga *Lophocladia* sp. *J. Nat. Prod.*, **69**, 640–644.
- Guardia, S.D., Valls, R., Mesguiche, V., Brunei, J.-M. and Gulioli, G. (1999) Enantioselective synthesis of (–)-bifuracadiol: a natural antitumor marine product; *Tetrahedron Lett.*, **40**, 8359–8360.
- Gulioli, G., Oratalo-Magne, A., Daoudi, M., Thomas Guyon, H., Vallis, R. and Pioveti, L. (2004) Trihydroxylated linear diterpenes from the brown alga *Bifurcaria bifurcata*. *Phytochemistry*, **65**, 2063–2069.
- Hall, S. and Strichartz, O. (1990) *Marine Toxins*. American Chemical Society, Washington, DC.
- Ham, Y.M., Baik, J.S., Hyun, J.W. and Lee, N.H. (2007) Isolation of a new phlorotannin, fucodiphlorethol G, from a brown alga *Ecklonia cava*. *Bull. Korean Chem. Soc.*, **28**, 1595–1597.
- Hamann, M.T. and Scheuer, P.J. (1993) Kahalalide F: a bioactive depsipeptide from the sacoglossan mollusk *Elysia rufescens* and the green alga *Bryopsis* sp. *J. Am. Chem. Soc.*, **115**, 5825–5826.
- Higa, T. (1989) Bioactive metabolites from marine organisms of Okinawa waters. In: *Studies in Natural Products Chemistry*. Vol 5 *Structure elucidation* (Part B). (ed. Attatur-Rahman). Elsevier, Amsterdam-Oxford-New York-Tokyo p. 341.
- Higa, T. (1985) 2-(1-Chloro-2-hydroxyethyl)-4,4-dimethylcyclohexa-2,5-dienone, precursor of 4,5-dimethylbenzo[b] furan from red alga *Desmia hornemanni*. *Tetrahedron Lett.*, **26**, 2335–2336.
- Higgs, M.D., Vanderah, D.J. and Faulkner, D.J. (1977) Polyhalogenated monoterpenes from *plocamium cartilagineum* from the British coast. *Tetrahedron*, **33**, 2775–2780.
- Hillison, C.I. (1977) *Seaweeds, a color-Coded, Illustrated Guide to Common Marine Plants of East Coast of the United States*. Keystone Books, The Pennsylvania State University Press, pp. 1–5.
- Hodgkin, J.H., Craigie, J.S., and McInnes, A.G. (1966) The occurrence of 2,3-dibromobenzyl alcohol dipotassium salt in *Polysiphonia lanosa*. *Can. J. Chem.*, **44**, 74–78.
- Ichiba, T. and Higa, T. (1986) New cuparene-derived sesquiterpenes with unprecedented oxygenation pattern from the sea hare *Aplysia dactylomela*. *J. Org. Chem.*, **51**, 3364–3366.
- Ilopoulou, D., Mihopoulos, N., Vigias, C., Papazafiri, P. and Roussis, V. (2003) Novel cytotoxic brominated diterpenes from the red alga *Laurencia obtuse*. *J. Org. Chem.*, **68**, 7667–7674.
- Ilopoulou, D., Vagias, C., Harvala, C. and Roussis, V. (2002) C<sub>15</sub> Acetogenins from the red alga *Laurencia obtuse*. *Phytochemistry*, **59**, 111–116.
- Ishii, T., Okino, T., Suzuki, M. and Machiguchi, M. (2004) Tichocarpols A and B, two novel phenylpropanoids with feeding-deterrent activity from the red alga *Tichocarpus crinitus*. *J. Nat. Prod.*, **67**, 1764–1766.
- Ishitsuka, M., Kusumi, T., Nomura, Y., Konno, T. and Kakisawa, H. (1979) New geranylgeranybenzoquinone derivatives from *Sargassum tortile*. *Chem Lett.*, 1269–1272.
- Ishitsuka, M., Kusumi, T. and Kakisawa, H. (1982) Acetylsanadaol, a diterpene having a novel skeleton, from the brown alga, *Pachydictyon coriaceum*. *Tetrahedron Lett.*, **23**, 3179–3180.
- Ireland, C. and Faulkner, D.J. (1977) Diterpenes from *Dolabella californica*. *J. Org Chem.*, **42**, 3157–3162.
- Ireland, C., Stallar, M.O., Faulkner, D.J., Finer, J. and Clardy, J. (1976) Some chemical constituents of the digestive gland of the sea hare *Aplysia californica*. *J. Org. Chem.*, **41**, 2461–2465.
- Irie, T., Suzuki, M., Kurosawa, E. and Masamune, T. (1966) Laurinterol and debromolaurinterol, constituents from *Laurencia intermedia*. *Tetrahedron Lett.*, 1837–1840.
- Irie, T., Suzuki, M., Kurosawa, E. and Masamune, T. (1970) Laurinterol, debromolaurinterol and isolaurinterol,

- constituents of *Laurencia intermedia* Yamada. *Tetrahedron*, **26**, 3271–3277.
- Iwamoto, C., Minoura, K., Hagishita, S., Nomoto, K. and Numata, A. (1998) Penostatins F-I, novel cytotoxic metabolites from a *Penicillium* species from an *Enteromorpha* marine alga. *J. Chem. Soc. Perkin Trans. 1*, **3**, 449–456.
- Iwamoto, C., Minoura, K., Hagishita, S., *et al.* (1999) Absolute stereostructures of novel penostatins A-E from a *Penicillium* species from an *Enteromorpha* marine alga. *Tetrahedron*, **55**, 14353–14368.
- Iwamoto, C., Yamada, T., Ito, Y., Minoura, K. and Numata, A. (2001) Cytotoxic cytochalasans from a *Penicillium* species separated from a marine alga. *Tetrahedron*, **57**, 2997–3004.
- Iwashima, M., Mori, J., Ting, X., *et al.* (2005) Antioxidant and antiviral activities of plastoquinones from the brown alga *Sargassum micracanthum*, and a new chromene derivative converted from the plastoquinones. *Biol. Pharm. Bull.*, **28**, 374–377.
- Jang, K.H.B., Lee, H., Choi, B.W., Lee, H.-S. and Shin, J. (2005) Chromenes from the brown alga *Sargassum siliquastrum*. *J. Nat. Prod.*, **68**, 716–723.
- Jiang, Z.-D., Jensen, P.R. and Fenical, W. (1999) Lobophorins A and B, New antiinflammatory macrolides produced by a tropical marine bacterium. *Biog. Med. Chem. Lett.*, **9**, 2003–2006.
- Jongaramruong, J. and Kongkam, N. (2007) Novel diterpenes with cytotoxic, anti-malarial and anti-tuberculosis activities from a brown alga *Dictyota* sp. *J. Asian Nat. Prod. Res.*, **9**, 743–751.
- Jung, H.A., Hyun, S.K., Kim, H.R. and Choi, J.S. (2006) Angiotensin-converting enzyme I inhibitory activity of phlorotannins from *Ecklonia stolonifera*. *Fish Sci.*, **72**, 1292–1299.
- Kang, H.S., Chang, H.Y., Jung, J.H., Son, B.W. and Choi, J.S. (2003) A new phlorotannin from the brown alga *Ecklonia stolonifera*. *Chem. Pharm. Bull.*, **51**, 1012–1014.
- Katsui, N., Suzuki, Y., Kitamura, S. and Irie, T. (1967) 5,6-Dibromoprotocatechualdehyde and 2,3-dibromo-4,5-dihydroxybenzyl methyl ether new dibromophenols from *Rhodomela larix*. *Tetrahedron*, **23**, 1185–1188.
- Kazlauskas, R., Murphy, P.T., Wells, R.J., Baired-Lambert, J.A. and Jamieson, D.D. (1983) Halogenated pyrrolo(2,3-d)pyrimidine nucleosides from marine organisms. *Aust. J. Chem.*, **83**, 165–170.
- Kim, J.-K., Noh, J.H., Lee, S., *et al.* (2002) The first total synthesis of 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (TDB) and its antioxidant activity. *Bull. Korean Chem. Soc.*, **23**, 661–662.
- Kim, Y.C., An, R.B., Yoon, N.Y., Nam, T.J. and Choi, J.S. (2005) Hepatoprotective constituents of the edible brown alga *Ecklonia stolonifera* on tacrine-induced cytotoxicity in Hep G2 cells. *Arch. Pharm. Res.*, **28**, 1376–1380.
- Kim, Y.H., Kim, E.-H., Lee, C., Kim, M.-H. and Rho, J.-R. (2007) Two new monogalactosyl diacylglycerols from brown alga *Sargassum thunbergii*. *Lipids*, **42**, 395–399.
- Kirkup, M.P. and Moore, R.E. (1983) Two minor diterpenes related to dictyodial A from the brown alga *Dictyota crenulata*. *Phytochemistry*, **22**, 2539–2541.
- Kladi, M., Xenaki, H., Vagias, C., Papazafiri, P. and Roussis, V. (2006) New cytotoxic sesquiterpenes from the red algae *Laurencia obtusa* and *Laurencia microcladia*. *Tetrahedron*, **62**, 182–189.
- Kladi, M., Vagias, C., Stavri, M., Mukhlesu Rahman, M., Gibbons, S. and Roussis, V. (2008) C15 Acetogenins with antistaphylococcal activity. *Phytochem. Lett.*, 31–36.
- Kladi, M., Vagias, C., Furnari, G., Morreau, D., Roussakis, C. and Roussis, V. (2005) Cytotoxic cuparene sesquiterpenes from *Laurencia microcladia*. *Tetrahedron Lett.*, **46**, 5723–5726.
- Knott, M.G., Mkwana, H., Arendse, C.E., Hendricks, D.T., Bolton, J.J. and Beukes, D.R. (2005) Plocoralides A–C, polyhalogenated monoterpenes from the marine alga *Plocamium corallorhiza*. *Phytochemistry*, **66**, 1108–1112.
- Koehn, F.E., Gunasekera, S.P., Niel, D.N. and Cross, S.S. (1991) Halitunal, an unusual diterpene Aldehyde from the marine alga *Halimeda tuna*. *Tetrahedron Lett.*, **32**, 169–172.
- Koing, G.M., Wright, A.D. and Linden, A. (1999) *Plocamium hamatum* and its Monoterpenes: chemical and biological investigations of the tropical marine red alga. *Phytochemistry*, **52**, 1047–1053.
- Kubaneck, J., Jensen, P.R., Keifer, P.A., Sullards, M.C., Collins, D.O. and Fenical, W. (2003) Seaweed resistance to microbial attack: a targeted chemical defense against marine fungi. *Proc. Natl Acad. Sci. USA* **100**, 6916–6921.
- Kubaneck, I., Prusak, A.C., Snell, T.W., *et al.* (2005) Antineoplastic diterpene-benzoate macrolides from the Fijian red alga *Callophycus serratus*. *Org. Lett.* **7**, 261–264.
- Kubaneck, J., Prusak, A.C., Snell, T.W., *et al.* (2006) Bromophycolides C-I from the Fijian red alga *Callophycus serratus*. *J. Nat. Prod.*, **69**, 731–735.
- Kuniyoshi, M., Yamada, K. and Higa, T. (1985) A biologically active diphenyl ether from the green alga *Cladophora fascicularis*. *Experientia*, **41**, 523–524.
- Kurata, K. and Amiya, T. (1977) Two new bromophenols from the red alga *Rhodomela larix*. *Chem. Lett.*, **6**, 1435–1438.
- Kurata, K. and Amiya, T. (1980) Bis(2,3,6-tribromo-4,5-dihydroxybenzyl) ether from the red alga, *Symphyocladia latiuscula*. *Phytochemistry*, **19**, 141–142.
- Kurata, K., Tanguchi, K., and Suzuki, M. (1996) Cyclozonarone, a sesquiterpene-substituted benzoquinone

- derivative from the brown alga *Dictyopteris undulate*. *Phytochemistry*, **41**, 749–752.
- Kurata, K., Amiya, T. and Nakano, N. (1976) 3,3'-Dibromo-4,4',5,5'-tetrahydroxybibenzyl, a new bromophenol from the red alga, *Polysiphonia urceolata*. *Chem. Lett.*, **5**, 821–822.
- Lane, A.L., Stout, E.P., Hay, M.E., *et al.* (2007) Callophycoic acids and callophycols from the Fijian red alga *Callophycus serratus*. *J. Org. Chem.*, **72**, 7343–7351.
- Laube, T., Beil, W. and Seifert, K. (2005) Total synthesis of two 12-nordrimanes and the pharmacological active sesquiterpene hydroquinone yahazunol. *Tetrahedron*, **61**, 1141–1148.
- Lee, H.-S., Lee, T.-H., Lee, J.H., *et al.* (2007) Inhibition of the pathogenicity of *Magnaporthe grisea* by bromophenols, isocitrate lyase inhibitors, from the red alga *Odonthalia corymbifera*. *J. Agric. Food Chem.*, **55**, 6923–6928.
- Lee, Y.S., Shin, K.H., Kim, B.K. and Lee, S. (2004) Anti-diabetic activities of fucosterol from *Pelvetia siliquosa*. *Arch. Pharmacol. Res.*, **27**, 1120–1122.
- Li, X.-C., Jacob, M.R., Ding, Y., *et al.* (2006) Capisterones A and B, which enhance fluconazole activity in *Saccharomyces cerevisiae*, from the marine green alga *Penicillus capitatus*. *J. Nat. Prod.*, **69**, 542–546.
- Li, K., Li, X.-M., Ji, N.-Y. and Wang, B.-G. (2007) Natural bromophenols from the marine red alga *Polysiphonia urceolata* (Rhodomelaceae): structural elucidation and DPPH radical-scavenging activity. *Bioorg. Med. Chem.*, **15**, 6627–6631.
- Lightowler, J.E. and Rylance, H.J. (1964) On the anti-inflammatory activity of some substituted phenolic compounds. *Br. J. Pharmacol.*, **22**, 221–227.
- Liu, Q., Xu, H., Zhang, T., Fan, X. and Han, I. (2008) *Huaxue Tongbao*. (2006) **69**, 708. In: *Nat. Prod. Rep. Marine. Nat. Prod.* **25**, 35–94.
- Lopez, A. and Gerwick, H. (1988) Ptiollodene, a novel eicosanoid inhibitor of 5-lipoxygenase and Na<sup>+</sup>/K<sup>+</sup> + ATPase from the red marine alga *Ptilota filicina*. *Tetrahedron Lett.*, **29**, 1505–1506.
- Ma, M., Zhao, J., Wang, S., *et al.* (2006) Bromophenols coupled with methyl gamma-ureidobutyrate and bromophenol sulfates from the red alga *Rhodomela confervoides*. *J. Nat. Prod.*, **69**, 206–210.
- Maeda, M., Kodama, T., Tanaka, T., *et al.* (1986) Structures of isodomic acids A, B and C novel insecticidal amino acids from the red alga *Chondria armata*. *Chem. Pharm. Bull.*, **34**, 4892–4895.
- Maier, I., Muller, D.G., Gassmann, G., Boland, W., Marner, E.I. and Jaenicke, L. (1984) Pheromone-triggered gamete release in *Chorda tomentosa*. *Naturwissenschaften*, **71**, 48–49.
- Mann, M.G., Mkwanzani, H.B., Antunes, E.M., *et al.* (2007) Halogenated monoterpene aldehydes from the South African marine alga *Plocamium corallorhiza*. *J. Nat. Prod.*, **70**, 596–599.
- Mao, S.-C., Guo, Y.-W. and Shen, X. (2006) Two novel aromatic valerenane-type sesquiterpenes from the Chinese green alga *Caulerpa taxifolia*. *Bioorg. Med. Chem. Lett.*, **16**, 2947–2950.
- Mori, J., Iwashima, M., Wakasugi, H., *et al.* (2005) New plastoquinones isolated from the brown alga, *Sargassum micracanthum*. *Chem. Pharm. Bull.*, **53**, 1159–1163.
- Moore, R.E. and Entzeroth, M. (1988) Majusculamide D and deoxymajusculamide D, two cytotoxins from *Lyngbya majuscula*. *Phytochemistry*, **27**, 3101–3103.
- Moon-Moo, K., Sang-Hoon, L. and Se-Kwon, K. (2009) Patent from PCT Int. Appl., WO 2009048195 A1 20090416. Language: English, Database: CAPLUS.
- Muller, D.G., Clayton, M.N., Gassmann, O., *et al.* (1985) Cystophorene and hormosirene, sperm attractants in Australian brown algae. *Naturwissenschaften*, **72**, 97–99.
- Muller, D.G., Boland, W., Becker, U. and Wahl, T. (1988) Caudoxirene, the spermatozoide-releasing and attracting factor in the marine brown alga *Perithalia caudate* (Phaeophyceae, Sporochneales). *Biol. Chem. Hoppe-Seyler*, **369**, 655–659.
- Mynderse, I., Moore, R., Kashiwagi, M. and Norton, T. (1997) Antileukemic activity in the Oscillatoriaceae: isolation of debromoaplysiatoxin from *Lyngbya*. *Science*, **196**, 538–540.
- Nahas, R., Abatis, D., Anagnostopoulou, M.A., Kefalas, P., Vagias, C. and Roussis, V. (2007) Radical-scavenging activity of Aegean Sea marine algae. *Food Chem.*, **102**, 577–581.
- Nishizawa, K. and Satoh, Y. (1975) Reaction of cycloalkanones with copper (II) halides. Reaction of cyclohexanones with copper (II) bromide. *Bull. Chem. Soc. Jpn.*, **48**, 1875–1877.
- Norte, M., Fernandez, J.J., Saouto, M.L., Gavin, J.A. and Garcia-Gravalos, M.D. (1997) Thyrsenols A and B, two unusual polyether squalene derivatives. *Tetrahedron*, **53**, 3173–3178.
- Numata, A., Kambara, S., Takahashi, C., *et al.* (1991) Cytotoxic activity of marine algae and a cytotoxic principle of the brown alga *Sargassum tortile*. *Chem. Pharm. Bull.*, **39**, 2129–2131.
- Numata, A., Kanbara, S., Takahashi, C., *et al.* (1992) A cytotoxic principle of the brown alga *Sargassum tortile* and structures of chromenes. *Phytochemistry*, **31**, 1209–1213.
- Numata, A., Takahashi, C., Ito, Y., *et al.* (1993) Communesins, cytotoxic metabolites of a fungus isolated from a marine alga. *Tetrahedron Lett.*, **34**, 2355–2358.

- Numata, A., Takahashi, C., Ito, Y., *et al.* (1996) Penochalasin, a novel class of cytotoxic cytochalasins from a *Penicillium* species separated from a marine alga: structure determination and solution conformation. *J. Chem. Soc. Perkin Trans.*, **1** 239–245.
- Ochi, M., Kotsuki, H., Muraoka, K. and Tokoroyama, T. (1979) The structure of yahazunol, a new sesquiterpene-substituted hydroquinone from the brown seaweed *Dictyopteris undulata*. *Okamura Bull. Chem. Soc. Jpn.*, **52**, 629–630.
- Ohata, K., Mizushima, Y., Hirata, N., *et al.* (1998) Sulphoquinovosyldiacylglycerol, KM043, A new potent inhibitor of eukaryotic DNA polymerases and HIV-reverse transcriptase type from a marine red alga *Gigartina tenella*. *Chem. Pharm. Bull.*, **46**, 684–686.
- Ohta, K. (1977) Antimicrobial compounds in the marine red alga *Beckerella subcostatum*. *Agric. Biol. Chem.*, **41**, 2105–2106.
- Ohta, K. and Takagi, M. (1977) Antimicrobial compounds of the marine red alga *Marginisporum aberrans*. *Phytochemistry*, **16**, 1085–1086.
- Osterhage, C., Kaminsky, R., Koeing, G.M. and Wright, A.D. (2000) Ascosalipyrrolidinone A, an antimicrobial alkaloid, from the obligate marine fungus *Ascochyta salicorniae*. *J. Org. Chem.*, **65**, 6412–6417.
- Park, H.J., Chung, H.Y., Kim, I. and Choi, I.S. (1999) Antioxidative activity of 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether from *Symphyocladia latiuscula*. *J. Fish. Sci. Technol.*, **2**, 1–7.
- Park, H., Kurokawa, M., Shiraki, K., Nakamura, N., Choi, I. and Hattori, M. (2005) Antiviral activity of the marine alga *Symphyocladia latiuscula* against herpes simplex virus (HSV-1) *in vitro* and its therapeutic efficacy against HSV-1 infection in mice. *Biol. Pharm. Bull.*, **28**, 2258–2262.
- Paul, V.J. and Fenical, W. (1983) Isolation of halimedtrial: chemical defense adaptation in the calcareous reef-building alga *Halimeda*. *Science*, **221**, 747–749.
- Paul, Y.J. and Fenical, W. (1984) Novel bioactive diterpenoid metabolites from tropical marine algae of the genus *Halimeda*. *Tetrahedron*, **40**, 3053–3062.
- Pec, M.K., Aguirre, A., Moser-Their, K., *et al.* (2003) Induction of apoptosis in estrogen dependent and independent breast cancer cells by the marine terpenoid dehydrothysiferol. *Biochem. Pharmacol.*, **65**, 1451–1461.
- Pereira, H.S., Leao-Ferreira, L.R., Moussatche, N., *et al.* (2004) Antiviral activity of diterpenes isolated from the Brazilian marine alga *Dictyota menstrualis* against human immunodeficiency virus type 1 (HIV-1). *Antiviral Res.*, **64**, 69–76.
- Perry, N.B., Bluent, J.W. and Munro, M.H.G. (1991) A cytotoxic and antifungal 1,4-naphthoquinone and related compounds from a New Zealand brown alga *Landsburgia quercifolia*. *J. Nat. Prod.*, **54**, 978–985.
- Puglisi, M.P., Tan, L.T., Jensen, P.R., and Fenical, W. (2004) Capisterones A and B from the tropical green alga *Penicillus capitatus*: unexpected anti-fungal defenses targeting the marine pathogen *Lindra thallasiae*. *Tetrahedron*, **60**, 7035–7039.
- Roh, Y.S., Son, B.W., Im, K.S. and Choi, H.D. (1994) Structure of floridoside, a glycerol glycoside from the marine red alga *Gracilaria verrucosa*. *Saengyak Hakhoechi*, **25**, 117–120.
- Rovirosa, J., Sepulveda, M., Quezada, E. and San-Martin, A. (1992) Isoepitaondiol, a diterpenoid of *Stypopodium flabelliforme* and the insecticidal activity of stypotriol, epitaondiol and derivatives. *Phytochemistry*, **31**, 2679–2681.
- Sakemi, S., Higa, T., Jefford, C.W. and Bernardinelli, G. (1986) Venustatriol: a new antiviral triterpene tetracyclic ether from *Laurencia venusta*. *Tetrahedron Lett.*, **27**, 4287–4290.
- San-Martin, A., Negrete, R. and Rovirosa, J. (1991) Insecticide and acaricide activities of polyhalogenated monoterpenes from Chilean *Plocamium cartilagineum*. *Phytochemistry*, **30**, 2165–2169.
- Seo, Y., Park, K.E., Kim, Y.A., *et al.* (2006) Isolation of tetraprenyltoluquinols from the brown alga *Sargassum thunbergii*. *Chem. Pharm. Bull.*, **54**, 1730–1733.
- Seo, Y., Park, K.E. and Nam Bull, T.J. (2007) Isolation of a new chromene from the brown alga *Sargassum thunbergii*. *Korean Chem. Soc.*, **28**, 1831–1833.
- Sheu, J.-H., Wang, G.-H., Sung, P.-J. and Duh, C.-Y. (1999) New cytotoxic oxygenated fucosterols from the brown alga *Turbinaria conoides*. *J. Nat. Prod.*, **62**, 224–227.
- Shimizu, Y. (2000) Microalgae as a drug source. In *Drugs from the Sea* (ed. N. Fusetani). Karger, Basel, pp. 30–45.
- Sims, J.J., Lin, G.H.Y. and Wing, R.M. (1974) Marine natural products, elatol, a halogenated sesquiterpene alcohol from the red alga *Laurencia elata*. *Tetrahedron Lett.*, **39**, 3487–3490.
- Smyrniotopoulos, V., Abatis L.-A., D., Tziveleka, C., *et al.* (2003) Acetylene sesquiterpenoid esters from the green alga *Caulerpa prolifera*. *J. Nat. Prod.*, **66**, 21–25.
- Solem, M.L., Jiang, Z.D. and Gerwick, W.H. (1989) Three new and bioactive icosanoids from the temperate red marine alga *Farlowia mollis*. *Lipids*, **24**, 256–260.
- Steierle, D.B., Wing, R.M. and Sims, J.J. (1979) Marine natural products-XVI: Polyhalogenated acyclic monoterpenes from the red alga *plocamium* of Antarctica. *Tetrahedron*, **35**, 2855–2859.
- Sun, Y., Xu, Y., Liu, K., Hua, H., Zhu, H. and Pei, Y. (2006) Gracilarioside and gracilamides from the red alga *Gracilaria asiatica*. *J. Nat. Prod.*, **69**, 1488–1491.

- Sun, J., Shi, D.Y., Li, S., *et al.* (2007) Chemical constituents of the red alga *Laurencia tristicha*. *J. Asian Nat. Prod. Res.*, **9**, 725–734.
- Suzuki, M., Yamada, H. and Kurata, K. (2002) Dictyterpenoids A and B, two novel diterpenoids with feeding-deterrent activity from the brown alga *Dilophus okamurae*. *J. Nat. Prod.*, **65**, 121–125.
- Suzuki, T., Furusaki, A., Matsumoto, T., Kato, A., Imanaka, Y. and Kurosawa, E. (1985) Teurilene and thyriferyl 23 acetate, meso and remarkably cytotoxic compounds from the marine red alga *Laurencia obtusa*. *Tetrahedron Lett.*, **26**, 1329–1332.
- Suzuki, T., Takeda, S., Suzuki, M., Kurosawa, E., Kato, A. and Imanaka, Y. (1987) Constituents of marine plants. Part 67 Cytotoxic squalene-derived polyethers from the marine red alga *Laurencia obtusa* (Hudson) Lamouroux. *Chem. Lett.*, 361–364.
- Takahashi, C., Numata, A., Ito, Y., *et al.* (1994a) Leptosins, antitumor metabolites of a fungus isolated from a marine alga. *J. Chem. Soc. Perkin Trans.*, **1**, **13**, 1859–1864.
- Takahashi, C., Numata, A., Matsumura, E., *et al.* (1994b) Leptosins I and J, cytotoxic substances produced by a *Leptosphaeria* species physico-chemical properties and structures. *J. Antibiotics*, **47**, 1242–1249.
- Takahashi, C., Takai, Y., Kimura, Y., Numata, A., Shigematsu, N. and Tanaka, H. (1995a) Cytotoxic metabolites from fungal adherent of a marine alga. *Phytochemistry*, **38**, 155–158.
- Takahashi, C., Minoura, K., Yamada, T., *et al.* (1995b) Potent cytotoxic from a *Leptosphaeria* species structure determination and conformational analysis. *Tetrahedron*, **51**, 3483–3498.
- Takahashi, C., Numata, A., Yamada, T., *et al.* (1996) Penostatins, novel cytotoxic metabolites from a *Penicillium* species separated from a green alga. *Tetrahedron Lett.*, **37**, 655–658.
- Takahashi, Y., Daitoh, M., Suzuki, M., Abe, T. and Masuda, M. (2002) Halogenated metabolites from the new Okinawan red alga *Laurencia yonaguniensis*. *J. Nat. Prod.*, **65**, 395–398.
- Talpir, R., Rudi, A., Kashman, Y., Loya, Y. and Hizi, A. (1994) Three new sesquiterpene hydroquinones from marine origin. *Tetrahedron*, **50**, 4179–4184.
- Tan, L.T., Williamson, R.T., Gerwick, W.H., Watts, K.H., McGough, K. and Jacobs, R. (2000) *Cis,cis* and *trans,trans*-ceratospongamide, new bioactive cyclic heptapeptides from the Indonesian red alga *Ceratodictyon spongiosum* and symbiotic sponge *Sigmadocia symbiotica*. *J. Org. Chem.*, **65**, 419–25.
- Tanaka, I. and Higa, T. (1984) Hydroxydictyodial, a new antifeedant diterpene from the brown alga *Dictyota spinulosa*. *Chem Lett.*, **2**, 231–232.
- Tang, H.-F., Yi, Y.-H., Yao, X.-S., Xu, Q.-Z., Zhang, S.-Y. and Lin, H.-W. (2002a) Bioactive steroids from the brown alga *Sargassum carpophyllum*. *J. Asian Nat. Prod. Res.*, **4**, 95–101.
- Tang, H.F., Yi, Y.H., Yao, X.S., Wu, J.H., Zhang, S.Y. and Xu, Q.Z. (2002b) Studies on the chemical constituents from marine brown alga *Ishige okamurai*. *Zhongguo Zhongyao Zazhi*, **27**, 269–273.
- Topeu, G., Aydogmus, Z., Imre, S., *et al.* (2003) Brominated sesquiterpenes from the red alga *Laurencia obtusa*. *J. Nat. Prod.*, **66**, 1505–1508.
- Trease, G.E. and Evans, W.C. (1996) *Pharmacognosy*, 14th ed.. W.B Saunders Company Ltd., London, Philadelphia, Toronto, Sydney, Tokyo, pp. 18–27.
- Tringali, C., Prattellia, M. and Nicols, G. (1984) Structure and conformation of new diterpenes based on the dola-bellane skeleton from *Dictyota* species. *Tetrahedron*, **40**, 799–803.
- Tziveleka, L.-A., Abatis, D., Paulus, K., Bauer, R., Viggias, C. and Roussis, V. (2005) Marine polyprenylated hydroquinones, quinones, and chromenols with inhibitory effects on leukotriene formation. *Chem. Biol.*, **2**, 901–909.
- Vairappan, C.S. (2003) Potent antibacterial activity of halogenated metabolites from Malaysian red algae, *Laurencia majuscula* (Rhodomelaceae, Ceramiales). *Biomol. Eng.*, **20**, 255–259.
- Vairappan, C.S., Kawamoto, T., Miwa, H. and Suzuki, M. (2004) Potent antibacterial activity of halogenated compounds against antibiotic-resistant bacteria. *Planta Med.*, **70**, 1087–1090.
- Wall, M.E., Wani, M.C., Manikumar, G., *et al.* (1989) Plant antimutagenic agents, structure and antimutagenic properties of cymobarbatol and 4-isocymobarbatol, new cymobols from green alga *Cymopolia barbata*. *J. Nat Prod.*, **52**, 1092–1099.
- Wang, R., Shimizu, Y., Rios-Steiner, J.R. and Clardy, J. (1993) The absolute configuration of bacillariolides I and N a new type of cyclopentane eicosanoids from a marine diatom. *J. Chem. Soc. Chem. Commun.*, 379–781.
- Wang, H., Li, Y.-L., Shen, W.-Z., Rui, W., Ma, X.-J. and Cen, Y.-Z. (2007) Antiviral activity of a sulfoquinovosyldiacylglycerol (SQDG) compound isolated from the green alga *Caulerpa racemosa*. *Bot. Marina*, **50**, 185–190.
- Wang, W., Okada, Y., Shi, H., Wang, Y. and Okuyama, T. (2005) Tasipeptins A and B: structures and aldose reductase inhibitory effects of bromophenols from the red alga *Symphyocladia latiuscula*. *J. Nat. Prod.*, **68**, 620–622.
- Watanabe, K., Umeda, K. and Miyakado, M. (1989a) Isolation and identification of three insecticidal principles

- from the red alga *Laurencia nipponica* Yamada. *Agric. Biol. Chem.*, **53**, 2513–2515.
- Watanabe, K., Miyakado, M., Ohno, N., Okada, A., Yanagi, K. and Moriguchi, K.A. (1988) A polyhalogenated isecitidal monoterpene from the red alga *Plocamium telfairiae*. *Phytochemistry*, **28**, 77–78.
- Wessels, M., Konig, G.M. and Wright, A.D. (1999) A new tyrosine kinase inhibitor from the marine brown alga *Styopodium zonale*. *J. Nat. Prod.*, **62**, 927–930.
- Wiemer, D.E., Idler, D.D. and Fenical, W. (1991) Vidalols A and B, new antiinflammatory bromophenols from the Caribbean marine red alga *Vidalia obtusiloba*. *Experientia*, **47**, 851–853.
- Williams, D.E., Sturgeon, C.M., Roberge, M. and Andersen, R.J. (2007) Nigricanosides A and B, antimitotic glycolipids isolated from the green alga *Avrainvillea nigricans* collected in Dominica. *J. Am. Chem. Soc.*, **129**, 5822–5823.
- Wu, C.X., Li, Z.G. and Li, H.W. (1990) Effect of berbamine on action potential in isolated human atrial tissues. *Asia Pac. J. Pharmacol.*, **5**, 191–193.
- Yamada, T., Iwamoto, C., Yamagaki, N., *et al.* (2002) Leptosins M-N, cytotoxic metabolites from a *Leptosphaeria* species separated from a marine alga., structure determination and biological activities. *Tetrahedron*, **58**, 479–487.

# 2

## Seaweeds: The Wealth of Oceans

**Upadhyayula Suryanarayana Murty and Amit Kumar Banerjee**

*Bioinformatics Group, Biology Division, Indian Institute of Chemical Technology (C.S.I.R),  
Hyderabad, India*

### 2.1 Introduction

As humans, we have always shown our ability to explore, exploit the gigantic resources we have in this planet. The ability to understand the proper use of resources has helped humans to attain the heights of civilization that any other species has failed to do on this planet. But, as a civilization grows needs arise and compel us to believe that “necessity is the mother of invention”. Population growth in the present era is enormous, which pushes us to seek more and more natural resources to meet the basic needs of human beings. Since the 20th century, the search for resources has gained momentum due to the advancement of technologies in all sectors of science. Therefore, today we are able to explore from space to the dark depth of the oceans for new knowledge and novel natural resources.

It is believed that so far, even after the drastic improvement of various technologies, we do not know much both about either space or marine life (Brook, 1949; Chapman, 1950; Bowen, 1956; Cantarow and Schepartz, 1962). According to global scientific research, life started in the ocean and the number of organic life forms present in the 71% marine resource of this planet is far more than the terrestrial life forms. We barely understand them so far and our knowledge is limited. The versatility and variety of life forms frame several scientific questions in our inquisitive minds.

The time has come where we need to look for more possible natural resources, not only for fulfilling our basic needs but also for advancement of our civilization. To establish the human race firmly for a longer duration and

make this planet safer for future generations, we need to explore new resources. Marine resources could be the best choice to hunt for future resources. Present scientific efforts are directed to marine biology, which has huge promise for the future. Several aspects are taken care of, such as new types of foods with better food values within a reasonable price, new medicines, new byproducts, etc.

### 2.2 Need for marine resources

The astonishing growth of human population raises the need to look for some other natural resources. Scarcity of land for cultivation, infertility of soil, limitation of various crops, requirement for more fodder, requisition of specific compounds for life-saving medicines, and so many other specialized requirements has forced mankind to jump into the depths of the ocean and look for alternatives. Even in mythology, ancient human beings believed that oceans had magical powers to meet any kind of needs of human being (Chapman, 1950; Bersamin, *et al.*, 1961; Citharel and Villeret, 1961).

### 2.3 Various marine resources

At a superficial level we harvest energies which are in use in many countries today. In a similar fashion, the complexity of oceanic ecology requires time to understand and look for our needs. Apart from animal resources, which are

brilliantly utilized in our daily life, the plant species that hail from the mysterious environment of these large water bodies hold tremendous promise to fulfill human necessities (Black and Mitchell, 1952; Ericson, 1952).

Various kinds of planktons, algae (Fogg, 1952) and other plants are regularly used as food sources, extraction of resources for candidate medicinal compounds, and other essential requirements (Cotton, 1915; Hendrick, 1916; Haug Larsen, 1955; Johnston, 1965).

## 2.4 Producers in the marine environment

Many producers are available in the marine environment. They are distributed from the surface to the bottom level of the ocean bed. Various kinds of grasses are found in the shallow areas or lagoons of the oceanic environment, which are unique in their own properties. Different kinds of protists are also observed that act as producers on the ocean bed. Though we have amazing numbers of larger producers in the terrestrial regions, still the major producers hail from marine life, the phytoplanktons. These tiny green cells also play a vital role in cooling our planet and in the reduction of global warming. Similarly, other marine algae such as green, brown, and red seaweeds are present in ample amounts and they play essential role in oxygen generation and act as vital producers.

## 2.5 Emergent plants

Extreme marine conditions also help in budding life forms especially different plant species. Plants that survive in excessive salt in the surrounding environment are basically known as emergent plants. To adapt to these harsh conditions, various adaptations and evolved characteristics are found in these marine life forms. These salt-tolerant plants such as mangroves have thick, waxy surfaces, which prevent water loss. Some have wooly coats of hair. Even in marshes and swamps, grasses such as salt grass (*Distichlis*), needle rush (*Spartina*), and cord grass (*Juncus*) survive easily. Other than the phenotypic differences, mentioned there are genotypic differences. These specially evolved life forms contain salt-tolerant genes, which are presently being isolated and cloned for the betterment of crop production in comparatively infertile soils.

## 2.6 Seaweed diversity

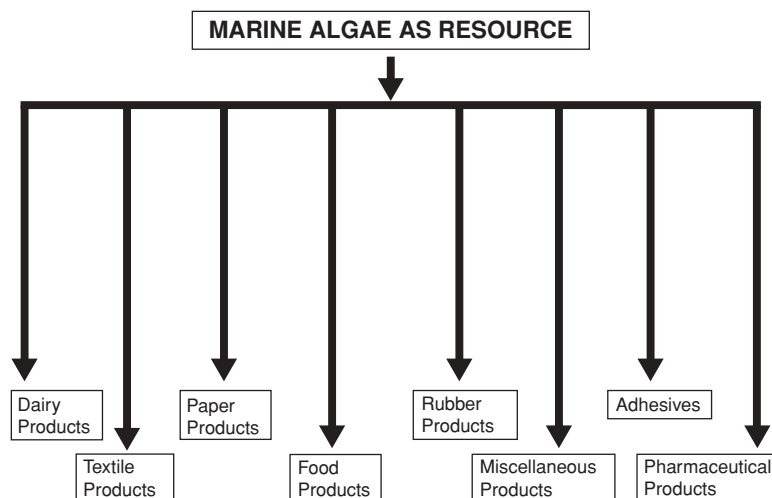
Seaweeds are crucial part of marine ecology and are essential for sustainability of several other organisms. They are present in a very diverse, colorful and ornamental manner.

Some float on the water surface and act as producers and form different single or multi-celled colonies. Others show very large forms such as kelps. They can remain attached to rocks or other supporting material or can be free floating. Some can also get attached to the ocean floor through root-like structures known as holdfasts. However, this holdfast does not play any role in the supply of nutrients or life of the seaweed, it is just used for anchorage. Another important and interesting structure observed in seaweeds is the presence of a stipe, which is a stem-like structure and holds the blades near to the water surface. Floating seaweeds occasionally contain an air sac to float on the water surface and expose the plant to sunlight for photosynthesis. Different kinds of pigmentation are observed in the seaweeds. Other than chlorophyll, sometimes they contain carotene (orange in color) or xanthophylls (brown in color) (Mautner, 1954). These are abundant in lagoons of Florida and several parts of the Pacific Ocean and other places. The green forms of seaweeds are predominant in fresh water rather than in the ocean. The sea lettuces, *Ulva* sp. are the most common green seaweeds observed in various habitats. The natural habitat of the Phaeophyta (brown seaweeds) is mostly in the coastal areas, preferably in cold water bodies. Physically, these are the largest seaweeds, which can even be up to 35 m (100 feet) in length. Kelps, *Macrocystis*, *Nereocystis*, and *Sargassum* are the most common. An extreme level of marine habitat is observed for the Rhodophyta (red seaweeds). They mostly grow on the dark ocean bed. Reddish-purple and reddish-brown varieties are also often observed on coral reefs and oyster beds.

## 2.7 Uses of seaweeds

Since time immemorial, humans have used seaweeds for different purposes from decoration to food materials. History shows that people of the Far East maintained and harvested seaweeds on a regular basis and they were an inseparable part of their mariculture practice. Brown seaweeds were converted into soda ash in ancient times and efficient applications were made by pottery and glass makers. Fertilizer such as potash was extracted from burnt or dried seaweeds.

Sea lettuce is sometimes consumed as food. Brown seaweeds maintain the surrounding ecology by providing food resources and shelters for shrimps, juvenile crabs, and fishes. These brown seaweeds are easily harvestable and they are used in industry as sources of iodine, trace minerals, fats, and sometimes vitamins. Red seaweeds are used in different countries as ingredients of soup and seasoning. Iodine is also mainly derived from seaweed sources. Seaweed products contribute in a great manner to the economy of the island like countries such as Japan and those



**Figure 2.1** Essential products obtained from marine algal resources including seaweeds used for human use.

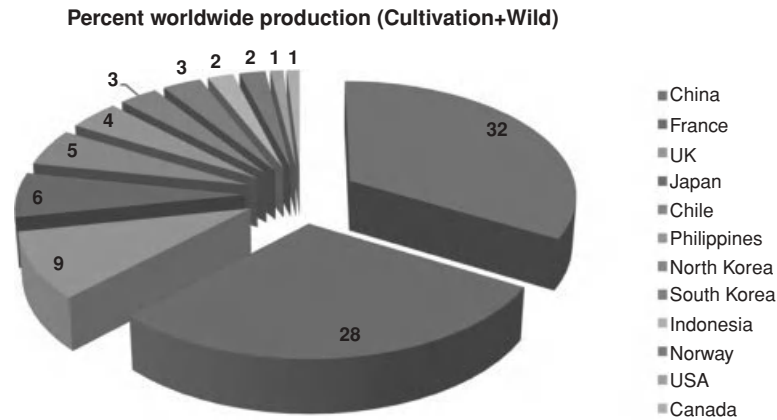
nations who are having large coastal areas. This marine resource has proved to be an enriched source of minerals and vitamins, which has helped us to consider it as an important food source for future. Seaweeds are outstanding source of vitamins like A, B1, B12, C, D, E, riboflavin, niacin, pantothenic acid and folic acid (Nisizawa, 1988). The specific broad areas of utilization of seaweeds are shown in Figure 2.1.

Details of the product types in Figure 2.1 are given in Table 2.1. The common products shown are extensively used in daily life and their demands are increasing almost on a daily basis. The commercial prospect is quite demanding and profitable.

During harvesting of the brown seaweeds, only the top portions are chopped out and pulled up onto a barge. Dried kelp or brown seaweed is used for production of

**Table 2.1** Seaweed product information

Kind of product	Specific uses
Dairy products	Ice cream; dry ice cream mix; sherbet; chocolate milk; chocolate; toddy; sterilized cream; cheese
Rubber products	Natural and synthetic latex creaming; Automobile carpeting; electrical insulation; babies' rubber pants; rubber coating; foam coating; tires
Miscellaneous products	Paints; ceramic glazes; porcelain ware; leather finishes; auto polishes; welding rod coating; boiler compounds; battery plate separators; wallboard joint cement; beet sugar processing; wax emulsions
Adhesives	Wallboard; paper bags; shipping containers; Gummed tape and decals
Pharmaceutical products	Aureomycin tablets; triple sulfa tablets; teramycin suspensions; penicillin suspensions; antacid tablets; sulfa suspensions; aspirin compound tablets; calamine lotion; hemostatic powder; bulking laxatives; toothpaste; dental impression compound; orthopedic impression; surgical jellies; Suppositories; mineral oil emulsions; Rubbing ointment; pharmaceutical soap
Miscellaneous food products	Bakery icings and meringues; salad dressings; Frozen foods; fountain syrups; orange concentrates; candy puddings
Paper products	Food packages; milk containers; butter cartons; Frozen food packages; insulation board; food wrappers; greaseproof paper; acoustical tile
Textile products	Textile print pastes; plastic laundry starch; size compounds for cotton and rayon



**Figure 2.2** Wild growth and cultivated seaweed production in percent worldwide top ranking countries.

carrageenan, algin, and agar. These products are widely used in ice cream, as suspended antibiotics in solution, and pigment in paints, canning meat and fish, and as glue.

The importance of seaweed lies in its great food value as it is rich in protein, lipids and carbohydrates (Dhargalkar and Pereira, 2005).

## 2.8 Marine farming: global scenario

Marine farming is gaining momentum due to the rise in need and requirement for cheap farming. As marine products are gaining popularity due to their enriched food values, simplicity and different taste, opportunities are rising for mass production. Seaweeds are definitely leading as the raw material resource. A worldwide market is available not only for the main products; demands are huge for the byproducts too. Asian countries such as China, Japan, Korea, and Philippines are leading in seaweed farming whereas European countries are collecting the raw material from natural resources.

Worldwide competition is on for gigantic production of useful seaweeds. South Asian countries are producing considerable amounts of seaweed. Across the globe, seaweed production is mostly divided into two types: natural abundance and production through artificial or marine farming. Asian countries follow the molecular farming strategy to obtain different types of algae whereas European countries are lucky enough to extract it from natural or wild habitats. Figures 2.2 and 2.3 depict the globally top ranked producers of seaweed.

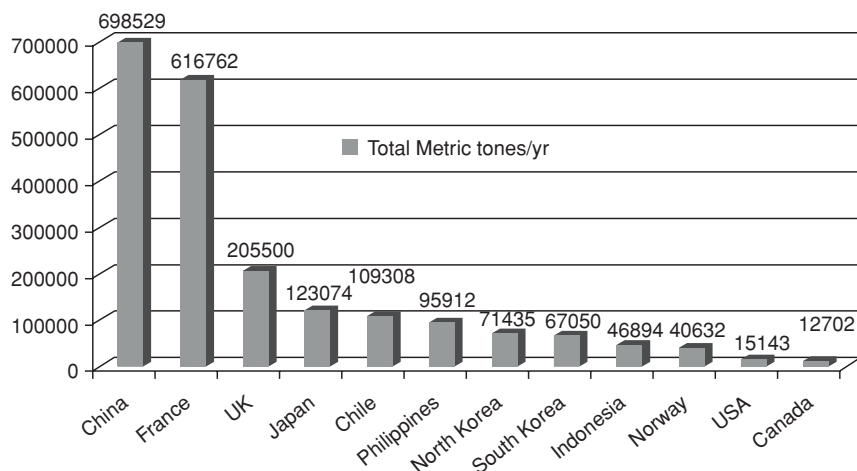
Figures 2.4 and 2.5 show the real time data in metric tonnes for different seaweed producing countries who are leading universal production.

## 2.9 SEAPURA: an EU effort

Marine farming, especially seaweed production, is gaining so much attention that several large-budget projects are coming up, SEAPURA is such a project, which is funded by the European Union (EU) (Lüning, 2001). In this



**Figure 2.3** Worldwide top ranking in percent production of cultivated seaweeds.

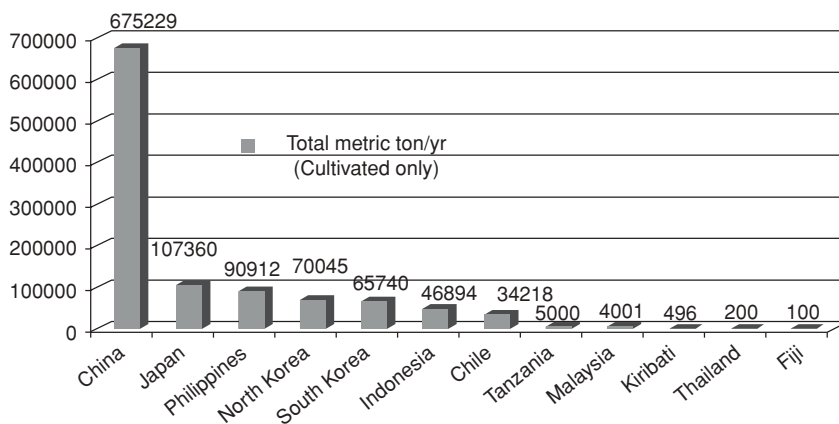


**Figure 2.4** Year wise total production of seaweeds (in metric tons). (Courtesy: Data Source [http://www.seavegetables.com/gis/stats\\_prod.asp](http://www.seavegetables.com/gis/stats_prod.asp))

interesting idea fish farming and seaweed production is taken care of concurrently. The whole project is divided into five important phases. In the first phase, nutrient-rich waste water obtained from fish farming is used for integrated seaweed aquaculture systems. Improvement of seaweed production and health of the weed inspection consist of the following two phases. Extracted high value algal biomass is used for fish feed, antibiotics for fishes and chemical and pharmaceutical product derivation in the later section. The second phase involves evaluation of environmental integrity and other socio-economical impacts from the output of the whole farming project. This kind of integrated and inter-related project will revolutionize the whole process and idea of farming and end up with great economical and product yield.

## 2.10 Seaweed farming: an Indian scenario

It has been noticed that despite being a large country with a vast coastline, India is not able to produce important marine weeds to a satisfactory level. Though the initiation of seaweed production in India was for promoting a livelihood option for those people who are on the economical margins, but slowly it got direction and self help groups (SHG) have done a tremendous job to increase the production, marketing, and community relationships (Krishnan and Narayanakumar, 2010). This small-scale industry has come out of its infancy and presently showing ray of light to becoming a bigger marine industry. The coastal areas of Orissa, Andhra Pradesh, Tamil Nadu, Kerala, and Goa



**Figure 2.5** Production of only cultivated seaweeds per year in metric tons. (Courtesy: Data Source [http://www.seavegetables.com/gis/stats\\_prod.asp](http://www.seavegetables.com/gis/stats_prod.asp))

have strong potential to become hub for seaweed production and their byproduct-related industry. Tamil Nadu has already shown progress on this issue. Seaweed farming of *Kappaphycus alvarezii* (Caddy and Santelices, 1988) was initiated by PepsiCo in the year 2000 and later on they supported the formation of SHG and obtaining bank loans, etc. The changing scenario of demand and supply and growing dependence on natural resources are showing lots of promises to develop a seaweed-based industry to an international level. In an Indian perspective, the predominant algal species are *Gracilaria*, *Gelidium* and *Gelidiella*. In *Gracilaria*, the common varieties found in the Indian coastal region are *G. crassa*, *G. corticata* and *G. multipartita* (syn. *G. folizfera*) (Coppen and Nambiar, 1991).

## 2.11 Expanding the existing knowledge base: current research trends in exploring seaweeds

The improvement in the technologies available in life sciences has provided the scientific community with a golden opportunity to understand and explore this wealth and obtain maximum benefit from it. Molecular techniques are expanding their horizons to extract valuable information from this outstanding resource. Various recent technologies are extensively being used for exploring more information about the seaweeds and their beneficiary products. Among these technologies, metagenomics and *in silico* biology are playing vital roles to understand the complex molecular issues involved in seaweed. Upstream and downstream processing technologies are aiding mass production and bulk product generation. Scientists from different quarters of the globe are involved in exploiting this wealth of the ocean with advanced techniques available.

### 2.11.1 Metagenomics in understanding seaweeds

In the recent past, various academic and private institutions are showing interest in the molecular aspects of seaweeds. Metagenomics has emerged as the recent tool for understanding the molecular aspects of seaweed colony formation, product development capability and understanding and increasing the inter-relation among different weeds living in a community or a specific environment. It has been a very successful approach for microbial communities (Hugenholtz and Tyson, 2008). Advances in the sequencing techniques have given the opportunity to move forward to a metagenomic approach and understand the environ-

mental or more specifically, community-related effects on an organism through DNA sequencing. Companies like Illumina, 454-Roche, and Pacific Biosciences are providing solutions where terabytes of genome data could be generated in a nominal time scale and this data could be explored to understand the organism and its behavior, thus enhancing the possibility of better yield for the existing products from the marine source as well as looking for novel products that could be used in future. Since 2006, metagenomic approaches have been used for marine-related information. Projects like marine RNA virome analysis, global ocean sampling (GOS) (Venter *et al.*, 2004; Yooseph *et al.*, 2007), coral holobiont analysis, and sampling of coral reefs are raising the hope for better products from the marine life.

### 2.11.2 Role of bioinformatics

Generating terabytes of raw data is one major aspect whereas analyzing those and mining the meaningful information is another important side of the whole scenario. Bioinformatics or computational biology provides the solution for these issues. Information technology has altered our way of life. It has also revolutionized the domain of biological sciences and made storage, deposition, retrieval, and analysis of huge amounts of data generated by next generation sequencing (NGS) technologies feasible.

### 2.11.3 Data storage and retrieval

Storage of generated data has long been an important aspect. Present advanced solutions provided by the software and hardware technologies are capable of managing data beyond our imagination. The latest developments in hardware technology replaced large cluster computers with graphical processing unit (GPU) card. Similarly Oracle, ASP.Net technologies have solved many technical problems of backend data storage, linking with front end databases and dynamic retrieval and analysis of the stored information and its regular updating. Recent development of a seaweed database paved a path for storing and analyzing future information. The Seaweed Metabolite Database (SWMD) (Davis and Vasanthi, 2011) (<http://www.swmd.co.in>) has been developed to share organized information about the compounds and their biological activity available in the literature. Apart from this prior information it also contains the geographical origin of the seaweed, method of compound extraction, and chemical description of the compounds. Some other databases that are useful for seaweed research are listed in Table 2.2.

**Table 2.2** Different types of databases involved in the research for understanding seaweeds at molecular level

Relevance of database	Name of the database	URL
Primary nucleotide and protein sequence database	National Centre for Biotechnological Information (NCBI) (Wheeler <i>et al.</i> , 2006)	<a href="http://www.ncbi.nlm.nih.gov/">http://www.ncbi.nlm.nih.gov/</a>
Primary nucleotide and protein sequence database	European Molecular Biology Laboratory database. (Tomé <i>et al.</i> , 1996)	<a href="http://www.ebi.ac.uk/embl/">http://www.ebi.ac.uk/embl/</a>
Primary nucleotide and protein sequence database	DNA Databank of Japan (Tateno <i>et al.</i> , 1998)	<a href="http://www.ddbj.nig.ac.jp/">http://www.ddbj.nig.ac.jp/</a>
Protein structure database	RCSB Protein Data Bank. (Bourne <i>et al.</i> , 2004)	<a href="http://www.rcsb.org/pdb/home/home.do">http://www.rcsb.org/pdb/home/home.do</a>
Protein structure database	Protein Information Resource. (Barker <i>et al.</i> , 2000)	<a href="http://pir.georgetown.edu/">http://pir.georgetown.edu/</a>
Protein classification database	CATH and SCOP (Pearl <i>et al.</i> , 2003) (Hubbard <i>et al.</i> , 1999)	<a href="http://www.cathdb.info/">http://www.cathdb.info/</a> <a href="http://scop.mrc-lmb.cam.ac.uk/scop/">http://scop.mrc-lmb.cam.ac.uk/scop/</a>
Metabolic pathway databases	Kyoto Encyclopedia for Genes and Genomes (Kanehisa <i>et al.</i> , 2008)	<a href="http://www.genome.jp/kegg/">http://www.genome.jp/kegg/</a>
Drug-related database	Drug Bank, PUBCHEM (Wishart <i>et al.</i> , 2006) (Richard <i>et al.</i> , 2006; Hur and Wild 2008)	<a href="http://www.drugbank.ca/">http://www.drugbank.ca/</a> <a href="http://pubchem.ncbi.nlm.nih.gov/">http://pubchem.ncbi.nlm.nih.gov/</a>

All websites accessed 29 March 2011.

#### 2.11.4 Different kind of information analysis

Computational biology plays an important role not only in storage but also in meaningful analysis for any organism. It is been observed that in the recent times it is being used extensively in seaweed-related research.

#### 2.11.5 Phylogeographical and evolutionary analysis

Samples collected from different locations are analyzed for their phenotypic and genotypic variations and their interaction with varying environmental conditions. During this kind of analysis the end step followed in modern biology is phylogenetic analysis, which involves intense mathematical and statistical calculations on information derived from the sequence information to understand the molecular proximity of organisms with respect to each other (Hoarau *et al.*, 2007). Tracing the phylogeny and evolutionary information holds promise for understanding the proper taxonomy, behavior and other specific species-related information. Phylogenetic analysis uses advanced computational approaches such as neighbor joining (NJ), minimal evolution (ME), maximum parsimony (MP) and maximum likelihood (ML)

methods to solve the complexity and intensity of an analysis and build a proper realistic phylogenetic tree. Advanced statistical approaches such as bootstrapping and jackknife methods are employed to eradicate the bias which can come through experimental or theoretical calculation error. On seaweeds such kind of studies has been performed with successful outcome (Cocquyt *et al.*, 2010).

### 2.12 Future prospects

So far, exploring and understanding marine life with specific reference to seaweed was not so easy due to the technical hurdles. Understanding these widespread life forms requires huge financing, and high throughput mass screening technologies to extract maximum information in less time period. Emergence of high throughput NGS techniques and efficient bioinformatics analysis with a reasonable amount of accuracy will definitely help us to mine the molecular products of interest from this marine goldmine. Exploitation of interdisciplinary approaches such as high throughput virtual screening (HTVS), advanced mathematical and statistical methods such as Bayesian methods, artificial intelligence supported techniques (artificial neural network, support vector machines, genetic algorithms, etc.) for

classifying the minute differences among the species will yield more benefit.

Stepping out from the classical approaches may provide better and valuable information. Modern techniques such as homology modeling, QSAR may hasten the process of compound screening for seaweed sources.

## 2.13 Conclusion

Although several countries are involved in commercialization of seaweed-derived products, such as the Philippines, and Japan is quite dependent on seaweed-derived products, still there is lack of global organization and interest from several countries. This natural resource has been neglected so far for some unknown reason, sometimes due to lack of interest for the project funding aspects. It has been a small-scale industry till now, but slowly the scenario is changing. The latest technologies are helping in speeding up the whole process, which in turn may yield more products and benefits to humans.

There are cautions we should learn from our past behaviors. Humans have exploited natural resources for its benefit but never took care of the resource itself. Extensive use of gasoline led to the pollution and global warming we are facing today. The whole marine ecology is very organized and intact. Minute disturbances may shift the balance to destruction, which in turn may lead to our abolition from this beautiful planet. Moreover, seaweeds are very sensitive in maintaining their micro and macro environment. Therefore, harvesting from natural or artificial marine farming should not effect this surrounding environment. This alternate marine resource may promise many things for human life and time has come when we can remove the tag of small scale industry. Proper organization, popularizing the products and proper scientific understanding and use of this natural resource will help us in building a better future.

## References

- Barker, G.M., Stephens, A., Hunter, C., *et al.* (2002) Biosecure – a model for analysis of biosecurity risk profiles (eds S.L. Goldson, and D.M. Suckling,). New Zealand Plant Protection Society Inc., pp. 73–91.
- Bersamin, S.V., Laron, S.V., Gonzales, F.R., and Banania R.B. *et al.* (1961) Some seaweeds consumed fresh in the Philippines. Proceedings of the Indo-Pacific Fisheries Council, 9th Session, Section 2, 115–119.
- Black, W.A.P. and Mitchell, R.L. (1952) Trace elements in the common brown algae and in sea water. *J. Mar. Biol. Assoc.*, **30**, 575–584.
- Bowen, H.J.M. (1956) Strontium and barium in sea water and marine organisms. *J. Mar. Biol. Assoc.*, **35**, 451–460.
- Bourne, P.E., Address, K.J., Bluhm, *et al.* (2004) The distribution and query systems of the RCSB Protein Data Bank. *Nucl. Acids Res.*, **32**, D223–D225.
- Brook, A.J. (1949) The seaweeds and their uses. *New Biol.*, **7**, 89–103.
- Cantarow, A. and Schepartz, B. (1962) *Biochemistry*, 3rd edn. Saunders, Philadelphia.
- Caddy, J.F. and Santelices, B. (eds) (1988) Case studies of seven commercial seaweed resources. *FAO Fish. Tech. Pap.*, **281**, 123–161.
- Chapman, V.J. (1950) *Seaweeds and Their Uses*. Methuen, London.
- Citharel, J., and Villeret, S. (1964) Researches on nitrogen metabolism of a number of marine algae from the Breton Coast. Fourth International Seaweed Symposium, Pergamon Press, London, pp. 291–300.
- Cocquyt, E., Verbruggen, H., Leliaert, F. and De Clerck, O. (2010) Evolution and cytological diversification of the green seaweeds (Ulvophyceae). *Mol. Biol. Evol.*, **27**(9), 2052–2061.
- Coppen, J.J.W. and Nambiar, P. (1991) Agar and alginate production from seaweed in India. Bay of Bengal Programme Post-Harvest Fisheries, **BOBP/WP/69**, 1–32.
- Cotton, A.D. (1915) Some Chinese marine algae. *Bull. Misc. Inform. Royal Botanical Gardens Kew*, **3**, 107–113.
- Davis, G.D.J. and Vasanthi, A.H.R. (2011) Seaweed metabolite database (SWMD): A database of natural compounds from marine algae. *Bioinformation*, **5**(8), 361–364.
- Dhargalkar, V.K. and Pereira, N. (2005) Seaweed: Promising plant of the millennium. *Science and Culture*, **71**, 3, 60–66.
- Ericson, L.E. (1952) Uptake of radioactive cobalt and vitamin B<sub>12</sub> by some marine algae. *Chem. Ind.*, **2**, 829–830.
- Fogg, G.E. (1952) *The Metabolism of Algae*. Methuen, London.
- Haug, A. and Larsen, B. (1956) Carotene content of some Norwegian seaweeds, and observations on the breakdown of carotene in seaweeds and seaweed meal. Second International Seaweed Symposium, Pergamon Press, London, pp. 16–22.
- Hendrick, J. (1916) The value of seaweeds as raw materials for chemical industry. *J. Soc. Chem. Indust.*, **35**, 565–574.
- Hoarau, G., Coyer, J.A., Veldsink, J.H., Stam, W.T. and Olsen, J.L. (2007) Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*. *Mol. Ecol.*, **16**, 3606–3616.
- Hubbard, T.J.P., Ailey, B., Brenner, S.E., Murzin, A.G. and Chothia, C. (1999) SCOP: A structural Classifications of Proteins Database. *Nucl. Acids Res.*, **7**(1), 254–256.
- Hugenholtz, P. and Tyson, G.W. (2008) Metagenomics. *Nature*, **455**, 481–483.

- Hur, J. and Wild, D.J. (2008) PubChemSR: A search and retrieval tool for PubChem. *Chem. Central J.*, **2**(11), 1–7.
- Johnston, H.W. (1965) The biological and economic importance of algae, Part I. *Tuatara*, **13**, **2**, 90–114.
- Kanehisa, M., Araki, M., Goto, S., *et al.* (2008) KEGG for linking genomes to life and the environment. *Nucl. Acids Res.*, **36**, D480–D484.
- Krishnan, M. and Narayanakumar, R. (2010) Structure, conduct and performance of value chain in seaweed farming in India. *Agric. Econ. Res. Rev.*, **23**, 505–514.
- Lüning, K. (2001) SEAPURA: seaweeds purifying effluents from fish farms. *Experimental Mariculture, Wadden Sea Newsletter*, **2001–2**, 20–21.
- Mautner, H.G. (1954) The chemistry of brown algae. *Econ. Bot.*, **8**, 174–192.
- Nisizawa, K. (1988) Production and utilization of products from commercial seaweeds (ed. D.J. Mchaugh) FAO, Rome, **299**, 147.
- Pearl, F.M.G., Bennett, C.F., Bray, J.E., *et al.* (2003) The CATH database: an extended protein family resource for structural and functional genomics. *Nucl. Acids Res.*, **31**(1), 452–455.
- Richard, A.M., Gold, L.S. and Nicklaus, M.C. (2006) Chemical structure indexing of toxicity data on the Internet: Moving toward a flat world. *Curr. Opin. Drug Dis. Dev.*, **9**(3), 314–325.
- Tateno, Y., Fukami-Kobayashi, K., Miyazaki, S., Sugawara, H. and Gojobori, T. (1998) DNA Data Bank of Japan at work on genome sequence data. *Nucl. Acids Res.*, **26**(1), 16–20.
- Tomé P.R., Stoehr, P.J., Cameron, G.N. and Flores, T.P. (1996) The European Bioinformatics Institute (EBI) databases. *Nucl. Acids Res.*, **24**(1), 6–12.
- Venter, J.C., Remington, K., Heidelberg, J.F., *et al.* (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science*, **304**, 66–74.
- Wishart, D.S., Knox, C., Guo, A.C., *et al.* (2006) DrugBank: a comprehensive resource for *in silico* drug discovery and exploration. *Nucl. Acids Res.*, **34**, D668–D672.
- Wheeler, D.L., Barrett, T., Benson, D.A., *et al.* (2006) Database resources of the National Center for Biotechnology Information. *Nucl. Acids Res.*, **33**, D39–D45.
- Yooseph, S., Sutton, G., Rusch, D.B., *et al.* (2007) The Sorcerer II Global Ocean Sampling Expedition: expanding the universe of protein families. *PLoS Biol.*, **5**(3), e16.

# 3

## Eco-Biochemical Studies of Common Seaweeds in the Lower Gangetic Delta

**Rajrupa Ghosh, Kakoli Banerjee and Abhijit Mitra**

*Department of Marine Science, University of Calcutta, Kolkata, India*

### 3.1 Seaweeds: an overview

The benthic environment of the marine and estuarine compartment supports and sustains a unique floral community. The near shore benthic habitats in the temperate zones often contain multicellular algae, large non-vascular plants known as seaweeds. Seaweeds refer to any large marine benthic algae that are multicellular, macrothallic, and thus differentiated from most algae that are of microscopic size. These plants form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial.

Seaweeds grow attached to rocks, shells, or any solid objects. Sometimes these are found in floating condition at the water's edge or scattered on the shore, but these are practically dislodged from their respective substrates. Seaweeds are attached by a holdfast that anchors the plant to a solid base or substrate. The holdfast is not a root in real sense; it does not absorb water or nutrients. Above the holdfast is a stem-like portion known as stipe. It acts as the flexible connection between the holdfast and the blade and ranges from very short length to approximately 35 m. The blades of the seaweeds are the photosynthetic portions, just like the leaves of the trees. The blades may be flat, ruffled, feathery, or even encrusted with calcium carbonate. Carbohydrates produced by these floral communities provide nutrition to faunal members of the benthic habitats. The large biomass

of seaweeds in the intertidal zone makes it an important primary producer of the marine and estuarine ecosystems.

Based on the color of their pigmentation, the seaweeds are broadly classified into various categories: Chlorophyceae (green), Phaeophyceae (brown), Rhodophyceae (red), etc.

- 1 **Chlorophyceae:** Algae are typically green in color due to the predominant presence of the green pigment, the chlorophyll *a*, contained in chloroplasts. Most of the green algae are found occurring in the littoral zones of the marine environment and produce motile reproductive bodies, e.g., *Codium* sp., *Enteromorpha intestinalis*, *Enteromorpha compressa*, *Ulva lactuca*, *Chaetomorpha* sp., *Cladophora* sp., etc.
- 2 **Phaeophyceae:** The brown algae are distinguished by their color, which varies from olive green through light golden to a deep shade of brown. Motile reproductive cells are commonly found in the brown algae, e.g., kelps like *Postelsia* sp., *Nereocystis* sp., *Macrocystis* sp., *Laminaria* sp., *Fucus* sp., *Padina gymnospora*, and *Rosenvingea intricata*. Since brown algae live primarily in shallow waters or on shoreline rocks, they exhibit adaptations that protect them from the constant pounding of the waves. The body of brown algae is very flexible, which allows them to bend or orient with the wave action. Unlike red

and green algae, brown algae usually do not reproduce by fragmentation because the tissues are too highly specialized to regenerate new parts. A major exception to this rule is *Sargassum* sp. Large masses of this seaweed are found floating in the Atlantic Ocean, where they tend to accumulate in an area known as the Sargasso Sea. These free-floating clumps of sargassum weed form a complex, three-dimensional habitat that sustains a diverse group of organisms.

- 3 **Rhodophyceae:** The marine forms are recognized by their bright pink color caused by the biloprotein pigments, r-phycoerythrin and r-phyocyanin. The freshwater forms, however, are bluish green. Red algae produce large amount of polysaccharides around their cells. Several of these polysaccharides are commercially important. The majority of the marine forms occur from low tide marks to greater depths up to 100 m beneath the surface of the sea, e.g., *Porphyra* sp., *Chondrus crispus*, *Catenella repens*, etc.

## 3.2 Commercial uses of seaweeds

The seaweeds are economically valuable resources. They are used as food, fodder, fertilizer, and medicine and thus useful to mankind in many ways. Agar and algin extracted from the seaweeds have a variety of industrial applications. The major uses of seaweeds are discussed here.

- 1 As food: In many countries like Malaysia, Indonesia, Korea, and Australia seaweeds are used for human consumption. These are used in the preparation of salads, soups, jellies, and vinegar. The common species utilized for the preparation of food are *Porphyra* sp., *Gracilaria* sp., *Laurencia* sp., *Caulerpa* sp., *Sargassum* sp., etc. These algae are rich in Vitamin B.
- 2 As fodder: Some important species of seaweeds that are used in cattle feed are *Ulva* sp., *Enteromorpha* sp., *Gracilaria* sp., *Padina* sp., *Sargassum* sp., etc. Poultry feed is also made in certain areas by mixing seaweeds with trash fishes.
- 3 As manure: Seaweeds have unique capacity to bioaccumulate trace elements from ambient media and therefore they are rich sources of macro- and microelements required for the growth of plants. Seaweeds like *Sargassum* sp. and *Gracilaria* sp. are used as manure for coconut plantation in India, particularly in the south Indian states.
- 4 As medicine: Seaweeds have wide applications in the sphere of medicine. *Sargassum* sp. and *Turbinaria* sp. are

good sources of alginates, which are known to prolong the period of activity of certain drugs. *Hypnea* sp. and *Acanthophora* sp. are the major sources of carrageenans, which have been found to be useful in ulcer therapy.

- 5 As industrial raw material: Agar-agar is a gelatinous colloidal carbohydrate present in the cell wall of algae and is extracted from some members of Rhodophyceae. In India, agar has wide use in the preparation of food, ice creams, jellies, soups, bacteriological samples and cosmetics. Being a non-toxic substance agar is also used for the preparation of wine, beer, hand lotions etc. Algin and alginates have extensive uses in the preparation of various medicines, cosmetics, paper products, textile products, paints, milk products, etc.
- 6 As biological treatment of wastes: Seaweeds can also be utilized for the biological treatment of industrial wastes by utilizing their unique bioaccumulation capacity. It has been found that *Catenella repens*, *Ulva* sp., and *Enteromorpha intestinalis* are unique absorbers of Zn, Cu, Fe etc. from the ambient aquatic medium. These algae, if cultured in the treatment plant of coastal industries can trap substantial amount of metals from the released industrial wastes.
- 7 Ecological value: The macroalgae are noted for their primary production. Efficiencies of solar energy trapped showed a maximum in *Enteromorpha intestinalis* (0.64%) and *Ulva lactuca* (0.43%) with an average of 0.35% by this group. A research conducted on this aspect indicates that in the deltaic complex of Indian Sundarbans, *Enteromorpha intestinalis* and *Ulva lactuca* are the most productive species, followed by *Enteromorpha prolifera* and *Rhizoclonium grande* (Chaudhuri and Choudhury, 1994). The gross and net primary productions and energetics of benthic macroalgae in this mangrove-dominated ecosystem are highlighted in Tables 3.1 and 3.2.

## 3.3 Indian scenario

In India, the total world seaweed production is estimated to be  $1821 \times 10^4$  t (wet weight) annually. Of this 4.83% is being harvested from the western and eastern Indian Ocean (Qasim, 1996).

The potential harvest of the seaweeds from the Indian Ocean is 870 kt of wet weight, out of which only 22 000 t are actually harvested from both western and eastern Indian Ocean which are largely red and brown seaweeds (Qasim, 1996).

Marine algae from Indian coasts have been fairly well surveyed since several decades. The latest systematic

**Table 3.1** Gross primary production (GPP) and energetics of benthic macroalgae

Species	GPP gC/m <sup>2</sup> /day	Glucose g/m <sup>2</sup> /day	Energy kcal/m <sup>2</sup> /day	Efficiency (%)
Chlorophyceae				
<i>Enteromorpha intestinalis</i>	3.84	9.60	35.91	0.64
<i>E. prolifera</i>	1.10	2.75	10.29	0.19
<i>Ulva lacuta</i>	2.54	6.35	23.75	0.43
<i>Rhizoclonium grande</i>	0.84	2.10	7.86	0.14
Rhodophyceae				
<i>Bostrychea radicans</i>	1.26	3.15	11.78	0.21
<i>Bostrychea</i> sp.	0.61	1.53	5.72	0.10
<i>Catenella nipae</i>	1.14	2.85	10.66	0.19
<i>C. adnata</i>	0.24	0.60	2.25	0.04
<i>C. lepriurii</i>	0.27	0.68	2.55	0.05
<i>Gracilaria verrucosa</i>	0.96	2.40	8.98	0.16
Total	12.80	32.01	119.72	2.14
Mean	1.28	3.20	11.97	0.22

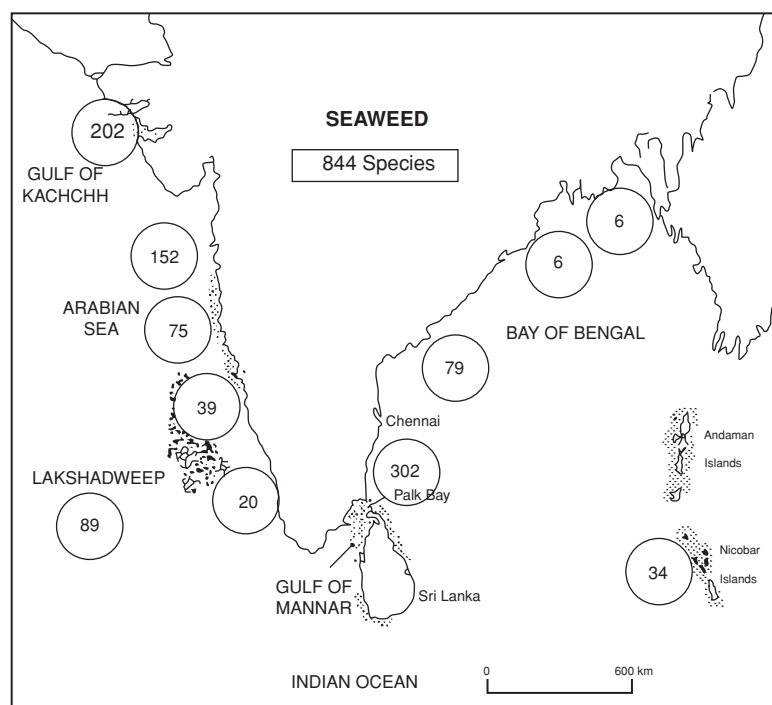
account (Oza and Zaidi, 2000) lists 844 species (including forma and varieties) distributed among 217 genera. The most abundant among them are Rhodophyta (434 species), followed by Chlorophyta (216 species), Phaeophyta (191 species) and Xanthophyta (3 species) (Figure 3.1).

The species grow abundantly along the Tamil Nadu and Gujarat coasts and around Lakshadweep and Andaman and

Nicobar islands. There are also rich seaweed beds around Mumbai, Ratnagiri, Goa, Karwar, Varkala, Vizhinjam, and Pulicat in Tamil Nadu, and Chilika in Orissa. However, on the West Bengal coast, the seaweed resource has not been quantified yet, although they are important mangrove associates. The taxonomic position and salient features of some common seaweeds found in the mangrove ecosystem of Sundarbans are listed here.

**Table 3.2** Net primary production (NPP) and energetics of benthic macroalgae

Species	NPP g/m <sup>2</sup> /day	Glucose g/m <sup>2</sup> /day	Energy kcal/m <sup>2</sup> /day	(%) of GPP	Net efficiency (%)
Chlorophyceae					
<i>Enteromorpha intestinalis</i>	3.26	8.15	30.48	85.00	0.55
<i>E. prolifera</i>	0.98	2.45	9.14	89.09	0.16
<i>Ulva lactuca</i>	2.25	5.63	21.04	88.58	0.38
<i>Rhizoclonium grande</i>	0.32	0.80	2.99	80.01	0.05
Rhodophyceae					
<i>Bostrychea radicans</i>	0.86	2.15	8.04	68.25	0.15
<i>Bostrychea</i> sp.	0.38	0.95	3.56	62.29	0.06
<i>Catenella nipae</i>	0.86	2.15	8.04	75.43	0.15
<i>C. adnata</i>	0.12	0.30	1.12	50.00	0.02
<i>C. lepriurii</i>	0.21	0.53	1.97	77.77	0.03
<i>Gracilaria verrucosa</i>	0.62	1.55	5.80	64.98	0.10
Total	9.86	24.66	92.18	76.99	1.65
Mean	0.97	2.47	9.22	77.18	0.17



**Figure 3.1** Seaweed diversity of India. Source: Venkataraman and Wafar, 2005.

#### Type I

##### Systematic position:

Division – Chlorophyta

Class – Chlorophyceae

Order – Ulvales

Family – Ulvaceae

Genus – *Enteromorpha*

Species – *intestinalis* (Link)

##### Salient features:

- 1 Plant body is tubular, more or less compressed, constricted, and coiled in the form of intestine.
- 2 Thallus dark green in color and found attached to the substratum with the help of a primary attaching cell.
- 3 Presence of numerous multinucleated rhizoids growing from the lower cell of the thallus.
- 4 Cells of the thallus are small and elongated.

#### Type II

##### Systematic position:

Division – Chlorophyta

Class – Chlorophyceae

Order – Ulvales

Family – Ulvaceae

Genus – *Enteromorpha*

Species – *compressa* (Link)

##### Salient features:

- 1 Plant body is tubular, more or less compressed, and constricted.
- 2 Thallus light green in color and found attached to the substratum with the help of a primary attaching cell.
- 3 Presence of numerous multinucleated rhizoids growing from lower cell of the thallus.
- 4 Cells of the thallus are small and round.

**Type III****Systematic position:**

Division – Chlorophyta

Class – Chlorophyceae

Order – Ulvales

Family – Ulvaceae

Genus – *Ulva*

Species – *lactuca* (Linnaeus)

**Salient features:**

- 1 Plant body is tubular, more or less compressed, flattened leaf like.
- 2 Thallus dark green in color and found attached to the substratum with the help of a primary attaching cell.
- 3 Presence of numerous multinucleated rhizoids growing from lower cell of the thallus.
- 4 Cells of the thallus are small and ovoid.

**Type IV****Systematic position:**

Division – Chlorophyta

Class – Chlorophyceae

Order – Cladophorales

Family – Cladophoraceae

Genus – *Rhizoclonium*

Species – *hookeri* (Kuetz)

**Salient features:**

- 1 Filaments rigid, dark green in color.
- 2 Thallus simple, intertwined to form a fleecy layer with numerous rhizoids.
- 3 Branches are formed at right angles to the main axis.
- 4 Cells measure about 170–182  $\mu\text{m}$  diameter and 72–105  $\mu\text{m}$  long.

**Type V****Systematic position:**

Division – Chlorophyta

Class – Chlorophyceae

Order – Cladophorales

Family – Cladophoraceae

Genus – *Rhizoclonium*

Species – *riparium* (Harvey)

**Salient features:**

- 1 Filaments pale green, expanded on the substrate.
- 2 Filaments flexuous intertwined into a fleece with thin cell wall.
- 3 Frequent rhizoid branches.
- 4 Vegetative cells measure about 23–26  $\mu\text{m}$ , diameter usually 1–2 times as long as broad.

**Type VI****Systematic position:**

Division – Chlorophyta

Class – Chlorophyceae

Order – Cladophorales

Family – Cladophoraceae

Genus – *Chaetomorpha*

Species – *aerea* (Kuetz)

**Salient features:**

- 1 Filaments are unbranched.
- 2 Filaments 50–55  $\mu\text{m}$  in diameter and 1–1.5 times long.
- 3 Rhizoids present at the base are unbranched and form disc-like in appearance.
- 4 Cell wall thick, hyaline, and lamellate.

**Type VII****Systematic position:**

Division – Chlorophyta

Class – Chrysophyceae

Order – Vaucheriaceae

Family – Cladophoraceae

Type – *Vaucheria* sp. (De cand)

**Salient features:**

- 1 Thallus filamentous, aseptate and laterally and irregularly branched.
- 2 Filaments usually attached by branched rhizoids.
- 3 Filaments cylindrical with numerous small chloroplasts towards the exterior bounding the central vacuole.
- 4 Chloroplasts are without pyrenoids.

**Type VIII****Systematic position:**

Division – Chlorophyta

Class – Rhodophyceae

Order – Gigartinales

Family – Rhabdoniaceae

Genus – *Catenella*

Species – *repens* (Batters)

**Salient features:**

- 1 Plants with repent and assurgent branches.
- 2 Branching is ditrichotomous below, but clearly pinnate above.
- 3 The axis and branches divided into dorsiventrally compressed ellipsoid to ovate segment 3–5 times longer than broad.
- 4 The haptera terminating into uncorticated flagellar outgrowth chiefly formed at forking points and not in the branching plane of the thallus.

**Type IX****Systematic position:**

Division – Chlorophyta

Class – Rhodophyceae

Order – Ceramiales

Family – Rhodomelaceae

Type – *Bostrychia* sp. (Montagne)

**Salient features:**

- 1 Filiform, black, or dull purplish.
- 2 Thallus with erect branches often distinguishable.
- 3 Rhizoids polysiphonous, ordinarily regular and bilaterally branched.
- 4 Branches with several cells of equal length being disposed about the central axis or these pericentral cells regularly transversely divided.

**Type X****Systematic position:**

Division – Chlorophyta

Class – Rhodophyceae

Order – Ceramiales

Family – Delesseriaceae

Genus – *Caloglossa*

Species – *leprieurii* (Montagne)

**Salient features:**

- 1 Plants dorsiventral, spreading or somewhat erect up to 4 cm across deep reddish violet in color.
- 2 Blades 1.5 mm broad, constricted at the forking and elsewhere.
- 3 Individual segments are lanceolate, 4–6 mm long, sometimes linear-attenuate, rarely ovate.
- 4 Rhizoids and secondary segments or blades formed at the constrictions; blades formed here are irregularly branched.

### 3.4 Biochemical composition of seaweeds with special reference to Indian Sundarbans

For the last few decades, scientists through the world have been searching for suitable and nutritional, healthy and readily available supplement to convention food. Worthy of mention here is that marine algae are considered to be a potentially good source of nutrients since they contain high amount of proteins, carbohydrates, significant amounts of vitamins A, B, C, especially B12 and lipids, important fatty acids, amino acids and pigments (Chakraborty and Santra, 2008).

In India a considerable amount of study on the nutrition component of macroalgae along the Indian coastline has been done (Dhargalkar *et al.*, 1980; Rao and Tipnis, 1964; Tewari *et al.*, 1968; Prasad *et al.*, 2006; Venkatesalu *et al.*, 2004; Muthuraman and Ranganathan, 2004). A significant study on fatty acid composition of estuarine algal species of Sundarbans was reported by Sen (Sen *et al.*, 2000, 2002).

Seaweeds are exposed to seasonal variations of abiotic factors that influence their metabolic responses (photosynthesis and growth rates) and levels of proximate constituents (Orduña-Rojas *et al.*, 2002). For subtropical species seawater temperature, light and nutrients have been shown to be the primary factors that modify the seasonal photosynthesis in *Hypnea musciformis* (Wulfen) Lamouroux (Durako and Dawes, 1980a) and *Gracilaria tikvahiae* McLachlan (Lapointe and Ryther, 1978; Penniman and Mathieson, 1985; Lapointe, 1987). Seasonal variations in the chemical composition and nutritive value have been reported in common marine seaweeds from different parts of the world; Kaehler and Kennish (1996) reported the same from Hong Kong, Kumar (1993) from coastal India and Mercer *et al.* (1993) from Ireland.

The seaweeds exhibit great variation in the nutrient contents, which are related to several environmental factors such as water temperature, salinity, light, and nutrients (Dawes, 1998). Most of the environmental parameters vary according to season and the changes in ecological conditions can stimulate or inhibit the biosynthesis of several nutrients in seaweeds (Lobban *et al.*, 1985). The nutritional properties of seaweeds and their seasonal oscillation are poorly known and normally are evaluated from the chemical composition (Mabeau and Fleurence, 1993). In this context study on the temporal variations in chemical composition of tropical seaweeds from the Gangetic delta of Sundarbans, West Bengal, India is very limited (Banerjee *et al.*, 2009a,b; Chakraborty and Santra, 2008). This mangrove-dominated deltaic lobe at the apex of the Bay of Bengal exhibits significant spatial and temporal variations of salinity

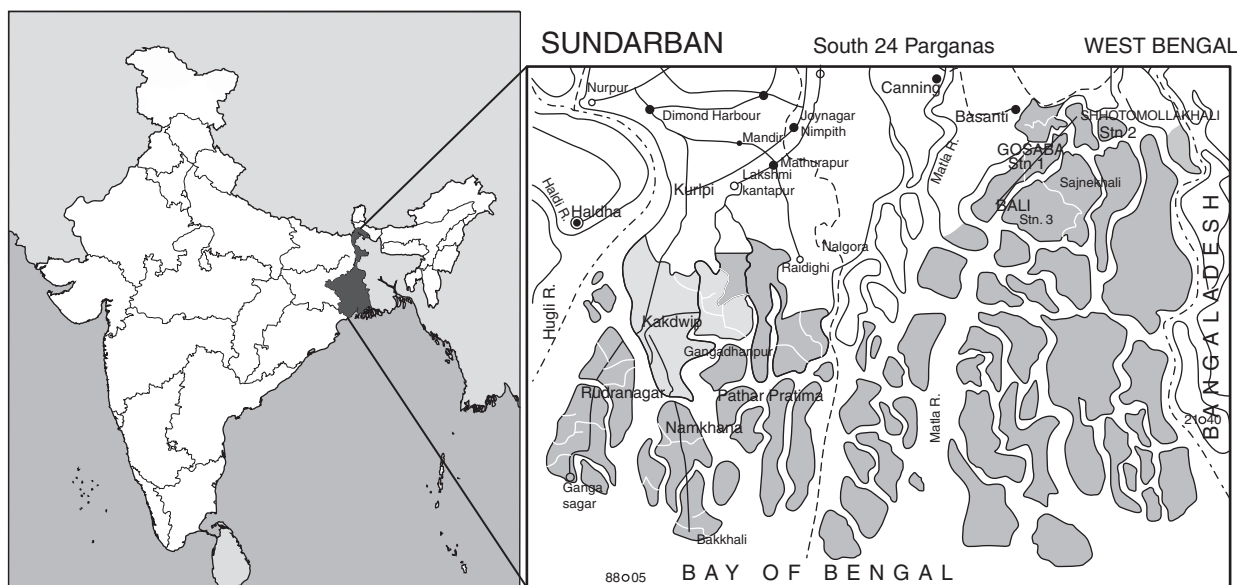
owing to varied geographic features. Recent studies suggest that mariculture of this species would require subtropical to tropical conditions and hence, this species can be considered as an important candidate for aquaculture due to its high carotenoid and protein content (Mitra *et al.*, 2006a).

Carbohydrates comprise 50–60% of the dry weight of seaweeds (Sekine *et al.*, 1965). Soluble carbohydrate ranged from 8.1 to 33.7% as reported in *Enteromorpha*, *Ulva*, and *Porphyra* (Kennish and Williams, 1997). Manivannan *et al.*, 2009 reported that carbohydrate concentration in seaweeds varied from 20 to 24% while working in Mandapam coastal regions along the south-east coast of India. The decrease in carbohydrates may be observed due to extensive growth of algal thalli (Dhargalkar, 1979). In general, green seaweeds are reported to have a higher carbohydrate level than those of red (Pattama and Chirapart, 2006).

Lipids represent only 1–5% of algal dry matter and exhibit an interesting polyunsaturated fatty acid composition particularly omega-3 and omega-6 acids, which play an important role in the prevention of cardiovascular diseases, osteoarthritis, and diabetes. The red and brown algae are rich in fatty acids with 20 carbon atoms: eicosapentaenoic acid (EPA,  $\omega$ -3 C 20:5) and arachidonic acid (AA,  $\omega$ -6 C 20:4).

Astaxanthin is a naturally occurring carotenoid pigment with unique antioxidant properties, which is present in both micro- and macroalgae. It is an important food ingredient both in the pisciculture and animal husbandry sectors owing to its wide application as an antioxidant.

The chemical composition of seaweeds varies with species, habitats, maturity, and environmental conditions (Ito and Hori, 1980). The seaweeds of Indian Sundarbans (Figure 3.2) is no exception to this. The present authors have recently completed a study on seaweed in the Indian Sundarbans region, which is the only mangrove forest in the world with three distinct saline conditions: the upper zone is dominated by freshwater, the mid zone with medium salinity, and the lower zone, which is adjacent to the Bay of Bengal, exhibits high salinity (Mitra, 2000). The central sector of Indian Sundarbans is also hypersaline due to the complete siltation of the Bidyadhari channel. Three stations were selected in this central sector: Gosaba (22°08'53.66"N; 88°56'34.20"E); Chotomollakhali (22°10'21.74"N; 88°53'55.18"E), and Bali (22°04'35.17"N; 88°44'55.70"E). Samplings were carried out at the low tide period through three seasons: premonsoon (March), monsoon (September), and postmonsoon (December) during 2008–2009. The average lipid, protein, carbohydrate and astaxanthin values of *Enteromorpha intestinalis*, *Ulva lactuca*, and *Catenella repens*, as observed in Indian Sundarbans are presented in Tables 3.3 to 3.5.



**Figure 3.2** Map of India and West Bengal showing the study site of Indian Sundarbans.

Macroalgae are unique sources of protein although the content varies with the types. Protein content of brown seaweeds are generally small (average: 5–15% dry weight), whereas higher protein contents are recorded in green and red seaweeds (average: 10–30% dry weight). The protein levels of *Ulva* and *Enteromorpha* spp. generally range between 5–20% of dry weight. Because of their high protein content, protein concentrates (PCs) of seaweeds have become more important for the food industry, especially in developed countries (Wong and Cheung, 2001). The recent utilization of macroalgae as a fish feed is also gaining momentum. The highest percentage of protein was recorded in *Enteromorpha intestinalis* (mean  $12.23 \pm 4.56\%$  dry weight, range  $5.46 \pm 0.60$  to  $18.92 \pm 0.24\%$  dry weight) (Table 3.3) followed by *Catenella repens* (mean  $10.98 \pm 4.31\%$  dry weight, range  $4.07 \pm 0.56$  to  $17.52 \pm 0.45\%$  dry weight) and *Ulva lactuca* (mean  $9.57 \pm 3.50\%$  dry weight, range  $3.53 \pm 0.39$  to  $14.89 \pm 0.14\%$  dry weight) (Tables 3.4 and 3.5). The protein content showed significant seasonal variation with higher values during monsoon (Tables 3.3–3.5) owing to the presence of high nitrate load in the ambient waters (Table 3.6). Similar results were observed by Bird in 1984 while working with *Gracilaria verrucosa*. The dependence of protein level in algae on available nitrogen was also pointed out by Lapointe (1981) and Dawes (1998). Protein values showed more or less similar trend in all the three stations and the values were in order Gosaba > Chhotomollakhali > Bali. The fluctuation in the protein values in all the three stations can be explained by variation in environmental

conditions such as temperature, salinity, and nutrients. This observation is confirmed by the positive correlation of protein content with dissolved nitrate and negative correlation with temperature and salinity. The significant positive correlations between nitrate level and protein content of three selected macroalgae [ $r_{\text{nitrate} \times \text{protein (Enteromorpha sp.)}} = 0.941$ ,  $p < 0.01$ ;  $r_{\text{nitrate} \times \text{protein (Ulva sp.)}} = 0.969$ ,  $p < 0.01$ ;  $r_{\text{nitrate} \times \text{protein (Catenella sp.)}} = 0.943$ ,  $p < 0.01$ ] statistically confirm our observation. The direct relationship of protein percentage in seaweeds with nitrate of the ambient water was reported by several workers (Oliveira *et al.*, 1997; Ovalle *et al.*, 1999; Banerjee *et al.*, 2009a). Gosaba being a sewage-contaminated zone (due to the presence of a fish landing station and tourism units) showed the maximum nitrate level in the water and subsequently the highest percentage of protein in the macroalgae. Data of protein content in macroalgae from the tropical and subtropical coastal environment frequently show lower concentrations (Kaehler and Kennish, 1996; Wong and Cheung, 2000). This is because of the predominantly oligotrophic marine environment with low availability of nitrogen as visualized in case of Brazilian marine environment (Oliveira *et al.*, 1997; Ovalle *et al.*, 1999). In the present study, our data of protein concentration in macroalgae are in accordance with the information available in the literature (Mcdermid and Stuercke, 2003; Munda and Gubensek, 1976; Munda and Gubensek, 1986; Owens and Stewart, 1983; Sauze, 1981; Tkachenko and Koval, 1990; Wheeler and Bjornseter, 1992).

**Table 3.3** The mean ( $\pm$ SD) values of protein, lipid, carbohydrate and astaxanthin in *Enteromorpha intestinalis* from Indian Sundarbans during 2008–2009

Stations	Premonsoon			
	Protein (% DW)	Lipid (% DW)	Carbohydrate (% DW)	Astaxanthin (ppm DW)
Gosaba	11.42 $\pm$ 0.28	4.45 $\pm$ 0.30	30.92 $\pm$ 0.23	113.80 $\pm$ 1.77
Chhotomollakhali	10.30 $\pm$ 0.17	3.78 $\pm$ 0.10	35.57 $\pm$ 0.11	133.53 $\pm$ 3.03
Bali	5.46 $\pm$ 0.60	2.86 $\pm$ 0.65	38.09 $\pm$ 0.06	188.77 $\pm$ 3.62
Stations	Monsoon			
	Protein (% DW)	Lipid (% DW)	Carbohydrate (% DW)	Astaxanthin (ppm DW)
Gosaba	18.92 $\pm$ 0.24	6.58 $\pm$ 0.41	28.28 $\pm$ 0.10	87.67 $\pm$ 1.59
Chhotomollakhali	17.39 $\pm$ 0.22	5.76 $\pm$ 0.08	32.63 $\pm$ 0.69	109.09 $\pm$ 2.29
Bali	10.59 $\pm$ 0.45	4.76 $\pm$ 0.18	34.04 $\pm$ 0.81	173.12 $\pm$ 2.12
Stations	Postmonsoon			
	Protein (% DW)	Lipid (% DW)	Carbohydrate (% DW)	Astaxanthin (ppm DW)
Gosaba	16.49 $\pm$ 0.37	5.52 $\pm$ 0.34	24.51 $\pm$ 0.43	96.73 $\pm$ 1.78
Chhotomollakhali	13.29 $\pm$ 0.19	5.13 $\pm$ 0.37	28.60 $\pm$ 0.42	117.97 $\pm$ 1.41
Bali	6.21 $\pm$ 0.15	4.98 $\pm$ 0.29	30.53 $\pm$ 0.29	181.18 $\pm$ 2.91
DW, dry weight.				

**Table 3.4** The mean ( $\pm$ SD) values of protein, lipid, carbohydrate and astaxanthin in *Ulva lactuca* from Indian Sundarbans during 2008–2009

Stations	Premonsoon			
	Protein (% DW)	Lipid (% DW)	Carbohydrate (% DW)	Astaxanthin (ppm DW)
Gosaba	10.16 $\pm$ 0.05	5.66 $\pm$ 0.47	35.64 $\pm$ 0.17	101.28 $\pm$ 1.51
Chhotomollakhali	8.56 $\pm$ 0.25	4.89 $\pm$ 0.42	37.61 $\pm$ 0.21	125.66 $\pm$ 5.08
Bali	3.53 $\pm$ 0.39	3.83 $\pm$ 0.21	38.34 $\pm$ 0.24	113.69 $\pm$ 1.72
Stations	Monsoon			
	Protein (% DW)	Lipid (% DW)	Carbohydrate (% DW)	Astaxanthin (ppm DW)
Gosaba	14.89 $\pm$ 0.14	7.27 $\pm$ 0.03	34.47 $\pm$ 0.34	78.60 $\pm$ 1.00
Chhotomollakhali	12.68 $\pm$ 0.03	6.26 $\pm$ 0.02	36.77 $\pm$ 0.15	90.42 $\pm$ 0.84
Bali	8.84 $\pm$ 0.15	5.23 $\pm$ 0.03	37.30 $\pm$ 0.11	112.72 $\pm$ 2.41
Stations	Postmonsoon			
	Protein (% DW)	Lipid (% DW)	Carbohydrate (% DW)	Astaxanthin (ppm DW)
Gosaba	12.27 $\pm$ 0.23	6.78 $\pm$ 0.13	33.17 $\pm$ 0.47	81.69 $\pm$ 1.03
Chhotomollakhali	10.30 $\pm$ 0.32	6.07 $\pm$ 0.06	34.87 $\pm$ 0.11	113.56 $\pm$ 0.11
Bali	4.87 $\pm$ 0.34	5.91 $\pm$ 0.03	35.56 $\pm$ 0.37	116.68 $\pm$ 1.05
DW, dry weight.				

**Table 3.5** The mean ( $\pm$ SD) values of protein, lipid, carbohydrate and astaxanthin in *Catenella repens* from Indian Sundarbans during 2008–2009

Stations	Premonsoon			
	Protein (% DW)	Lipid (% DW)	Carbohydrate (% DW)	Astaxanthin (ppm DW)
Gosaba	10.58 $\pm$ 0.14	2.27 $\pm$ 0.03	26.58 $\pm$ 0.73	120.32 $\pm$ 1.58
Chhotomollakhali	9.71 $\pm$ 0.38	1.20 $\pm$ 0.04	29.64 $\pm$ 0.45	143.89 $\pm$ 1.67
Bali	4.07 $\pm$ 0.56	1.19 $\pm$ 0.02	36.37 $\pm$ 0.28	198.66 $\pm$ 3.12
Stations	Monsoon			
	Protein (% DW)	Lipid (% DW)	Carbohydrate (% DW)	Astaxanthin (ppm DW)
Gosaba	17.52 $\pm$ 0.45	3.27 $\pm$ 0.03	24.53 $\pm$ 1.53	107.58 $\pm$ 1.75
Chhotomollakhali	15.65 $\pm$ 0.20	3.04 $\pm$ 0.02	27.96 $\pm$ 0.98	117.12 $\pm$ 1.30
Bali	9.31 $\pm$ 0.83	2.23 $\pm$ 0.03	29.31 $\pm$ 0.29	182.75 $\pm$ 3.56
Stations	Postmonsoon			
	Protein (% DW)	Lipid (% DW)	Carbohydrate (% DW)	Astaxanthin (ppm DW)
Gosaba	14.32 $\pm$ 0.87	3.98 $\pm$ 0.03	22.27 $\pm$ 0.78	110.33 $\pm$ 1.58
Chhotomollakhali	12.10 $\pm$ 0.61	2.51 $\pm$ 0.01	24.39 $\pm$ 1.92	129.74 $\pm$ 1.02
Bali	5.54 $\pm$ 0.73	2.76 $\pm$ 0.03	27.48 $\pm$ 1.66	192.90 $\pm$ 2.41
DW, dry weight.				

**Table 3.6** The mean ( $\pm$ SD) results of physicochemical parameters in surface waters from Indian Sundarbans during 2008–2009

Stations	Premonsoon				
	Salinity (‰)	Temperature (°C)	Nitrate ( $\mu$ g/l)	Phosphate ( $\mu$ g/l)	Silicate ( $\mu$ g/l)
Gosaba	16.45 $\pm$ 0.03	32.37 $\pm$ 0.06	28.55 $\pm$ 0.30	2.75 $\pm$ 0.01	99.46 $\pm$ 0.34
Chhotomollakhali	20.64 $\pm$ 0.02	32.63 $\pm$ 0.05	26.28 $\pm$ 0.19	1.73 $\pm$ 0.05	92.37 $\pm$ 0.33
Bali	25.30 $\pm$ 0.10	32.73 $\pm$ 0.06	22.34 $\pm$ 0.16	1.04 $\pm$ 0.03	120.82 $\pm$ 0.53
Stations	Monsoon				
	Salinity (‰)	Temperature (°C)	Nitrate ( $\mu$ g/l)	Phosphate ( $\mu$ g/l)	Silicate ( $\mu$ g/l)
Gosaba	12.32 $\pm$ 0.24	30.00 $\pm$ 0.01	30.48 $\pm$ 0.30	2.86 $\pm$ 0.02	101.20 $\pm$ 0.59
Chhotomollakhali	17.29 $\pm$ 0.26	30.20 $\pm$ 0.05	29.32 $\pm$ 0.19	2.52 $\pm$ 0.03	99.95 $\pm$ 1.96
Bali	21.14 $\pm$ 0.02	30.43 $\pm$ 0.15	25.91 $\pm$ 0.34	1.12 $\pm$ 0.01	125.70 $\pm$ 1.39
Stations	Postmonsoon				
	Salinity (‰)	Temperature (°C)	Nitrate ( $\mu$ g/l)	Phosphate ( $\mu$ g/l)	Silicate ( $\mu$ g/l)
Gosaba	14.14 $\pm$ 0.02	28.37 $\pm$ 0.06	29.42 $\pm$ 0.14	2.52 $\pm$ 0.16	94.43 $\pm$ 0.27
Chhotomollakhali	19.24 $\pm$ 0.08	28.63 $\pm$ 0.15	28.58 $\pm$ 0.52	2.33 $\pm$ 0.17	92.66 $\pm$ 0.15
Bali	23.38 $\pm$ 0.22	28.80 $\pm$ 0.06	22.33 $\pm$ 0.14	1.19 $\pm$ 0.01	132.43 $\pm$ 0.74

The major biochemical component in the selected seaweeds was carbohydrate. The percentage of soluble carbohydrate in rhodophytes, *Catenella repens* (overall mean  $27.62 \pm 3.99\%$  dry weight; range  $22.27 \pm 0.78$  to  $36.37 \pm 0.28\%$  dry weight) (Table 3.5) was lower than the chlorophytes (mean  $31.46 \pm 3.99\%$  dry weight; range  $24.51$ – $38.09 \pm 0.06\%$  dry weight) in *Enteromorpha intestinalis* and mean  $35.97 \pm 1.62\%$  dry weight; range  $33.17 \pm 0.47$  to  $38.34 \pm 0.24\%$  dry weight in *Ulva lactuca* (Tables 3.3 and 3.4). Seasonal variations in carbohydrate content of the seaweed were also observed in the present study, irrespective of stations (Tables 3.3 to 3.5). The values were highest in the premonsoon and lowest in the postmonsoon periods indicating the role of surface water temperature to be vital for photosynthesis. The present study area at the apex of the Bay of Bengal enjoys bright sunshine and high tropical temperatures almost throughout the year. This may be a reason behind the higher carbohydrate value in the seaweeds. The significant positive relationships between ambient water temperature and carbohydrate content of the seaweeds [ $r_{\text{temperature} \times \text{carbohydrate}} (\text{Enteromorpha sp.}) = 0.784$ ,  $p < 0.01$ ;  $r_{\text{temperature} \times \text{carbohydrate}} (\text{Ulva sp.}) = 0.737$ ,  $p < 0.01$ ;  $r_{\text{temperature} \times \text{carbohydrate}} (\text{Catenella sp.}) = 0.705$ ,  $p < 0.01$ ] confirm the view of synthesis of organic carbon (through photosynthesis) under optimum solar radiation and temperature. The positive influence of water temperature, salinity, and pH on carbohydrate synthesis was confirmed by several workers (Munda and Kremer, 1977; Perfeto, 1998). The inverse relationship between carbohydrate and protein with temperature and salinity correspond to a pattern observed for several species of seaweeds (Mourandi-Givernaud *et al.*, 1993; Banerjee *et al.*, 2009a). The trend may be attributed to the positive role of light intensity, temperature and decrease of nitrogen for carbohydrate synthesis, while for the proteins these parameters act inversely (Rosemberg and Ramus, 1982; Rotem *et al.*, 1986). Thus, the active period of carbohydrate synthesis coincides with the decrease in protein concentration in seaweed and vice versa.

The lipid content was highest in *Ulva lactuca* (mean  $5.77 \pm 1.03\%$  dry weight; range  $3.83 \pm 0.21$  to  $7.27 \pm 0.03\%$  dry weight) (Table 3.4), followed by *Enteromorpha intestinalis* (mean  $4.87 \pm 1.09\%$  dry weight; range  $2.86 \pm 0.65$  to  $6.58 \pm 0.41\%$  dry weight) (Table 3.3), and *Catenella repens* (mean  $2.49 \pm 0.91\%$  dry weight; range  $1.19 \pm 0.02$  to  $3.98 \pm 0.03\%$  dry weight) (Table 3.5). In comparison to protein and carbohydrate, lipid exhibited relatively low proportion in *Enteromorpha intestinalis*, *Ulva lactuca*, and *Catenella repens*. Significant variation in lipid percentage was observed between the stations (Tables 3.3 to 3.5). The mangrove-dominated Gangetic delta enjoys a tropical climate and therefore temperature plays a major role in the

variation of the lipid content in macroalgae. Significant negative correlation between lipid content and water temperature has also been observed in our study for all the three selected macroalgae [ $r_{\text{temperature} \times \text{lipid}} (\text{Enteromorpha sp.}) = -0.703$ ,  $p < 0.01$ ;  $r_{\text{temperature} \times \text{lipid}} (\text{Ulva sp.}) = -0.713$ ,  $p < 0.01$ ;  $r_{\text{temperature} \times \text{lipid}} (\text{Catenella sp.}) = -0.806$ ,  $p < 0.01$ ]. This observation is in alignment with the works of Jones and Harwood (1993) who concluded that temperature increases the level of unsaturation of acyl chains that slows down both metabolism and transport of lipid.

Astaxanthin is a powerful antioxidant. Many studies have demonstrated the antioxidant properties of algal carotenoids and the role they play in preventing many diseases linked to oxidative stress (Okuzumi *et al.*, 1993). In the present study, astaxanthin value was highest in *Catenella repens* (mean  $144.81 \pm 35.38$  ppm dry weight; range  $107.58 \pm 1.59$  to  $198.66 \pm 3.12$  ppm dry weight) (Table 3.5). The next position was occupied by *Enteromorpha intestinalis* (mean  $133.54 \pm 36.60$  ppm dry weight; range  $87.67 \pm 1.59$  to  $188.77 \pm 3.62$  ppm dry weight) (Table 3.3) followed by *Ulva lactuca* (mean  $103.81 \pm 16.12$  ppm dry weight; range  $78.60 \pm 1.00$  to  $116.68 \pm 1.05$  ppm dry weight) (Table 3.4). The synthesis of astaxanthin enhances with the increase in environmental stresses as revealed through a study in mangroves by Mitra *et al.* (2006b). In this study, the astaxanthin load showed spatial variation with respect to all the three macroalgae (Tables 3.3 to 3.5), which may be due to variation in salinity amongst the selected stations. The significant positive correlation between salinity and astaxanthin level in the selected macroalgal species [ $r_{\text{salinity} \times \text{astaxanthin}} (\text{Enteromorpha sp.}) = 0.940$ ,  $p < 0.01$ ;  $r_{\text{salinity} \times \text{astaxanthin}} (\text{Ulva sp.}) = 0.845$ ,  $p < 0.01$ ;  $r_{\text{salinity} \times \text{astaxanthin}} (\text{Catenella sp.}) = 0.922$ ,  $p < 0.01$ ] speaks in favor of astaxanthin synthesis under stressful conditions, posed by high aquatic salinity.

## References

- Banerjee, K., Ghosh, R., Homechaudhury, S. and Mitra, A. (2009a) Biochemical composition of marine macroalgae from Gangetic Delta at the Apex of Bay of Bengal. *Afr. J. Basic App. Sci.*, **1**(56), 96–104.
- Banerjee, K., Ghosh, R., Homechaudhury, S. and Mitra, A. (2009b) Seasonal variation in the biochemical composition of seaweed (*Catenella repens*) from Gangetic delta, northeast coast of India. *J. Earth Syst. Sci.*, **118**(5), 497–505.
- Bird, K.T. (1984) Seasonal variation in protein:carbohydrate ratios in a subtropical estuarine alga, *Gracilaria verrucosa*, and the determination of nitrogen limitation status using these ratios; *Bot. Mar.*, **27**(3), 111–115.

- Chakraborty, S. and Santra, S.C. (2008) Biochemical composition of eight benthic algae collected from Sunderban. *Ind. J. Mar. Sci.*, **37**(3), 329–332.
- Chaudhuri, A.B. and Choudhury, A. (1994) *Mangroves of the Sundarbans*. Vol.1: India. The IUCN Wetlands Programme, Bangkok, Thailand, IUCN.
- Dawes, C.J. (1998) *Marine Botany*. John Wiley & Sons, Inc., New York.
- Dhargalkar, V.K. (1979) Biochemical studies on *Ulva reticulata* Forsskal. *Proceedings of the International Symposium on Marine algae of Indian Ocean region*, CSMCRI, Bhavnagar, p. 40.
- Dhargalkar, V.K., Jagtap, T.G. and Untawale, A.G. (1980) Biochemical constituents of seaweeds along the Maharashtra coast. *Ind. J. Mar. Sci.*, **9**, 297–299.
- Durako, M.J. and Dawes, C.J. (1980a) A comparative seasonal study of two populations of *Hypnea musciformis* from the East and West Coasts of Florida, USA. II. Photosynthetic and respiratory rates. *Mar. Biol.*, **59**, 157–162.
- Ito, K. and Hori, K. (1980) Seaweed: chemical composition and potential uses. *Food Rev. Int.*, **5**, 101–144.
- Jones, A.L. and Harwood, J.L. (1993) Lipids and lipid metabolism in the marine alga *Enteromorpha intestinalis*. *Phytochemistry*, **34**(4), 969–972.
- Kaehler, S. and Kennish, R. (1996) Summer and winter comparisons in the nutritional value of marine macroalgae from Hong Kong. *Bot. Mar.*, **39**, 11–17.
- Kennish, R. and Williams, G.A. (1997) Feeding preference of the herbivorous crab *Grapsus albolineatus*: the differential influence of algae nutrient content and morphology. *Mar. Ecol. Progr. Ser.*, **147**, 87–95.
- Kumar, V. (1993) Biochemical constituents of marine algae from Tuticorin coast. *Ind. J. Mar. Sci.*, **22**, 138–140.
- Lapointe, B.E. (1981) The effects of light and nitrogen on growth, pigment content, and biochemical composition of *Gracilaria foliifera* v. *angustissima* (Gigartinales, Rhodophyta). *J. Phycol.*, **17**, 90–95.
- Lapointe, B.E. (1987) Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. *Mar. Biol.*, **93**, 561–568.
- Lapointe, B.E. and Ryther, J.H. (1978) Some aspects of the growth and yield of *Gracilaria tikvahiae* in culture. *Aquaculture*, **15**, 185–193.
- Lobban, C.S., Harrison, P.J. and Duncan M.J. (1985) *The Physiological Ecology of Seaweeds*. Cambridge University Press, Cambridge.
- Mabeau, S. and Fleurence J. (1993) Seaweed in food products: biochemical and nutritional aspects; *Trends Food Sci. Technol.*, **4**, 103–107.
- Manivannan, K., Karthikai Devi, G., Thirumaran, G. and Anantharaman, P. (2009) Mineral composition of marine macroalgae from Mandapam coastal regions; southern coast of India. *Am. Eur. J. Bot.*, **2**(1), 42–51.
- Mercer, J.P., Mai, K.S. and Donlon, J. (1993) Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* Linnaeus and *Haliotis discus hananai* Ino. I. Effects of algal diets on growth and biochemical composition. *Invert. Reprod. Develop.*, **23**, 2–3.
- Mcdermid, K.J. and Stuercke, B. (2003) Nutritional composition of edible Hawaiian seaweeds. *J. Appl Phycol.*, **15**, 513–524.
- Mitra, A. (2000) The Northwest coast of Bay of Bengal and Deltaic Sundarbans, In: *Seas at the Millennium, An Environmental Evaluation*. Charles R C Sheppard, ed. Pergamon Press, Oxford, p. 145.
- Mitra, A., Banerjee, K. and Banerjee, A. (2006a) Screening mangroves in search of Astaxanthin. *Seshaiyana*, **14**(1), 1–2.
- Mitra, A., Basu, S., Banerjee, K. and Banerjee, A. (2006b) Impact of tidal submergence on astaxanthin content of mangroves. *Ultra Sci.*, **18**(2), 117–122.
- Mourandi-Givernaud, A., Givernaud, T., Morvan, H. and Cosson, J. (1993) Annual variations of the biochemical composition of *Gelidium latifolium* (greville) Thuret et Bornet. *Hydrobiologia*, **260/261**, 607–612.
- Munda, I.M. and Gubensek, F. (1976) The amino acid composition of some common marine algae from Iceland. *Bot Mar.*, **19**, 85–92.
- Munda, I.M. and Gubensek, F. (1986) The amino acid composition of some benthic marine algae from the Northern Adriatic. *Bot Mar.*, **29**, 367–372.
- Munda, I.M. and Kremer, B.P. (1977) Chemical composition and physiological properties of fucoids under conditions of reduced salinity. *Mar. Biol.*, **42**, 9–15.
- Muthuraman, B. and Ranganathan, R. (2004) Biochemical studies on some green algae of Kanyakumari coast. *Seaweed Res. Utilization*, **26**(1&2), 69–71.
- Okuzumi, J., Takahashi, T., Yamane, T., et al. (1993) Inhibitory effects of fucoxanthin, a natural carotenoid, on *N*-thyl-*N*'-nitro-*N*-nitrosoguanidine induced mouse duodenal carcinogenesis. *Cancer Lett.*, **68**, 159–168.
- Oliveira, E.C., Corbisier, T.N., De Eston, V.R. and Ambrosio, O. (1997) Phenology of a seagrass (*Halodule wrightii*) bed on the southeast coast of Brazil. *Aquatic Bot.*, **56**, 25–33.
- Orduña-Rojas, J., Robledo, D. and Dawes, C.J. (2002) Studies on the Tropical agarophyte *Gracilaria cornea* J. Agardh (Rhodophyta, Gracilariales) from Yucatán, Mexico. I. Seasonal physiological and biochemical responses. *Bot. Mar.*, **45**, 453–458.
- Ovalle, A.R.C., Rezende, C.E., Carvalho, C.E.V., Jennerjahn, T.C. and Ittekkot, V. (1999) Biogeochemical characteristics of coastal waters adjacent to small river-mangrove systems, East Brazil. *Geo-Marine Lett.*, **19**, 179–85.

- Owens, N.J.P. and Stewart, W.D. (1983). *Enteromorpha* and the cycling of nitrogen in a small estuary. *Estuar Coast Shelf Sci.*, **17**(3), 287–296.
- Oza, R.M. and Zaidi, S.H. (2000) *A Revised Checklist of Indian Marine Algae*. Central Salt and Marine Chemicals Research Institute, Bhavanagar, India, pp. 296.
- Pattama, R. and Chirapart, A. (2006) Nutritional evaluation of tropical green seaweeds *Caularпа lentillifera* and *Ulva reticulata*. *Kasetsart J. Nat. Sci.*, **40**(1), 75–83.
- Penniman, C.A. and Mathieson, A.C. (1985) Photosynthesis of *Gracilaria tikvahiae* McLachlan (Gigartinales, Rhodophyta) from the Great Bay Estuary, New Hampshire. *Bot. Mar.*, **28**, 427–435.
- Perfeto, P.N.M. (1998) Relation between chemical composition of *Grateloupia doryphora* (Montagne) Howe, *Gymnogongrus griffithsiae* (Turner) Martius, and abiotic parameters; *Acta. Bot. Bras.*, **12**, 77–88.
- Prasad, K., Goswami, A.M., Meena, R., Ramavat, B.K., Ghosh, P.K. and Siddhanta, A.K. (2006) Superior quality agar from red algae. *Gelidiella acerosa* (Rhodophyta, Gelidiales) from Gujarat coast of India: An evaluation. *Ind. J. Mar. Sci.*, **35**(3), 268–274.
- Qasim, S.Z. (1996) Indian Ocean and seaweeds. *J. Ind. Ocean Studies*, **3**(2), 117–126.
- Rao, P.S. and Tipnis, U.K. (1964) Protein content of marine algae from Gujarat coast. *Curr. Sci. (India)*, **33**(1), 16–17.
- Rosemberg, G. and Ramus, J. (1982) Ecological growth strategies in the seaweeds *Gracilaria follifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): soluble nitrogen and reserve carbohydrates. *Mar. Biol.*, **66**, 251–259.
- Rotem, A., Roth-Bejeranu, N. and Arad, S.M. (1986). Effect of controlled environmental conditions on starch and agar contents of *Gracilaria* sp. (Rhodophyceae). *J. Phycol.*, **22**, 117–121.
- Sauze, F. (1981). Chemical and energetic potential of aquatic biomass. *Tech. Eau. Assain.*, **413**, 7–23.
- Sekine, T., Sasakawa, T., Morita, S., Kimura, T. and Kuratom, K. (1965) In: *Laboratory manual for Physiological Studies of Rice* (eds S. Yoshida, D. Forno, J.B. Cook and K.A. Gomez). International Rice Research Institute, Manila, India.
- Sen, N., Naskar, K., Bhattacharya, D.K. and De, B.K. (2000). Fatty acid composition of the lipids of different halophytic algae from estuarine habitats of the Indian Sunderbans. *J. Oil Technol. Ass. Ind.*, **32**(3), 120–123.
- Sen, N., Naskar, K. and De, B.K. (2002) Fatty acid composition of the lipids of different halophytic algae from estuarine habitats of the Indian Sunderbans – II. *J. Oil Technol. Ass. Ind.*, **34**(1), 13–16.
- Tewari, A., Rao, M.P. and Krishnamurthy, V. (1968) Chemical composition of a species of *Porphyra* from Visakhapatnam, S. India. *Curr. Sci.*, **37**, 138–139.
- Tkachenko, F.P. and Koval, V.T. (1990) Biochemical composition of abundant benthic seaweeds of the Black Sea. *Hydrobiologia*, **26**(6), 39–43.
- Venkataraman, K. and Wafar, M. (2005) Coastal and marine biodiversity of India. *Ind. J. Mar. Sci.*, **34**, 57–75.
- Venkatesalu, V., Sundaramoorthy, P., Anantharaj, M., Gopalakrishnan, M. and Chandrasekaran, M. (2004) Studies on the fatty acid composition of marine algae of Rameshwaram coast. *Seaweed Res. Utilin.*, **26**, 83–86.
- Wheeler, P.A. and Bjornsater, B.R. (1992) Seasonal fluctuations in tissue nitrogen, phosphorus and N:P for five macroalgal species common in the Pacific Northwest coast. *J. Phycol.*, **28**, 1–6.
- Wong, K.H. and Cheung, P.C.K. (2000) Nutritional evaluation of some subtropical red and green seaweeds part I – proximate composition, amino acid profiles and some physico-chemical properties. *Food Chem.*, **71**, 475–482.
- Wong, K.H. and P.C.K. Cheung, (2001). Nutritional evaluation of some subtropical red and green seaweeds part II – in vitro protein digestibility and amino acid profiles of protein concentrate. *Food Chem.*, **72**, 11–17.

# 4

## Chemodiversity and Bioactivity within Red and Brown Macroalgae Along the French coasts, Metropole and Overseas Departements and Territories

**Nathalie Bourgougnon<sup>1</sup> and Valérie Stiger-Pouvreau<sup>2</sup>**

<sup>1</sup>*Université Européenne de Bretagne (UEB), Laboratoire de Biotechnologie et Chimie Marines, France, Université de Bretagne-Sud, Campus de Tohannic, Vannes*

<sup>2</sup>*Laboratoire des Sciences de l'Environnement Marin (LEMAR)-IUEM-UBO, Plouzané, France*

### 4.1 Introduction

The exhaustion of some terrestrial natural resources or their progressive degradation is actually problematic and interests every country in the world. The economic and ecological management of these natural resources has become a vast problem as stated during the World Summit on Sustainable Development (Johannesburg, 2002), for example “sustainable management of natural and environmental resources are the 3rd priority objective” (towards a global partnership for sustainable development). Terrestrial natural resources were widely exploited, but marine resources, although they represent 71% of the globe, are not yet valorized. Europe is boarded by marine coasts and thus constitutes a large reserve of potentially natural products, in particular those which can be extracted from marine plants.

In parallel, industries are nowadays faced with several problems: the majority of consumers yearn for a higher living standard and quality; some synthetic products are toxic to the environment, as well as to the health of human

beings, while more and more contaminations have occurred in the medical sectors.

Therefore, a number of uses may be found for those marine products. For example, in cosmetics, research on novel antibacterial compounds is done to stabilize some creams which are subjected to contaminations. Moreover, fouling of ships is a major economic problem that represents billions of dollars a year to the global economy, due to the high cost and short half life of metal-based antifouling paints. In this context, an alternative technology to these metal-based technologies could be the development of coatings whose active ingredients are extracted from marine organisms. In agriculture and aquaculture, the use of some antibiotics (fungicides, bactericides) follows strict legislation, so that more natural compounds are preferred to pharmaceutical and synthesized products. In medicine, particularly in hospitals, bacteria have become more and more resistant to common antibiotics, and trigger postoperative infections (i.e. nosocomial illness). Then, industries are more and

more interested in developing new natural products that have the same efficiency of action, but are harmless to the environment and health, and thus submitted to a less strict legislation.

France possesses an exceptional situation. Present on two continents, in the three oceans (Atlantic, Indian, and Pacific) and in the Mediterranean Sea, this country is rich with various ecosystems, populations, and organisms that live in metropolitan and overseas territories. As a consequence of its large presence all around the world, 17 ecoregions belong to France. Fourteen are present in overseas regions: 4 in New Caledonia, 4 in the Western Atlantic, 2 in Polynesia, 1 in Wallis and Futuna, 2 in the Indian Ocean (la Réunion and Mayotte) and 1 in St-Pierre et Miquelon. Three ecoregions are present around the metropolitan part: in the Channel, along the Atlantic coast and in the Mediterranean Sea. In terms of biological diversity, France is the only country in the world in 5 (Mediterranean, New Caledonia, Polynesia, Caribbean, Indian Ocean) of the 34 known hot spots of biodiversity in the world. Moreover, with its large diversity, this country is a source, and therefore, a treasure trove of terrestrial and marine organisms.

The marine environment represents a mine of useful products awaiting discovery for several reasons. Ecological pressures, including competition for space, the fouling of surfaces, predation, and successful reproduction, have led marine organisms to the evolution of unique secondary metabolites with various biological activities (Kornprobst, 2005). Marine organisms are widely recognized sources of structurally novel secondary metabolites (Blunt *et al.*, 2007). These natural products have provided promising drug leads, offered targets for synthetic organic chemists, and afforded opportunities for elucidation of unusual biosynthetic pathways. Secondary metabolic pathways probably evolved as a result of complex interactions between organisms in their native habitats, but the role of natural products in mediating such interactions remains poorly understood in the majority of cases. Marine ecosystems are a unique resource that provide a diverse array of natural products, primarily from invertebrates such as sponges, tunicates, bryozoans, and mollusks, from marine algae such as micro- and macroalgae, and from marine bacteria and cyanobacteria.

Among marine resources, we decided to focus on macroalgae. In addition to their ecological importance as primary producers, macroalgae also represent an important bioresource for industrial uses. Along coastal ecosystems where the biodiversity is often high, and then where intractability is also high, macroalgae produce, most of the time with the help of an associated microflora, various molecules essentially for their defense, the colonisation of their surface and intraspecific communication, and for which the

production is variable according to biotic and abiotic fluctuations (Kübanek *et al.*, 2003).

This type of molecule is of great importance for the organism in terms of interactions with its environment. We will then present the use of this kind of metabolite in the chemical ecology of seaweeds. Moreover, some metabolites are specifically produced and can be used as chemomarkers. We will then present some examples of genera where metabolites can be used as specific chemomarkers. Finally, the discovery of new biologically active secondary metabolites is a goal of many industrial domains and among marine organisms, macroalgae are more and more harvested as a novel source of active biomolecules as described in numerous and growing scientific papers (De Roeck-Holtzhauer, 1991; Kornprobst, 2005; Ioannou & Roussis, 2009).

During the last 20 years, the study of the chemistry of natural products from biodiversity became dominated by the search for active molecules directed towards drug production. This has sometimes sidetracked the scientific investigation from solving crucial questions in various scientific areas such as: ecological interactions between species, implication of the chemical environmental structure of molecules, the understanding of biodiversity at a molecular scale, etc. The challenge for the next years will then be to explore how model organisms vary their production of metabolites in interaction with the environment and as a response to environmental biotic and abiotic changes. For this, it will be necessary to study the role of the bioactive molecules within communities, their roles in competition for space and resources, and their role in defence against predators and pathogens. This will promote parallel studies in taxonomy and chemistry and clarify the link between biodiversity and chemodiversity.

In this review, we focus on French research work on the domain of natural compounds extracted from diverse macroalgae collected in French territories, to present the richness of active molecules with properties including ecological roles such as herbivore deterrence and antimicrobial defenses, taxonomical markers, as well as pharmacological activities, such as antitumor, antimicrobial, and antiviral effects. The high level of biodiversity of marine macroalgae collected from French territories makes them a prime target for bioprospecting: a wide range of novel biomolecules are produced by these organisms, ranging from bioactive molecules and enzymes of interest for medicine to biopolymers with diverse industrial applications. This chapter thus contextualizes recent studies on marine macroalgal natural products isolated from French teams, with particular attention on structurally diverse natural products with ecological relevance, taxonomical importance and pharmaceutical potential.

## 4.2 Exploitation of marine algal resources

### 4.2.1 International context

Seaweed represents 23.4% of the tonnage and 9.7% of the value of the global (marine, brackishwater, and freshwater) aquaculture production, estimated at 59.4 million tonnes and US\$70.3 billion in 2004. Considering only mariculture (50.9% of the global aquaculture, estimated at 30.2 million tonnes and US\$28.1 billion), aquatic plants represent 45.9% of the tonnage and 24.2% of the value. The seaweed aquaculture production (92% of the world seaweed supplies) is estimated at 11.3 million tonnes and US\$5.7 billion (99.7% being provided by Asian countries). Approximately 220 species of algae are cultivated; however, six genera (*Laminaria* (kombu; 40.1%), *Undaria* (wakame; 22.3%), *Porphyra* (nori; 12.4%), *Eucheuma/Kappaphycus* (11.6%), and *Gracilaria* (8.4%)) provide 94.8% of the seaweed aquaculture production, and four genera (*Laminaria* (47.9%), *Porphyra* (23.3%), *Undaria* (17.7%), and *Gracilaria* (6.7%)) provide 95.6% of its value. The main components of the seaweed-derived industry are the sea-vegetable sector (76.1% of the tonnage and 88.3% of the value), the phycocolloid sector (11.2% of the tonnage and 10.8% of the value), and the emerging phycosupplement sector (10.8% of the tonnage and a presently underestimated value of 0.9%, but with promised expansion in the near future with new high value-added products) (FAO, 1994; Chopin & Sawhney, 2009).

Asian countries are the main markets for the use of seaweeds, such as marine vegetables. Japanese people are the first consumers with an average of 1.6 kg (dry weight) per year per capita (Fleurence, 1999). Three quarters of this production is done in Asian countries, such as China, Korea, and Japan, and are intended mainly for food. In the last few decades, emphasis has moved from wild harvesting to farming and controlled cultivation for the production of valuable new products on a large scale (Ioannou & Roussis, 2009). The aquaculture sector produces large amounts of seaweeds, such as *Laminaria*, *Porphyra*, and *Gracilaria*, and microalgae, including *Dunaliella* and *Spirulina*. In many countries, the food industries use a wide range of algae, which are well known to have high contents of fibers, minerals, vitamins, and different antioxidants. The use of microalgae has great potential for the production of food ingredients, as they are photoautotrophic microorganisms that can grow on a very simple culture medium containing seawater, nitrate, phosphate, trace amounts of certain metals, and carbon dioxide. Excluding the bioactive secondary metabolites that target the pharmaceutical market, the most common compounds from algae which already have commercial appli-

cations other than phycocolloids include polyunsaturated fatty acids, steroids, carotenoids and lectins (Tseng, 2001; FAO, 2004; Ioannou & Roussis, 2009).

### 4.2.2 French and Breton context

France is a country with an exceptional situation in the three oceans and its richness is due to the diversity of territories, populations and organisms which compose its overseas territories. The French offshore area (Exclusive Economic Zone, EEZ) is the second largest worldwide, with about 11 million km<sup>2</sup>. Brittany presents almost 800 islands and small islands with 2700 km of rocky coasts. Brittany is one of the largest fields of algae in Europe. The marine algae of the Brittany coasts thus constitute an extremely significant and diversified natural vegetable production. Algae proliferate on these coasts due to the optimal conditions for their development, light immersion of the rocky sea beds and constant drainage by the currents. Thus, nearly 700 species of algae are currently listed on the Breton littoral (Arzel, 2000; Dizerbo & Herpé, 2007). The use of algae has existed for centuries in Brittany; their harvest belongs to the patrimonial economy of the littoral. Regarded a long time as marginal, marine algae have helped many populations live and survive for several centuries. Exploited initially by the habitants of the littorals for their personal use, they become a source of income for many families. Indeed, algae are used to enrich fields and as fuel. Families then resold the surplus of their harvests to the inland habitants. Indeed, the latter were not authorized to collect this resource; the exploitation of seaweed was reserved to the habitants of littorals. Established on tradition and customs that were progressively put into law, the harvest of algae has undergone numerous and drastic transformations from the industrial era on. The improvement of the outputs and the professionalization of this activity led the maritime office to regulate it. The *goemon* trade increased when sodium carbonate and later iodine industries solicited the inhabitants of the coast for their harvests. Since the 1920s, marine macroalgae have been used by more and more companies (Arzel, 1987, 2000; Arzel & Barbaroux, 2003).

The extraction of sodium carbonate, iodine and then the discovery of colloids – agars, alginates, and carrageenans – gradually implied the establishment of numerous companies along the Brittany coast. Indeed, marine algae had to be processed as soon as they were harvested to preserve all their qualities; some companies cultivate the algae in the natural environment or outside in ranks before the transformation of the resource. Since the 1980s, cosmetic, food, thalassotherapy, and the pharmaceutical fields have been interested in algal resources.

Currently, the French and European seaweed industry relies on macroalgae collected from the wild with the exception of carrageenan extraction plants, which extract *Kappaphycus* and *Eucheuma* farmed in South-East Asia and Eastern Africa.

In Brittany, about 60 equipped boats collect the *Laminaria* species between Pleubian and Plouguerneau and around the Penmarc'h Pointe and Audierne.

Fifty thousand to 60 000 tons of collected algae each year in Brittany are transformed into colloids, are intended for agri-food or cosmetic industries, or are employed as organic compounds. The diverse phyla of marine macroalgae (red, brown, and green seaweeds) produce a number of molecules that are attractive for diverse industries. Applications of algal products range from simple biomass production for food, feed, and fuels to valuable products such as sugar polymers, cosmetics, pharmaceuticals, pigments, and food supplements. They also have the potential to be used as a source of new bioactives for human, animal or plant health, as well as a source of new synthons and biocatalysts in sustainable chemistry.

France is the seventh largest producer of marine algae in the world and the first in Europe. Moreover, 90 % of the French production is harvested in Brittany. Among macroalgae present in Brittany, brown algae are the main group which is harvested, following by red and green algae (Figure 4.1).

Among brown algae, *Ascophyllum nodosum*, *Laminaria digitata* and *Fucus serratus* are the most harvested species. As for red and green algae, harvesters collect mainly *Palmaria palmata*, the two allied morphological species *Chondrus crispus*/*Mastocarpus stellatus* and *Ulva* sp.

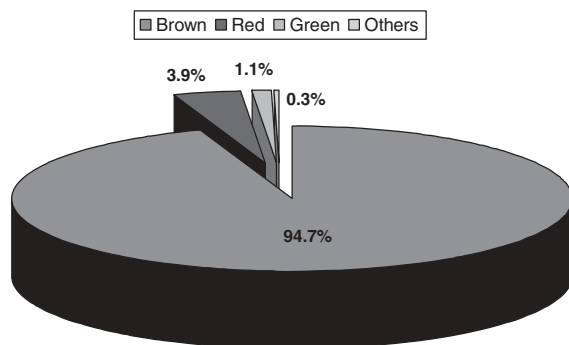
The growing demand worldwide for raw materials for food, cosmetics, bioactives, and more recently for chemistry and bioenergy, raises the issue of the sustainability of

the French and European industry. Presently, most of the algal biomass used by the French industry is harvested from wild populations with concerns about sustainability and biodiversity conservation.

In parallel, proliferations of green and red algae appear along the north Breton shallow sandy bays, which represent a true economic constraint for the affected communities: in addition to the nuisance for residents and the tourist activity, the communities must carry out systematic collecting. The algae are used thereafter in agricultural spreading or composting, but these solutions reach their limits quickly and bring only little added value to the collected algae. For example, each year in Morbihan, the peninsula of Rhuy must face a surge of algae and more precisely of red algae, thus obliging the communities to collect it urgently from their shores, under the pressure of the summer visitors. During the summertime, it is then a question of removing more than 2000 t of red algae (mainly *Solieria chordalis*) from the high part of the beaches of the communities of Sarzeau, Saint-Jacob, Kerfontaine and Penvins. *S. chordalis* (Rhodophyta, Gigartinales) is an invasive species that grows on sandy bottoms and stones. It is detached easily and then taken to sea or the shore. In recent years, algal stranding has appeared more and more precociously and the phenomenon has gained in significance.

On the other hand, some European countries are faced with the invasion of some algal species; these invasive seaweeds cause economic problems and represent large biomasses which could be valorised (Plouguerné, 2006; Sangiardi 2010). For example, the valorization of the invasive *Sargassum muticum* began after eradication failures and progress in its geographic areas. This valorization was achieved in different domains, including alginate extraction, fertilizers and pharmacological screening (Pruja, 1986). The following sections explain how researchers have found a way to valorize some invasive macroalgal species.

Harvest of marine macroalgae in Brittany (2009)



**Figure 4.1** Percentages of the three main groups of macroalgae harvested in Brittany (France) in 2009 (from CSAVM 2010).

#### 4.2.3 French research network on marine bioactive compounds extracted from macroalgae

Different categories of actors working on seaweeds can be distinguished: collectors, companies, organizations, and laboratories.

##### Laboratories working on natural compounds from marine macroalgae

The main laboratories working on natural substances extracted from macroalgae are localized along the Atlantic coast (Table 4.1) and more precisely in Brittany.

**Table 4.1** French laboratories currently working on natural compounds extracted from macroalgae

Laboratory	Institution	Location and studied area(s)	Research area
Biochimie et molécules marines	IFREMER Institut Français de recherche et d'Exploitation de la Mer	Plouzané, Brittany Atlantic and Pacific coasts	Polysaccharide structural elucidation and their valorization
EA 3877: Laboratoire d'Ecophysiologie et de Biotechnologie des Halophytes et Algues Marines (LEBHAM)	Institut Universitaire Européen de la Mer (IUEM) – Université de Bretagne Occidentale (UBO)	Plouzané, Brittany Atlantic and Pacific coasts	Natural compounds from brown and red seaweeds in relation with ecological monitoring of populations
UMR 6539 CNRS IRD UBO : Laboratoire Environnement Marin (LEMAR)			
UMR 7139 CNRS UPMC: Végétaux Marins et Biomolécules	Marine Station of Roscoff, CNRS/Université Pierre et Marie Curie – Paris VI	Roscoff, Brittany	
*Equipe Défense des Algues			
*Equipe Structure des polysaccharides marins			
CEVA	Transfer Center	Pleubian, Brittany	Valorisation of seaweeds
EA 3884 : Laboratoire de Biotechnologie et Chimie Marines (LBCM)	Université de Bretagne Sud (UBS)	Vannes and Lorient Atlantic coast	Antiviral, antifouling and cytotoxic activities of macroalgal extracts
EA 2160: Mer Molécules Santé (MMS)	Université de Nantes	Nantes, Pays de la Loire Atlantic coast	Pigments extracted from seaweeds
Département Biotechnologie marine	IFREMER NANTES	Nantes, Pays de la Loire Atlantic coast	Polysaccharides from brown algae
Département Sciences et Techniques alimentaires marines			
UMR CNRS 6144: Génie des Procédés Environnement Alimentaire (GPEA)	Univ-Nantes, IUT St Nazaire, UBS	Nantes, Lorient, Vannes, South Brittany Atlantic coast	
UMR CNRS 6250 Littoral Environnement et Sociétés	Université de La Rochelle	La Rochelle Atlantic coast	Valorization of macroalgal extracts
Equipe Molécules à Activités Biologiques			
EA 4323: laboratoire des Matériaux – Polymères – Interfaces – Environnement Marin (MAPIEM)	Université du Sud Toulon-Var	Toulon Atlantic and Mediterranean coasts	Chemical elucidation of terpen from brown algae

To be efficient in the domains touching marine resources, researchers decided to create networks to bring together all the competencies to efficiently work on the better understanding of and the valorisation of marine organisms. Various research groups, *Groupe de Recherches* (GDR) in French, were created. Various international players are currently working on finding solutions for improved management of and upgrading of marine biomasses.

### **National scale**

In France, for 4 years now, the members of the scientific SEAPRO network (Sustainable Exploitation of Aquatic PROducts) have been involved in different programs to promote new industrial upgrading solutions or to improve the existing ones. The general strategy retained by the SEAPRO participants is the following: (1) to locate and quantify the marine biomasses (raw and processed)

and to establish dynamic maps in order to study different upgrading strategies; (2) to transfer (bio)technological solutions to industries for the better management and use of their by-products and to reinforce links between academic and professional players; (3) to open research paths to find new upgrading solution or new derived products (<http://www.seapro.fr>).

GDR BioChiMar (Biodiversity and Marine Chemodiversity) includes several research groups (chemists and biologists) which work on marine ecosystems, more precisely on the diversity of marine organisms and on the isolation of molecules produced by marine organisms. The objectives followed by the GDR are to animate, modernize and consolidate collaboration within the scientific community on the sustainable management of marine resources. All the teams from the GDR are engaged in pluridisciplinary topics implying chemistry, biology, chemical ecology and valorisation (<http://biochimar.icsn.cnrs-gif.fr>).

Within the GDRs, we have selected two research projects, whether originating from the CRISP group or from the BIOTECMAR group, on the valorization of seaweed from Atlantic and Mediterranean parts of France.

The CRISP/IRD-Pacific project (2005–2008) takes place within the general CRISP project (Coral Reef InitiativeS for the Pacific, [www.crisponline.net](http://www.crisponline.net))

The aims of this project were to improve the understanding and development of marine benthic organisms found in reef and coral ecosystems from the South Pacific region; these organisms are known to contain pharmacologically active substances. This project was divided into four actions: (1) legislative aspects (analyzing and improving regulations on accessing marine genetic heritage and developing its economic potential for sustainable development); (2) knowledge about ecosystems (improving inventories, prospecting and taxonomy of species of interest); (3) isolation of marine substances (research on chemical substances that could be of interest in various health areas); and (4) training/information (capacity building and workshops in partner countries).

The Interreg IVB-BIOTECMAR (2009–2011) is an integrated transregional project for communication, technical information and technology transfer in the domain of biotechnological exploitation of marine products and by-products. This network supports the development of an integrated chain using marine by-products and products derived from fishing, aquaculture, seaweed and food industry for the production of valuable ingredients for food, animal and human nutrition, cosmetics and health (antioxidants, antistress, antihypertensives, collagen, pigment, etc.). The website presents the network, its actions and its events. It especially fosters links between all the “Sea product” players and R&D centers. (<http://www.biotechmar.eu>)

### Regional scale

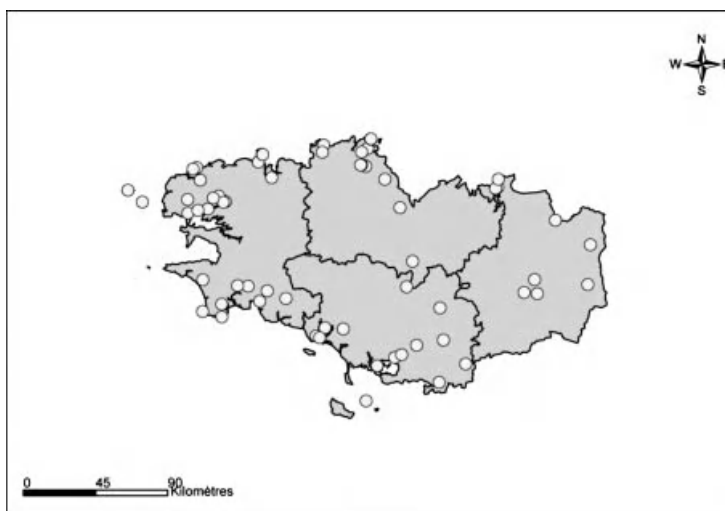
Marine research institutes and universities located in Brittany have joined together in a “Blue Network” (20 members including some of the Breton laboratories presented in Table 4.1) to create an informal coordination structure called GIS Europôle Mer in 2004. This network plays a key role within the region to purchase specific equipment or shared equipment. The aim of Europôle Mer is to become a research center of excellence, in which the central activity is to boost the scientific potential of the teams involved, to strengthen interactions between teams, in particular interdisciplinary links between the disciplines, and to support research projects around five research areas: (1) genomics and “blue chemistry”; (2) global change – ocean – marine ecosystems interactions; (3) observation and dynamics of coastal systems; (4) deep sea exploration and understanding; and (5) complex systems for observation, measures and intervention.

The Service des Affaires Maritimes has as a field of competence: legal and economic aspects of the environment, marine environment, and the risk/health aspect, the economic development of the activities related to fishing, with the marine culture, the definition of the maritime regulations.

The interprofessional organization of maritime fishing and the marine breeding includes various committees with national, regional and departmental competences. The “seaweed” commission, created in 1992, is supposed to manage the activity of fishing and harvesting of the marine plants. This commission includes all of the players of the sector.

The *Région Bretagne* includes numerous companies (67 establishments accounting for 1635 jobs reported in 2007 by CCI Brest), which valorize seaweeds in several sectors (cosmetic, agricultural and/or food uses). Two international groups, CARGILL and DANISCO, also exploit the algal biomass harvested in Brittany for the extraction of hydrocolloids. These companies use macroalgae harvested from natural populations (70 000–80 000 t) or imported from Asian countries. Companies are distributed all around the coasts of Brittany as shown in Figure 4.2. They belong to several application domains, such as the agrifood, nutraceutical and cosmetic fields.

Despite the availability of a nearly unpolluted and abundant natural resource, the French seaweed industry faces major challenges due to the lack of comprehensive scientific and technical data on which to base the development of new products and also to the limitation in the amounts of sustainable biomass. Only a high potential for innovation can ensure the development of this industry, which must also deal with environmental issues and sustainable



**Figure 4.2** Distribution of companies using macroalgae along the coasts of Brittany (France) (Sangiardi 2010).

development, particularly in terms of biodiversity conservation and environmentally friendly industrial processes.

Created in 1982 with the support of the Breton local authorities and the SMEs of the seaweed sector (network), the CEVA (Centre d'Etude et de Valorisation des Algues) operates an applied research on seaweeds (microalgae and macroalgae), marine halophytes, and marine biotechnologies. CEVA assures in particular the transfer of scientific knowledge from the academic world towards the industrial domain (<http://www.ceva.fr>).

The network of professional organizations including 21 companies of transformation or upgrading, the Chambre Syndicale des Algues et des Végétaux Marins (CSAVM) aims to protect and to represent its members in the profession in Brittany. It is trying to set up a status for harvesting people, management sustainability of the resource, the development of seaweed cultivation, together with the certification of seaweeds for the "Organic farming" label.

### 4.3 Why a focus on red and brown seaweeds?

Our choice to present only red and brown macroalgae is threefold: (1) an evolution point of view, (2) French productivity and (3) literature data.

- 1 Seaweeds are not all closely related and then do not form a single evolutionary lineage (which is the case for other organisms). Using cladistic analysis, the green algae should be grouped with the land plants and present taxa in ocean, freshwater, and seawater. Conversely, red and brown seaweeds have remained essentially marine organisms, have

no representative in terrestrial environments, and evolved totally independently as mentioned by Dawson (1966). These two biological groups are then promising because they synthesize originally metabolites, which are impossible to cultivate.

- 2 The production of macroalgae in France is composed of 98% and 1.6% respectively brown and red macroalgae (Chambre Syndicale des Algues et Végétaux Marins, 2009, com. pers.). We then decided to focus only on red and brown algae, the two main groups harvested along French coasts, by presenting the main classes of bioactive metabolites and then their potentiality in several industrial applications, studied and isolated from French teams.
- 3 In their review on natural products from seaweeds, Ioannou & Roussis (2009) clearly showed that among macroalgae, brown and red algae significantly appear richer in secondary metabolites than others taxa.

### 4.4 Marine red seaweeds and biological activities

In red seaweeds, two main classes of molecules are studied by French researchers: polysaccharides and proteins. Thus, only these two kinds of molecules are presented in detail in this section. Other types of molecules were punctually isolated and are presented in Sections 4.7, 4.8, and 4.9.

#### 4.4.1 Polysaccharides

##### Properties

The cell walls of Rhodophyta are extremely variable. Cellulose is believed to be the cell wall skeletal material in the majority of species. However, the cellulose was not detected in some representatives of Bangiophyceae, but they were shown to contain a linear  $\beta(1 \rightarrow 4)$  mannan (Usov, 1992). Floridean starch, a branched  $\alpha$ -glucan similar to amylopectins of higher plants, is a storage product in the cytoplasm. Some species of red seaweeds contain considerable amounts of neutral linear xylans built of  $\beta$ -D-xylopyranose residues linked by  $1 \rightarrow 3$  and  $1 \rightarrow 4$  glycosidic bonds. Most marine red algae elaborate high-molecular-weight, water-soluble sulfated galactans as the major, matrix-phase components of their cell walls (Craigie, 1990; Usov, 1992). These polysaccharides from red seaweeds include different sulfated galactans, sulfated rhamnans or mannans, complex hybrid galactans, carrageenans and agars. These polysaccharides are made up of a repeating disaccharide backbone of 3-linked  $\beta$ -D-galactopyranose (G units) and 4-linked  $\alpha$ -D-galactopyranose (D units) or 3,6-anhydrogalactose (DA units) in alternation. Different carrageenan repeating units have been identified on the basis of the sulfation pattern anhydrogalactose content and of the substitution by methoxyl, glycosyl, and/or pyruvate groups (Craigie, 1990; Usov, 1992; Knutsen *et al.*, 1994; Lahaye, 1995; Bourgougnon, 2003). Carrageenan is a collective term and covers several families denoted as  $\lambda$ -,  $\kappa$ -,  $\beta$ - or  $\omega$ -carrageenan. The corresponding IUPAC nomenclature allotted names to Greek symbols such as  $\kappa$ -carrageenan (carrabiose 4'sulfate, DA-G4S),  $\iota$ -carrageenan (carrabiose 2,4'-disulfate, DA2S-G4S) or  $\lambda$ -carrageenan (carrabiose 2,6,2'-trisulfate, D2S6S-G2S) (Bondu *et al.*, 2010).

Complex hybrid galactans, with both agar- and carrageenan-like structures, have also been reported (Haslin *et al.*, 2000, 2001, Haslin & Pellegrini, 2001.). The ability to synthesize acid polysaccharides is the most interesting property of red seaweeds. Several species produce complex heteropolysaccharides containing uronic acids together with neutral or sulfated monosaccharides or galactans (Bourgougnon, 1993; Bourgougnon *et al.*, 1994, 1996a,b,d; Lahaye, 1995). Red algal galactans constitute a spectrum of polysaccharides bearing with a variety of non-glycosyl substitutions (methoxyl, pyruvate, sulfate groups, other sugar residues (galactose, xylose) or uronic acids. The chemical structure of agars and carrageenans vary between and within species. In some carrageenophytes, the structure of carrageenan depends on the life stage of the algae (gametophyte, sporophyte) or presents seasonal variations (Craigie, 1990; Craigie and Rivero-Carro, 1992; Haslin *et al.*, 2000).

Agars are typically low in sulfate ester substitution, but those from numerous sources are rich in methyl ether or pyruvate acetal substitution. Conversely, carrageenans are comparatively rich in sulfate ester substitution but poor pyruvate acetal- and methyl ether-substitutions (Craigie, 1990).

The sulfated galactans from some species, such as agarocolloids and carrageenans, represent a growing industry, with more than 1 million tonnes of seaweeds extracted annually for hydrocolloid production (FAO, 2004). The wide use of these compounds is based on their gelling, viscosifying and emulsifying properties, which have generated an increasing commercial and scientific interest in the food, cosmetic and medical/pharmaceutical industries.

##### Biological functions and variation

Pharmaceutical compounds constitute one of the most important potential markets for algal products. Prior to 1950, the use of seaweed extracts and microalgae as drugs or drug sources was restricted to folk medicine. It has been proposed that they are involved in recognition mechanisms between seaweeds and pathogens (Potin *et al.*, 1999). Although several polysaccharides have been described with antiviral (Gonzales *et al.*, 1987, 1991; Bourgougnon *et al.*, 1993; 1994, 1996c; Mazumder *et al.*, 2002; Pujol *et al.*, 2006; Bourgougnon, 2003; Damonte *et al.*, 2004; Talarico *et al.*, 2004, 2005, Kornprobst, 2005; Salvador *et al.*, 2007; Karabayyavasoglu *et al.*, 2007; Mayer *et al.*, 2009, Monthana *et al.*, 2009; Bouhlal *et al.*, 2010a, b), anticoagulant (Carlucci *et al.*, 1997; Opoku *et al.*, 2006), anti-tumoral (Alves de Sousa *et al.*, 2007; Mayer *et al.*, 2009), antimetastatic (Xuelian *et al.*, 2006) and anti-inflammatory effects (Solimabi and Das, 1980), worthwhile for clinical uses (Bondu *et al.*, 2010).

##### Two examples of biological polysaccharides extracted from invasive French seaweeds

###### *Solieria chordalis* (Gigartinales, Solieriaceae)

Among the commercially exploited carrageenophytes, the most numerous ones belong to the Solieriaceae family, which is part of the order Gigartinales (Doty, 1988) known to contain the highest number of genera (Gabrielson & Hommersand, 1982; Womersley, 1994). Among them, the genus *Solieria* is acknowledged to contain  $\iota$ -carrageenan as a main component (Deslandes *et al.*, 1985; Saito and De Oliveira, 1990; Murano *et al.*, 1997).

*S. chordalis* is present in large quantities in the subtidal zone of the Brittany shoreline. Because of its great production of  $\iota$ -carrageenan (Bellion *et al.*, 1983; Deslandes, 1988; Deslandes *et al.*, 1985, Claude *et al.*, 2009), this seaweed constitutes an interesting material for investigations about the metabolic pathway of carrageenan biosynthesis

(Fournet *et al.*, 1999; Goulard *et al.*, 2001) and the chemical structure of this polysaccharide.

Bondu *et al.* (2010) show that the structural components of this polysaccharide are mainly a (DA2S-G4S)-type structure in association with methylated  $\iota$ -carrageenan, pyruvated  $\alpha$ -carrageenan and the minor precursor,  $\nu$ -carrageenan, in small amounts. The relative molecular weight of the native polysaccharide was estimated by LP-GPC as 913 kDa. The low molecular weight fractions (LMWF) (below 20 kDa) obtained by free-radical depolymerization and mild-acid hydrolysis presented substitution patterns similar to those of the native polysaccharide.

These fractions proved to be devoid of direct cytotoxicity on Daudi (human Burkitt's lymphoma), Jurkat (human leukemic T-cell lymphoblast) and K 562 (human chronic myelogenous leukemia) cell lines. On the other hand, they showed great immunostimulating properties: enhancement of neutrophil phagocytosis, cytotoxicity by natural killer cells, antibody-dependent cell cytotoxicity and stimulation of lymphocyte proliferation. The immunological assays showed an enhancement of the innate immunity further to an *in vitro* exposure of immune cells to high concentrations of the low molecular weight fractions LMWFs from *S. chordalis* carrageenan through natural killer (NK) cell- and neutrophil-dependent mechanism and antibody-dependent cell cytotoxicity (ADCC). Moreover, no marked direct toxic effects on tumoral cells lines were noticed. It is worth recalling that the LMWFs of carrageenans of *S. chordalis* were obtained by free-radical depolymerization and mild-acid hydrolysis. The free OH radical led to a non-selective degradation of the polysaccharide, contrary to the acidic hydrolysis, more directed towards the less stable 3,6-anhydrogalactosyl bonds. The free-radical depolymerization generated products with identical chemical structures (sulfate, methyl and pyruvate substitutions), but different molecular weights. On the other hand, the products arising from acid hydrolysis exhibited heterogeneous chemical structures together with different molecular weights. By showing that the highest activities were always produced by the lowest molecular weight fractions, immunological assays carried out in this study suggest an implication of the molecular weight in the direct cytotoxicity by NK cells. Furthermore, they also indicate an impact by the molecular weight and structure on the ADCC mechanism. In conclusion, according to the adhered-immunity cells under study, these investigations provided evidence of a synergistic, or not, impact by the molecular weight and the structure of oligo-carrageenans on the immune response. Further to these investigations, it could be worth using the low molecular weight fractions of carrageenan from *S. chordalis*, in immunotherapeutic approaches to cancer treatment.

*Asparagopsis armata* (Bonnemaisoniales, Bonnemaisoniaceae)

Red marine algae from the Bonnemaisoniaceae family have been shown to produce a wide range of halogenated metabolites, including butenones, acetones, acrylic and acetic acids, pyranones, octenones, furanones (Codomier *et al.*, 1977, 1981; Combaut *et al.*, 1978; 1979). The genus *Asparagopsis* is considered as a particularly prolific source of halogenated metabolites with biological activities (Kladi *et al.*, 2004).

The ontogenesis of the Mediterranean invasive species *Asparagopsis armata* is an alternation of morphologically dissimilar gametic, carposporic, and tetrasporic reproductive phases which produced complex sulfated galactans composed of galactose: anhydrogalactose: sulfates in molar ratios of 1/0.01/1.23, 1/0.04/0.47 and 1/0.01/1.13, respectively (Haslin *et al.*, 2000, 2001). These peculiar polymers of 1,3- and 1,4-linked galactose units, containing uronic acids (3.5–15.9% dry wt.) and in which D-galactose content (77.6–92.6% of total galactose) exceeds L-galactose content, agree neither with ideal carrageenan nor agar-type polysaccharides. These water-soluble polysaccharides were studied for their *in vitro* activity against human immunodeficiency virus (HIV)-1. Syncytia formation was completely suppressed after the 7th day of infection by gametic and tetrasporic polysaccharides at 10  $\mu\text{g/ml}$  ( $\text{IC}_{50} = 9 \mu\text{g/ml}$ ) and 8  $\mu\text{g/ml}$  ( $\text{IC}_{50} = 7 \mu\text{g/ml}$ ), respectively. No cytotoxicity towards MT4 cells was found at these concentrations. The carposporic polysaccharide is ineffective, even at the concentration of 100  $\mu\text{g/ml}$ . Inhibition of virus-induced cytopathogenicity on MT4 cells might be related to the sulfate content of the polysaccharides from *Asparagopsis armata*, since the activity increased from the less sulfated and inactive carposporic galactan (10% dry wt.  $\text{SO}_3^-$ : 0.47 sulfate ester per monosaccharide), to the higher sulfated tetrasporic and gametic ones (23.8% and 30.1% dry wt.  $\text{SO}_3^-$ : 1.13 and 1.23 sulfate ester per monosaccharide). The dosage of the Reverse Transcriptase activity on CEM cell lines supernatant confirmed the protective effect of the gametic polysaccharide from *A. armata* against HIV-1 replication. Virus production was highly delayed with 10  $\mu\text{g/ml}$  of sulphated polysaccharide and completely blocked with 50  $\mu\text{g/ml}$ . The chemical desulfated gametic polysaccharide lost its anti-HIV activity even at 50  $\mu\text{g/ml}$ , thus confirming the relationship between the degree of sulfation and the antiviral activity (Haslin *et al.*, 2001). The time of action of the gametic polysaccharide from *A. armata* was studied by adding the compound at different times of the experiment. The maximal antiviral effect involves the presence of the polysaccharides after or during infection but not before infection. Activity of the polysaccharide after infec-

tion demonstrates that the compound blocks the replication of HIV and then the syncytia formation between uninfected and infected cells. This time of action suggests an inhibition of an early step of HIV infection by an interaction of the polysaccharide with the viral enzymes (probably on the RT step (Haslin *et al.*, 2001).

#### 4.4.2 Phycoerythrin

In addition, the Rhodophyceae contain a particular protein particularly major light-harvesting pigment called phycoerythrin (PE). In 1843, Kützing described a pigment isolated from *Rytiphlaea tinctoria* as phycohaematin or fluoridorubin, in addition to describing phycoerythrin. Rowan (1989) and the French team in Potier (Chevelot-Magheuer *et al.*, 1976) has continued to support the earlier claims that floridorubin was a light-harvesting pigment in *Rytiphlaea tinctoria*.

##### Properties

R-phycoerythrin (R-PE) is a major light harvesting pigment in red seaweeds; it has an important role in photosynthesis (Glazer, 1989) and belongs to the family of phycobiliproteins. It is a hydrosoluble oligomeric red protein of 240 kDa with interesting fluorescent properties; the single fluorescence maximum is located at 580 nm. PE is commonly used for different applications, such as immunology, cell biology, flow cytometry, food and cosmetics (Sekar and Chandramohan, 2008). PE is classically purified by a combination of several techniques: ammonium sulfate precipitation, and different chromatography techniques, such as ion-exchange, gel filtration and absorption (Denis *et al.*, 2009) (Figure 4.3).

##### Biological functions and variation

In reality, PE used for this particular application is obtained from Cyanobacteria and the microalgae *Porphyridium cruentum*, in which the pigment can represent up to 50% of the protein fraction (Bermejo Román *et al.*, 2002). The main problem in the use of PE as a food dye is the relative insta-

bility of this chromoprotein to heat and pH variations. In 2002, these authors obtained by an inexpensive and simple process B-PE and R-phycoerythrin in its native state, from the red alga *Porphyridium cruentum*. The best results of this purification procedure were scaled up by a factor of 13 to a large preparative level using an anionic chromatographic column of DEAE cellulose. Gradient elution with acetic acid–sodium acetate buffer (pH 5.5) was used. In these conditions both 32% of B-phycoerythrin and 12% of R-phycoerythrin contained in the biomass of the microalgae was recovered. B-PE was homogeneous as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), yielding three migrating bands corresponding to its three subunits, consistent with the  $(\alpha\beta)_6\gamma$  subunit composition characteristic of this biliprotein and the spectroscopic characterization of B-PE (UV–visible absorption and emission spectroscopy; steady-state and polarization fluorescence), is accompanied (Bermejo Román *et al.*, 2002). In association with this application, the use of enzymatic liquefaction of red seaweeds already described in the literature could be an alternative procedure to improve protein solubilization, especially PE, in mild conditions (Lahaye and Vigouroux, 1992; Fleurence *et al.*, 1995). Studies are being performed to understand the physical conditions needed to improve the stability of PE especially that extracted from *Palmaria palmata*.

##### Phycoerythrin extracted from the invasive French Grateloupia turuturu

*Grateloupia turuturu* Yamada (Halymeniaceae), an invasive red macroalga, represents an unexploited important biomass found all year round along the shore lines of Brittany. The chemical composition and the seasonal variation of *Grateloupia turuturu* were investigated by Denis *et al.* (2010). Size, ash, protein, lipid, dietary fibre (soluble, insoluble and total), protein pigment (R-PE, R-phycoerythrin), and fatty acid content were measured in *G. turuturu* samples collected over 1 year (2006). The average size of this seaweed was 32.0 cm long and approximately 5.0 cm wide, while the size of the thallus was maximal in June (in length and width). On the dry weight basis, this alga was constituted of more than 18% ash, about 23% protein, 2.6% lipids, and approximately 60% dietary fiber. The most abundant fatty acids were palmitic acid and eicosapentaenoic acid (52% and 12% of the fatty acid fraction, respectively). The study of seasonal variations showed that the best period to collect the seaweed for food use is between February and June. This seaweed is rich in proteins (23% DW) more particularly R-PE (Denis *et al.*, 2009). More recently, the same researchers have proposed a membrane screening realized at a lab-scale (Amicon stirred cell) to determine a

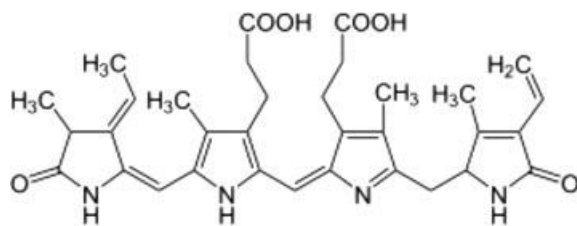


Figure 4.3 Structure of phycoerythrin.

suitable membrane molecular weight cut-off and a material allowing for an optimal concentration and pre-purification of R-PE contained in a hydrosoluble extract (Denis *et al.*, 2010). The best adapted membrane turned out to be a polyethersulfone 30 kDa one. An up-scaling methodology was then realized on a PCI Microlab40 pilot plant equipped with an industrial type polyethersulfone 25–30 kDa membrane. The results show that R-PE was concentrated without denaturation and accumulation of undesired molecules. Ultrafiltration up to a volume reduction factor of 5 was effective for R-PE concentration and pre-purification: 100% of R-PE was recovered, 32.9% of other proteins and 64.6% of sugars passed through membrane. Based on these results, a techno-economic study was investigated on an ultrafiltration unit at an industrial scale (Denis *et al.*, 2010).

In association with this application, the use of enzymatic liquefaction of red seaweeds already described in the literature could be an alternative procedure to improve protein solubilization, especially PE, in mild conditions (Lahaye and Vigouroux, 1992; Fleurence *et al.*, 1995a). Studies are being performed to understand the physical conditions needed to improve the stability of PE especially that extracted from *Palmaria palmata*.

The use of high protein level seaweeds in feed for fish farming or food dye seems to be a promising way to use this plant as a marine resource in Europe. This would also satisfy industrial needs which require a partial substitution of animal meal by plant meal in fish feed. This new requirement, which should enable improved exploitation of seaweeds in the western world, is a new challenge for research (Fleurence, 1999).

## 4.5 Marine brown seaweeds and biological activities

We selected three classes of compounds known for their biological functions and their potential valorisation in several industrial domains and studied by French researchers. These molecules are (1) polysaccharides, (2) phenolic compounds and finally (3) the interesting class of terpenes; the last two classes being the predominant metabolite classes found in brown algae (Blunt *et al.*, 2006, 2007).

### 4.5.1 Polysaccharides

#### Properties

Cell walls of brown algae are constituted of a fibrillar compartment formed mainly of cellulose microfibrils, which is embedded in an amorphous matrix of acid polysaccharides linked to each other by proteins (Kloareg *et al.*, 1986). The acid polysaccharides are mainly composed of alginic acids and sulfated fucans (also known as fucoidan).

Alginic acid or alginate is the common name given to a family of linear polysaccharides containing 1,4-linked  $\beta$ -D-mannuronic (M) and  $\alpha$ -L-guluronic acid (G) residues arranged in a non-regular, blockwise order along the chain (Andrade *et al.*, 2004). Alginate is located in the cell wall and in the matrix of the algae cementing the cells together and giving certain mechanical properties to the algae (Kloareg and Quatrano, 1988).

The physical properties of alginates are largely determined by the relative amounts of the three types of blocks (M, G and MG) present in the co-polymer (Kloareg and Quatrano, 1988; Indegaard and Minsaa, 1991). The mannuronic acid to guluronic acid ratio is an index of the nature of gel. In general, alginic acid with a low M/G ratio and a large proportion of guluronic blocks form a strong and rigid gel. Alginic acid with a low number of guluronic blocks and a high M/G ratio produce soft and elastic gel.

The other family of acids and soluble polysaccharides are represented by fucans which are sulfated and ramified polysaccharides constituted by a large variety of sugars, as L-fucose, D-galactose (reviewed in Berteau and Mulloy, 2003). In agreement with Berteau and Mulloy (2003), the term fucoidan has been used for fucans extracted from algae and will be used in the rest of the text. Fucoidans appear to play a role in the cell-wall organization (Kloareg and Quatrano, 1988; Bisgrove and Kropf, 2001) and could be involved in the cross-linkage of alginate and cellulose (Mabeau *et al.*, 1990).

#### Biological functions and variation

Alginate has a considerable technological importance for its solution properties and as a gelling agent (Larsen *et al.*, 2003). Alginate produced by brown seaweed is used widely in the food and pharmaceutical industries due to its ability to chelate metal ions and to form a highly viscous solution. The gelation of alginates is dependent on the number and length of polyguluronate sequences (Miller, 1996). The ratio of M:G units varies between 0.25 to 2.25 and is known to vary between species but also to some extent between parts of the plant, season of collection, age of the plant and by implication between plants (Haug *et al.*, 1969; Miller, 1996).

In temperate areas, they are mainly extracted from the brown seaweeds *Macrocystis pyrifera*, *Ascophyllum nodosum*, and *Laminaria* spp., whereas in tropical regions (China, Philippines, India, and Vietnam), *Sargassum*, *Turbinaria*, and *Padina* are the major sources (Critchley and Ohno, 1998).

### Examples of polysaccharides from French models

An interesting study was carried out in French Polynesia. With the aim to valorize brown seaweeds which proliferate on coral reefs (Stiger and Payri, 1999, 2005), a valorization campaign was led to use the high biomass of *Sargassum pacificum* (previously known as *S. mangarevense*, Mattio *et al.*, 2008) and *Turbinaria ornata*, as published by Zubia *et al.* (2003).

The study shows that alginate yields in Polynesian seaweeds were in the range of 6.0 to 21.1% DW, with the highest values shown in *Turbinaria ornata*. These ratios are comparatively lower than those from other alginophytes used in the industry (13–38% DW; Perez *et al.*, 1992). *Sargassum pacificum* showed a lower alginate yield than *T. ornata* (6.0–12.4% DW against 16.8–21.1% DW). In both species studied, M:G ratios were quite high (1.25–1.42) in comparison with the literature data (Zubia *et al.*, 2008). These observations favor the use of Polynesian seaweed alginates for the manufacturing of strong gels as reported previously for *Sargassum* and *Turbinaria* species (Jothisarawathi *et al.*, 2006).

Another study reported interesting fucans extracted from *Turbinaria ornata* from Tahiti (French Polynesia) (Deslandes *et al.*, 2000). These fucans showed an antiproliferative effect on the asynchronous cells of a human non-small-cell bronchopulmonary carcinoma line (NSCLC-N6).

### 4.5.2 Phenolic compounds (phloroglucinol and derived products)

Among the arsenal of molecules produced by brown macroalgae, phenolic compounds can be accumulated at high levels, up to nearly 20% DW in Fucales and 30% DW in Dictyotales (e.g., Ragan and Glombitza 1986; Targett *et al.*, 1995; Amsler and Fairhead, 2006).

Phenolic compounds constitute a class of molecules separated into phloroglucinols (mono-, di-, tri-, tetra- and oligomeric) and phlorotannins (Singh and Bharate, 2006). Halogenated monomeric phenolics are occasionally found in brown algae, as well as in a few red algae.

#### Properties

Phenolic compounds possess a large type of chemical structures comprising always one or several aromatic cycle(s) and one or several hydroxyl radicals (Singh and Bharate, 2006). Some of them are water-soluble (essentially small molecules) while others are partially liposoluble (polymerized forms of phenolic compounds). Brown algae, but also green and red algae, can synthesize this type of compound. Among phenolic compounds produced by brown macroalgae, a particular focus is made on phlorotannins.

**Table 4.2** Types of phlorotannins encountered within brown macroalgae

Type of linkage	Class of phlorotannin
Ether	Fuhalols and phlorethols
Phenyl	Fucols
Both ether and phenyl	Fucophlorethols
Dibenzodioxin	Eckols and carmalols

Phlorotannins are chains of high molecular weight derived from phloroglucinol (Singh and Bharate, 2006); the different units of phloroglucinol are linked by a carbon-carbon linkage or by a diaryl ether linkage. The size variation of phlorotannins is large: from 126 Da (free phloroglucinol) to 650 000 Da (polymer of 4800 units).

Four types of phlorotannins, based on the type of linkage present within the molecule, could be produced by brown macroalgae, as presented in Table 4.2. Most of these four types of compounds have halogenated representatives in brown algae. No carmalols were actually identified in Sargassaceae, which are present in France. Molecules can have a linear or polycyclic structure as shown in Figure 4.4 for the structure of phlorotannins that could be isolated from French brown macroalgae.

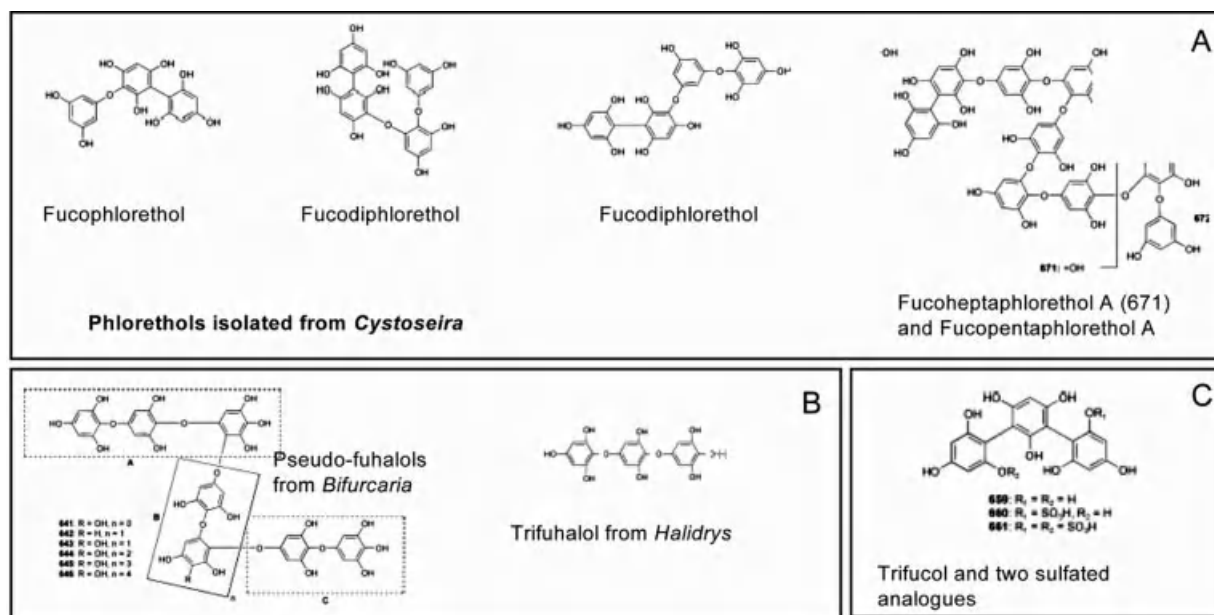
Several fuhalols, phlorethols and hydroxyphlorethols were isolated from *Sargassum muticum*, *Halidrys siliquosa* and *Bifurcaria bifurcata*, and also from different species of *Cystoseira*. Each time, compounds were isolated as peracetates (Figure 4.4).

Diphlorethol and trifuhanol (Figure 4.4A) were isolated from *Halidrys siliquosa*. Some polyprenylated cyclic polyketides of the enaimenones family (not shown), together with the trifuhanol B were isolated from *Sargassum muticum*. Both bifuhanol and pentafulanol were isolated from *Bifurcaria bifurcata*. These compounds are constituted of three parts (Figure 4.4B): an initial part (A) with three loops, a terminal part (C) with two loops, and an intermediate part (B) with two loops, which could occur several times. Some compounds possess only part B, while some possess only part B but repeated several times (up to four times).

The trifucol and its two sulfated analogs were reported in numerous brown algae and are known in *Bifurcaria bifurcata* (Figure 4.4C). The fucophlorethol A and B Fucodiphlorethols B and D were isolated from *Cystoseira baccata*.

#### Biological functions, variation and valorization

Phlorotannins have various putative roles (see Amsler and Fairhead, 2006 for a review).



**Figure 4.4** Examples of phlorethols (A), fuhalols (B) and fucols (C) isolated from brown macroalgae (from Singh and Barhate, 2006).

Phlorotannins play a primary role as integral structural components of cell-walls in Fucales (Schoenwaelder and Clayton, 1999; Schoenwaelder, 2002; Amsler and Fairhead, 2006). The structural role of these compounds essentially distributed in peripheric cells, was demonstrated in several studies and indicates the contribution of apoplastic haloperoxidases (Potin and Leblanc, 2006) in numerous events, such as cuticular protection, the reparation of lesions and mostly the bioadhesion of zygotes from brown macroalgae on the substrate. Phlorotannins, which are liberated in the seawater (then soluble), are not polymerized and are normally secreted as halogenated monomers (Shibata *et al.*, 2006). The roles and the variability of phlorotannins, as primary and secondary metabolites, are reviewed by Amsler and Fairhead (2006).

Moreover, published reports show that the production of phenolic compounds on marine algae is usually associated with a chemical defense and have been proved to be involved in various protection mechanisms, such as against grazing (Pavia and Toth, 2000; Lüder and Clayton, 2004; Stiger *et al.*, 2004; Hemmi *et al.*, 2005; Fairhead *et al.*, 2006; Svensson *et al.*, 2007), pathogen attack (Hay, 1996; Potin *et al.*, 2002), epiphytism (Amsler and Fairhead, 2006; Brock *et al.*, 2001, 2007; Wikström and Pavia, 2004), antifouling (Fusetani, 2004; Maréchal and Hellio, 2009) and UV damages (Swanson and Druehl, 2002; Henry and Van

Alstyne, 2004; Bjerke *et al.*, 2005; Fairhead *et al.*, 2006). Moreover, these compounds exhibit antioxidative properties (Nakai *et al.*, 2006; Kuda *et al.*, 2007; Kumar Chandini *et al.*, 2008; Le Lann *et al.*, 2008a) and are also involved in photoprotection mechanisms, particularly to counteract the cytotoxic effects of UV radiation (Swanson and Druehl, 2002).

Phenolic content in Phaeophyceae is governed by both intrinsic and extrinsic factors. The former includes life history stage (Van Alstyne *et al.*, 2001; Stiger *et al.*, 2004), plant size (Denton *et al.*, 1990; Stiger *et al.*, 2004; Plouguerné, 2006) and tissue age (Pedersen, 1984), as well as maturity of the thallus (Van Alstyne *et al.*, 1999; Stiger *et al.*, 2004; Plouguerné *et al.*, 2006a; Le Lann and Stiger-Pouvreau, 2009). Toth and Pavia (2002) demonstrated that meristematic and cortical tissues of brown seaweeds usually contain more phenolic compounds than the cells of medulla; this is due to the differential abundance of physodes, *i.e.* cellular vacuoles accumulating mainly phenolic compounds (Ragan and Glombitza 1986).

Various environmental factors such as season (Connan *et al.*, 2004; Plouguerné *et al.*, 2006a; Le Lann, 2009), light (Abdala-Díaz *et al.*, 2006; Plouguerné *et al.*, 2006a) and nutrient concentration (Yates and Peckol, 1993) can be responsible for variations in the phenolic content of brown macroalgae.

Polyphenolic compounds are not stable and the structural and conformational analyses are difficult due to the rapid oxidation and the nucleophile reactivity of these compounds. The pioneering work of Ragan and Jensen (1978) and Ragan and Glombitza (1986) remained unquestioned for a long time. Recently, novel analytical techniques were developed by Koivikko *et al.* (2007, 2008) and Cérantola *et al.* (2006), which made it possible to isolate pure or semipure phenolic compounds.

In terms of valorization, the potentials of phlorotannins are multiple, even if their reactivity (instability, complexation with proteins, carbohydrates and others molecules) is problematic during their extraction and purification. Nevertheless, recent works encouraged researchers in the exploration and isolation of bioactive compounds within phlorotannins. As an example, during the European project SeaHealth, Zaragoza *et al.* (2008) demonstrated interesting activities (preventive and curative activities against atheromas of a hydroalcoholic extract from *Fucus*. Several antibacterial and antifungal (Hellio *et al.*, 2001; Sandsalen *et al.*, 2003; Plouguerné, 2006), antilarval and antialgal (Ragan and Glombitza, 1986; Hellio *et al.*, 2004), UV-protection (Connan, 2004; Le Lann *et al.*, 2008b) activities were highlighted for phlorotannins isolated from macroalgae. From a pharmacological point of view, phlorotannins oligomers also present numerous properties. As an example, the phloroglucinol which is the base unit of phlorotannins is used in the drug Spasfon<sup>®</sup>, against human digestive troubles.

### Examples of phlorotannins extracted from French models

French researchers worked on the variability of the pool of phlorotannins extracted from brown macroalgae. Phenolic contents were determined on methanolic extracts and shown to vary according to several environmental parameters. Some researchers were interested in the spatio-temporal variations of phenolic contents. Connan *et al.* (2004) worked on seaweeds forming belts on a sheltered rocky shore in Brittany. They demonstrated that the highest contents were found in species growing at midtide level, with a decrease above and below this shore level. Phenolic contents presented a seasonal pattern, with a summer maximum for the Fucales and winter maximum for a member of the Laminariales. Stiger *et al.* (2004), working on tropical macroalgae demonstrated high phenolic content during the austral season (in relation with the reproductive period of concerned algae). Plouguerné *et al.* (2006a) and Le Lann and Stiger-Pouvreau (2009) demonstrated that *Sargassum muticum* produced more phenolic compounds in spring which correspond to the growth period of the algae. Phenolic contents are also known to vary in relation with the developmental stage (Stiger *et al.*, 2004), the part of the thallus (Connan *et al.*, 2006) together with the maturity of the thallus (Stiger *et al.*, 2004; Plouguerné *et al.*, 2006a; Le Lann and Stiger-Pouvreau, 2009).

Other studies were interested in the variability of phenolic content in relation with the conditioning of algae (Connan, 2004; Le Lann *et al.*, 2008b), and the extraction processes (Connan, 2004; Zubia *et al.*, 2009). More recent studies focused on the identification of the phlorotannin pool of *Fucus spiralis*. Cérantola *et al.* (2006) showed that these species have a simultaneous production of fucol and fucophlorethol classes, with both, higher antioxidative activity (DPPH) than positive control (ascorbic acid and phloroglucinol). Then, high molecular weight phlorotannins exhibit higher antioxidant activity than the monomer phloroglucinol. Moreover, this activity was not necessarily linked to the structural type of the polymer. More effort was done in the purification process of phenolic compounds. Audibert *et al.* (2010) described a procedure to obtain from *Ascophyllum nodosum*, purified oligophenolic fractions (molecular weight <2 kDa) to perform RP-HPLC analysis. Among purified fractions, the ones containing phenolic compounds >50 kDa seemed to be the most active (DPPH, ABTS), in relation with the content of phenolic compounds (Audibert *et al.*, 2010).

Currently, research is focused on the use of innovative techniques to extract and identify phlorotannins and to better understand the biosynthesis pathway of these compounds. In this context, authors have tested several innovative techniques, such as pressurized-liquid and solid-phase extraction techniques (Onofrejová *et al.*, 2009), supercritical fluid extraction (Herrero *et al.*, 2006) or accelerated solvent extraction followed by a solid phase extraction purification procedure (Zubia *et al.*, 2009) as examples of research development. Other researchers have used innovative methods to obtain the phenolic content of extracts or have tested the radical-scavenging potential of phenol pools in algae using electrochemistry (Keyrouz *et al.*, 2011; Blanc *et al.*, 2011).

Concerning the biosynthesis pathway of phenolic compounds, authors have hypothesized on the precursors of phenolic compounds. El Hattab *et al.* (2009) hypothesized from a study on the brown alga *Zonaria tournefortii*, that eicosapentaenoic acid could be a possible precursor of the phloroglucinol derivatives. Previous studies on this species have shown that it could accumulate phenolic compounds such as acylphloroglucinols (Amico *et al.*, 1981). These phloroglucinol derivatives have been isolated, not only from Pacific members of the Dictyotaceae, but also from the taxonomically unrelated Sargassaceae (order

Fucales) of the genus *Cystophora* (El Hattab *et al.*, 2009). These acylphloroglucinol derivatives could be generated by a polyketide-type biosynthesis beginning with the condensation of malonyl-CoA and acyl-CoA, in order to furnish a tetraketide or a pentaketide intermediate. A research program was started in 2009 with the aim to understand the biosynthesis pathway of phlorotannins using functional genomic and metabolic profiling (Project Phlorotan-ING 2009–2012).

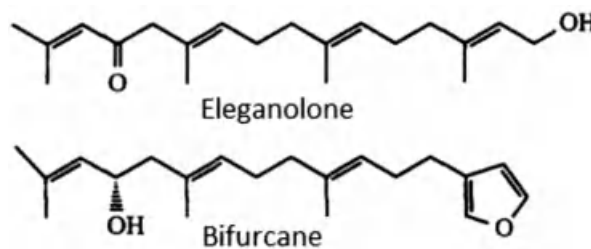
### 4.5.3 Terpenes

Only classes of terpenes and meroditerpenes isolated from French teams are presented here.

#### Properties

The chemical structure of terpenes is relatively complex. They can have a linear, monocyclic, bicyclic or rearranged (cyclization by a heteroatom) structure. All terpenes are built from a similar ramified hydrocarbonated chain: isoprene (2methylbuta-1,3-diene), which gives the molecule an apolar character. In brown algae, terpenes are known in two main orders: Fucales and Dictyotales. Fucales possess only linear diterpenes and Dictyotales only cyclic diterpenes and sesquiterpenes (Kornprobst, 2005). Terpenes may be classified by the number of terpene units in the molecule; a prefix in the name indicates the number of terpene units needed to assemble the molecule. In this part, we identified diterpenes, sesquiterpenes, and halogenated terpenes, which can be isolated from brown seaweeds. We also included the interesting class of meroditerpenes, which is composed of a complex of terpenic and aromatic parts and which can be used in the chimiotaxonomy of the genus *Cystoseira* (see Section 4.8).

Diterpenes are composed of four isoprene units and they derive from geranylgeranyl pyrophosphate. Among brown seaweeds, diterpenes were isolated from lipid extracts and are present in Fucales and Dictyotales species (Kornprobst, 2005). Several acyclic diterpenes were isolated from the Fucales *Bifurcaria bifurcata* (Biard *et al.*, 1980; Combaut and Piovetti, 1983; Culioli *et al.*, 1999a,b, 2000, 2001, 2004; Hougaard *et al.*, 1991; Semmak *et al.*, 1988; Valls *et al.*, 1986, 1993; Valls *et al.*, 1995). Among all diterpenes isolated from *B. bifurcata*, we can cite: geranylgeraniol and geranylgeraniol-derived diterpenes of which the principal ones are (S)-13-hydroxygeranylgeraniol, 13-ketogeranylgeraniol, the (S)-13-hydroxyfuranoditerpene, (S)-12-hydroxygeranylgeraniol, eleganolone (13-oxogeranylgeraniol) and eleganediol (13-hydroxygeranylgeraniol). Some of them showed a cytotoxic effect to fertilized sea urchin eggs (Valls *et al.*,



**Figure 4.5** Example of two types of diterpenes produced by *Bifurcaria bifurcata* (from Le Lann, 2009).

1995). Figure 4.5 illustrates two of the several types of diterpenes produced by *Bifurcaria bifurcata*.

In Dictyotales species, the vast majority of terpenes are cyclic diterpenes generally classified into three groups: xenicanes, dolabellanes and dilophanes. French teams isolated some diterpenes from *Dictyota* species (Viano *et al.*, 2009, 2011).

Many diterpenes have been demonstrated to possess antimicrobial and antifungal (Enoki *et al.*, 1983; Tringali *et al.*, 1985), antifeedant (Hay and Steinberg, 1992; Paul *et al.*, 2001; Vallim *et al.*, 2005), antifouling (Schmitt *et al.*, 1998; Barbosa *et al.*, 2007; Viano *et al.*, 2009), antitumour (Di Guardia *et al.*, 1999), antiviral (Pereira *et al.*, 2004) and cytotoxic (Kolesnikova *et al.*, 2006) activities.

Sesquiterpenes consist of three isoprene units and have the molecular formula  $C_{15}H_{24}$ . The sesqui- prefix means one and a half. Some sesquiterpene compounds were isolated from ether extracts of *Dictyopteris* species (Kurata *et al.*, 1996; El Hattab *et al.*, 2007).

Meroditerpenes, as presented before, are constituted of two parts: (1) a terpenic part which present a high variability, from hemiterpenes (only one isoprenic unit) to diterpenes; with linear or cyclic structure and (2) an aromatic part often constituted by quinone and sometimes by carboxylic acids or methoxylated derivated quinones (Kornprobst, 2005). These particular compounds can be isolated from Fucales (meroditerpenes) and Dictyotales (merosesquiterpenes and meroditerpenes) species.

#### Biological functions, variation and examples of French studies

Geographical variations in the diterpenoid composition of *Bifurcaria bifurcata* was highlighted by Valls *et al.* (1993a, 1995), Culioli *et al.* (1999a,b, 2000, 2001) and El Hattab *et al.* (2008). These studies revealed that the polar fraction of the lipid extract of this species collected from the Moroccan coast (Oualidia part), was clearly distinguishable from the extracts obtained from other zones of collection (Spanish, French, and Irish Atlantic coasts). Moreover, most

recent studies of the polar fraction of the crude extract obtained from two specimens of *B. bifurcata* collected at Oualidia (Morocco) and Quiberon (Atlantic coast of southern Brittany), respectively, confirm this chemical characteristic (Culioli *et al.*, 2004; Ortalo-Magné *et al.*, 2005).

Valls *et al.* (1995) showed the existence of chemical types in *Bifurcaria bifurcata* from French coasts. Type I is characterized by the presence of Eleganolone as the main diterpene while type II presents Bifurcane (Figure 5) as the main diterpene. Types I and II depend on biotic and abiotic factors and can be isolated from specimens of *B. bifurcata* collected in two geographical areas (Valls *et al.* 1995): type I isolated from St Brieuc and Lorient and type 2 isolated from Roscoff. Further investigations will be interesting to study the cause of this chemical variability within *Bifurcaria bifurcata*.

Concerning meroditerpenes which can be found in brown algae, we can cite the work of Mokrini *et al.* (2008) who isolated meroditerpenes from *Cystoseira baccata*. Some of them presented an antimicrobial activity against only one strain (*E. gayraliae*), and also promising antimicrobial and anti-invertebrate activities. Moreover, none of the meroditerpenes tested showed significant toxicity toward larvae of sea urchins (*Echinus esculentus*) or oysters (*Crassostrea gigas*) ( $LC_{50} > 100 \mu\text{g/mL}$ ) (Mokrini *et al.*, 2008).

## 4.6 The use of metabolites from marine red and brown algae for their chemical defense

### 4.6.1 Biotic interactions of marine red and brown algae (pathogens, grazing, etc.)

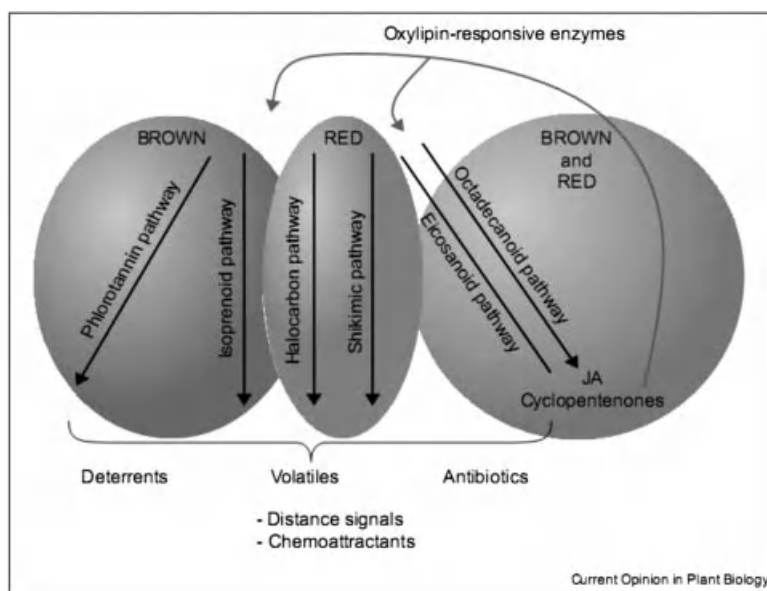
Research in marine chemical ecology continues to focus on predator–prey, competitive interactions, settlement cues, and potential defenses against infection by microorganisms. Both autotrophic sessile terrestrial organisms and marine plants are continuously being challenged by microorganisms (including viruses, bacteria, fungi, and other algae) and by grazers, which may cause wounds and diseases. Competition to find appropriate substrates for the settlement of propagules is of particular importance in the sea. Hence, epiphytism, which reduces the availability of light and increases hydrodynamic drag, provides a permanent threat. To survive in such a dangerous environment, marine plants obviously had to evolve defenses such as the production of chemical deterrents (Hay, 1996; Potin *et al.*, 2002). Studies of the defense responses of marine plants against parasitism and grazing at various biological levels (i.e. molecular, cellular, within individuals, within populations and community-wide), are necessary to determine the

specific rules that govern the biotic interactions of plants in the marine environment, and to address general questions such as how immunity evolved in eukaryotes. Due to the wide range of competitive environments they survive in, marine organisms have developed unique defense strategies and bioactive compounds that, in some cases, are unparalleled by their terrestrial counterparts (Barros *et al.*, 2005).

The Station Biologique de Roscoff (SBR) is engaged in a number of genome sequencing projects as the leading partner, including the first genome for the multicellular brown alga, *Ectocarpus siliculosus* (Cock *et al.*, 2010), a close relative of large kelp species. Both projects were supported by BiogenOUEST, GIS Europole Mer, National GIS Institut de la Génomique Marine and European level. These recent efforts, focused on acquiring and analyzing seaweed genome sequences, have generated a considerable amount of data that provide an in-depth, comprehensive view of the carbohydrate metabolism of brown seaweeds (Michel *et al.*, 2010ab). The *Chondrus crispus* genome and the upcoming *Porphyra* genome will provide access to the genes involved in these novel functions. There are also other red algae for which full or near-full genome sequences have been secured; these genomes include those of the Atlantic nori species *Porphyra umbilicalis* (Gantt *et al.*, 2010) and the coralline red alga *Calliarthron* sp.

Potin *et al.* (2002) indicate that marine plants are not passive participants in biotic interactions, and that they can actively alter their susceptibility to various attackers. Although a large body of evidence to show that algae are endowed with chemical defenses has been available for some time, the idea that many of these defenses are induced following challenges by bioaggressors has emerged only recently. Potin *et al.* (2002) summarize biosynthetic pathways by a model of the integration that results in the production of defense metabolites and putative distance signals in marine algae (Figure 4.6). Oxylipins are thought of as endogenous chemical signals that induce the production of phlorotannins in fucoid algae in response to grazing. The isoprenoid pathway is also activated in response to grazing in brown algae. In red algae, the shikimic pathway and both the octadecanoid and the eicosanoid pathways are activated by elicitor treatment, and are responsive to C20- and C18-fatty-acid-derived oxylipins. In fucoid brown algae, waterborne or airborne cues that induce phlorotannin accumulation may derive from a lipoxygenase pathway.

Alternatively, they may be composed of a mixture of volatile or non-volatile compounds originating from several biosynthetic pathways. The role of these volatile compounds as chemoattractants for gametes is well established, but the nature of the warning distance signals is not yet elucidated. The function of these bioactive compounds and the



**Figure 4.6** Model of the integration of the biosynthetic pathways that result in the production of defense metabolites and putative distance signals in marine algae (Potin *et al.*, 2002).

nature of specific pathways involved in their synthesis have hardly been investigated in marine algae, which emerged as independent lineages early in the evolution of eukaryotes (Gaquerel *et al.*, 2007).

A good illustration of the biosynthetic pathways concern the example of association in which the host is the parenchymatous red alga *Chondrus crispus*, a common species in the low intertidal zone of North Atlantic rocky shores, and the pathogen a filamentous green alga, *Acrochaete operculata* provides a natural model of compatible and incompatible interactions that is based on the life-history phase of the host. *C. crispus* has an isomorphic life history, in which the haploid gametophytic and diploid sporophytic generations differ only by minor traits, such as the sulfate-ester group distribution of their matrix polysaccharides,  $\kappa$ - and  $\lambda$ -carrageenans. Few spots of infection were observed on the thallus surface of the gametophytic fronds, whereas zoospores settled at high densities at the surface of sporophytic fronds. Recognition by the pathogen of the carrageenan oligomeric degradation products of the host cell walls governs this interaction. Irrespective of the pathogen behavior, however, the first line of defense of the red-algal host is recognition of the attacker (Potin *et al.*, 2002). More recently, Bouarab *et al.* (2004) continued the study with *C. crispus*. These authors demonstrated that *C. crispus* uses both oxygenated derivatives of C18 (octadecanoids) and C20 (eicosanoids) PUFAs as developmental or defense hormones. A pathogen extract triggers in *C. crispus* a cascade

of fatty acid oxidations leading to the synthesis of several eicosanoids and octadecanoids including precursors of jasmonic acid (JA). However, attempts to identify jasmonic acid in *C. crispus* cell homogenates have remained unsuccessful. Furthermore, treatments of the red alga with fatty acid hydroperoxides and MeJA upregulated defense enzyme activities, such as fatty acid oxygenases, but also PAL and shikimate deshydrogenase, potentially involved in the secondary metabolism leading to an induced resistance to the pathogen attack. In 2007, Gaquerel *et al.* provide a detailed kinetic study by LC-MS profiling of the *in vivo* activation of fatty acid oxidative cascades by exogenous MeJA at various concentrations and of the consecutive upregulation of defense responses. These authors demonstrate that methyl jasmonate (MeJA) triggers a cascade of oxidation of PUFAs leading to the synthesis of prostaglandins and other oxygenated fatty acids. As a result of a lipoxygenase-like activation, MeJA induces a concomitant accumulation of 13-hydroxy-9Z, 11E-octadecadienoic acid (13-HODE) and 13-oxo-9Z, 11E-octadecadienoic acid (13-oxo-ODE) in a dose-dependent manner in *C. crispus*. Furthermore, MeJA increases the level of mRNA encoding a glutathione S-transferase and induces the Activity of a new enzyme catalyzing the region- and stereo-selective bisallylic hydroxylation of polyunsaturated fatty acids from C18 to C22. The enzyme selectively oxidized the omega 7 carbon position ( $\omega$ -7) and generated the stereo-selective (*R*)-hydroxylated metabolites with a large enantiomeric excess. The enzyme

specificity for the fatty acid recognition was not dependent on the position of double bonds but at least requires a methylene interrupted double bond 1,4-pentadiene motif involving the  $\omega$ -7 carbon.

In the case of brown macroalgae, *Laminaria digitata* was the most studied species in terms of associated bacterial flora at the Marine Station of Roscoff. Corre and Prieur (1990) showed that *L. digitata* is able to control its bacterial flora. They highlighted, using electron microscopy and a microbiological approach, that the density of bacteria varied in relation to the part of the algae with a maximal density in the meristematic part and a decrease of the density to the median and apical parts of the thallus (Corre and Prieur, 1990). Some seasonal variations were also demonstrated with a high density during summertime for the meristematic part of the thallus. The bacterial flora present at the surface of algae is mainly composed of Gram-negative bacteria (Corre and Prieur, 1990) with coccoidal forms in the meristematic area and bacilli forms on the surface of the old part of the thallus.

Over the last decade, numerous studies involving brown algal models observed oxidative bursts in response to oligosaccharide elicitors, in most cases to control the growth of bacterial biofilms (Küpper *et al.*, 2001, 2002; Weinberger and Friedlander 2000a, b; Weinberger *et al.*, 1999) or the attack of eukaryotic endophytes (Küpper *et al.*, 2002).

As an example of a study, Kupper *et al.* (2001) showed that sporophytes of the brown algal kelp *L. digitata* respond to incubation in the presence of alginate oligosaccharides by a sudden increase in oxygen consumption, concomitant with a marked release of hydrogen peroxide. The hydrogen peroxide concentrations generated in the surroundings of the algae appear sufficient to exert an inhibitory effect toward potentially harmful micro-organisms. Other defenses may involve the oxidation of intracellular iodide, leading to the release of toxic, iodinated compounds (Potin *et al.*, 1999; Ar Gall *et al.*, 2004), fatty acids or methyl jasmonate (Kupper *et al.*, 2009) or aldehydes (Goulitquer *et al.*, 2009). Nevertheless, the role of iodine as potential defensive compounds is not sure as demonstrated by Kupper *et al.* (2008) who showed that iodine is stored by algae under its inorganic form in the extracellular matrix of cells from *L. digitata*.

In this last study, authors showed that *L. digitata* is able to produce aldehydes under biotic (elicitation with GG) and abiotic (exposition to copper and increase of UV, temperature and salinity) stresses. More recently, Kupper *et al.* (2009) reported for the first time an oxidative burst in an algal system induced by free fatty acids (arachidonic and linolenic acids).

The exploration of this chemical diversity for pharmaceutical and cosmetic purposes has revealed important chemical prototypes for the discovery of new agents, stim-

ulating the use of sophisticated spectroscopic methods and development of new synthetic methodology (Kornprobst, 2005; Mayer 2002; Mayer and Hamann 2004, 2005; Mayer *et al.*, 2007; Ioannou and Roussis, 2009; El Gamal, 2010). Since 1960, till today more than 15,000 novel compounds have been isolated from marine organisms (Cardozo *et al.*, 2007). Modern analytical techniques for the characterization and quantification of macroalgal chemical defenses were reported by La Barre *et al.* (2004). Several comprehensive reviews of natural products and the chemical ecology of macroalgae have been published recently and the medicinal and pharmaceutical uses of natural seaweed products that show the broad range of bioactivities of macroalgal metabolites has been reviewed by Smit (2004).

## 4.6.2 Biofouling

### Context

Engineered structures such as ships and marine platforms, as well as offshore rigs and jetties, are under constant attack from the marine environment. These structures need to be protected from the influences of the key elements of the marine environment such as saltwater, biological attack and temperature fluctuations. The settlement and accumulation of marine organisms on an inanimate substrate can cause a large amount of difficulties to engineered structures. In heat exchangers, biofouling can clog systems and on ship hulls it can increase the hydrodynamic drag, lowering the maneuverability of the vessel and increasing the fuel consumption. This leads to an increase in costs within the shipping industry through the increased use of manpower, fuel, material and dry docking time (Abarzua and Jakubowski, 1995; Chambers *et al.*, 2006).

The process of biological fouling is often grouped in the literature into key growth stages which include an initial accumulation of adsorbed organics, the settlement and growth of pioneering bacteria creating a biofilm matrix and the subsequent succession of micro and macrofoulers (Wahl, 1989; Abarzua and Jakubowski, 1995; Yebra *et al.*, 2004). Methods for inhibiting both organic and inorganic growth on wetted substrates are varied but most antifouling systems take the form of protective coatings. Unfortunately, operational profiles vary; hence the application of one universal coating to ship hulls is unlikely and specific coatings designed for the particular needs of certain exposure and operational profiles may need to be targeted individually. Antifouling paints have been widely used to prevent and control biofouling and, therefore, their economic, as well as ecological relevance, is unquestionable. Among the several antifouling biocides used until now, tributyltin (TBT)-based coatings, introduced in the early 1970s,

are the most effective and successful at reducing biofouling due to their high toxicity to fouling organisms. However, deleterious consequences derived from TBT environmental stability and extreme toxicity to non-target organisms was soon detected, with ecotoxicological effects such as imposex and decreased reproductive viability in gastropods, or increased shell thickness in oysters (Alzieu, 2000; Evans *et al.*, 2000; Bellas, 2006, 2007, 2008). These environmental problems resulted in the development of legislation by the International Maritime Organization (IMO) to ensure the international prohibition of the application of organotin-based coatings on ships by 1 January 2003, and a complete prohibition of the presence of organotin compounds in antifouling systems by 1 January 2008 (IMO Resolution A. 895 21, 25/11/1999). The Regulation 782/2003 of the European Parliament also prohibits the use of organotin compounds on ships registered in the European Union with the same enforcement dates. As a result of the existing legislation, an intense effort has been devoted to the development of alternative biocides. The global phase out of organotin-based antifouling coatings requires the development of environmentally acceptable antifouling compounds which maintain the same efficiency against fouling as TBT, often by including the use of booster biocides, chemicals added to antifouling paints to improve their overall efficacy. Several booster biocides (e.g. Irgarol 1051, Diuron, copper thio-cyanate and Tolyfluanid) have thus been approved for use during the last few years and replaced TBT in antifouling products. However recently, some of the booster biocides have also been banned or their use regulated in Europe because of their environmental persistence and toxicity to non-target organisms (Thomas *et al.*, 2002; Voulvoulis *et al.*, 2002; Konstantinou and Albanis, 2004; Bazes *et al.*, 2008; Maréchal and Hellio, 2009), thus stressing the need to perform adequate risk assessment procedures for antifouling biocides. The recent restrictions on the use of traditional toxic antifouling paints, also evidenced a growing need for new alternative compounds that ensure good performance without polluting the marine ecosystem (Pérez *et al.*, 2006).

### Organisms and biofouling

The biomimetics approach implies the use of the natural world as a model on which to base an engineering development. Marine organisms have been shown to use both physical and chemical methods to protect themselves from biofouling (Bakus *et al.*, 1986; Davis *et al.*, 1989; Steinberg *et al.*, 1998; Fusetani, 2004; Bazes *et al.*, 2006). Since many sessile marine organisms have developed efficient defense mechanisms against microbial epibionts, there is an increasing interest in such organisms as a source of naturally released antifouling substances Ioannou and Roussis, 2009;

Maréchal and Hellio, 2009). Seaweeds are known to produce inhibitory allelochemicals interfering with epiphytes and other epibionts, competing for light, nutrients and/or space (Le Gal, 1988; Harlin, 1996; Ioannou and Roussis, 2009). Allelopathy was defined to include all biochemical interactions among higher plants and microorganisms, both stimulatory and inhibitory actions (Potin *et al.*, 1999). Furthermore, allelopathic compounds are often defined as chemical agents produced by one organism to affect the health, growth, behaviour, or population biology of organisms of other species (Engel and Pawlik, 2000).

Algae produce a wide range of secondary metabolites which have shown biological activities against bacteria, fungi, diatoms, macroalgae, mussels and barnacles (De Nys *et al.*, 1995; Devi *et al.*, 1997; Hellio *et al.*, 2000a,b; Da Gama *et al.*, 2002). Secondary metabolites which can function in multiple defensive roles (Engel and Pawlik, 2000) are often extracted in combination with organic solvents, such as dichloromethane, methanol or ethanol (De Nys *et al.*, 1995; Da Gama *et al.*, 2002). The production and secretion of allelochemicals by aquatic macrophytes could be an effective defense strategy against other photosynthetic organisms competing for light and nutrients. Although bacteria do not compete with macroalgae for light or most nutrients, they may enhance the attachment of primary producing microalgae (Silkina *et al.*, 2009).

### *Antifouling activities from French red seaweed compounds Screening In red macroalgae (Université de La Rochelle, Muséum National d'Histoire Naturelle)*

Thirty marine algal species (14 Rhodophyta) were collected in April 1998 from the West Coast of France (Concarneau bay, Brittany) (Table 4.3).

Extractions of compounds were performed as previously described (Hellio *et al.*, 2000a), resulting in aqueous (A), ethanolic (B) and methylene chloride (C) extracts. The extracts were tested in laboratory assays against species which are representative of major groups of fouling organisms: Thirty five marine bacteria strains obtained from the Culture Collection of the LUBEM (Laboratoire Universitaire de Biodiversité, d'Ecologie et de Microbiologie, Quimper) from Université de Bretagne Occidentale (Hellio *et al.*, 2001). Test organisms were collected from various substrates, from the tidal zone, in the Glénan Islands (Brittany, France); five strains of marine fungi (unidentified marine fungi F1 and F2 respectively from sand collected from Malaysia and from driftwood collected from the Fleet estuary, United Kingdom, *Corollospora maritima* (F3), *Lulworthia* sp. (F4) and *Dendryphiella salina* (F5) isolated from driftwood collected respectively from Dinas (Wales, United Kingdom), Denmark and Galway, Ireland), benthic

**Table 4.3** Macroalgae identification and location

Class	Family	Name	Exposition	Zonation
Rhodophyceae	Ceramiaceae	<i>Bornetia secundiflora</i>	S	L
		<i>Ceramium rubrum</i>	S	M
		<i>Griffithsia flocculosa</i>	S/E	L
		<i>Halurus equisetifolius</i>	S/E	M
		<i>Plumaria elegans</i>	E	L
	Delesseriaceae	<i>Cryptopleura ramosa</i>	S/E	L
		<i>Delesseria sanguinea</i>	E	L
	Dumontiaceae	<i>Dilsea carnosa</i>	E	M
	Gelidiaceae	<i>Gelidium latifolium</i>	S	L
	Gigartiniaceae	<i>Chondrus crispus</i>	E	M
		<i>Gigartina stellata</i>	S	M
	Palmariaaceae	<i>Palmaria palmata</i>	S/E	L
	Rhodomelaceae	<i>Laurencia pinnatifida</i>	E	M
		<i>Polysiphonia lanosa</i>	S	M

S: Sheltered shore; E: exposed shore; S/E: sheltered and exposed shore; U: upper shore; M: medium shore; L: low shore.

microalgae (*Amphora coffeaformis*, *Chlorococcum submarinum*, *Cylindrotheca closterium*, *Dunaliella tertiolecta*, *Micromonas pusilla*, *Phaeodactylum tricornutum*, *Porphyridium cruentum*, *Pyramimonas amyliifera*, *Rhodella maculata*, *Rhodorus marinus*, *Tetraselmis levis*, and *Tetraselmis* sp.) and against the adhesion of the spores of two species of macroalgae (*Enteromorpha intestinalis* and *Ulva lactuca*) and the zygotes of *Sargassum muticum*. The organisms chosen are representative of the algal fouling community. Toxicity was evaluated against the larvae of oysters and sea urchins. The activity of several extracts was comparable to that of heavy metals and biocides (such as TBTO and CuSO<sub>4</sub>) currently used in antifouling paints. We obtained 90 extracts from 30 marine seaweeds and tested them on 82 bioassays (78 biofoulers and 4 toxicity variables).

This work was very largely published (Hellio *et al.*, 2000a,b,c, 2002a,b; Hellio, 2001). Starting from these results, a research project presented by Bergé *et al.* (2002) aimed to establish, by the statistical treatment, a global screening procedure on a laboratory scale including the most representative biofoulers for the isolation of biogenic compounds devoid of toxicity and which will be able to repel colonizers. Statistical analysis were then realised on those 7380 results. All the variables on which none algal extracts were found active were removed, the remaining ones were thus standardized before any statistical analysis.

Starting from 78 bioassays associated to the different living epibionts found in the fouling community and 4 biotests related to toxicity, the author has reduced them, by

statistical treatments, to 14 variables for biofouling and one for toxicity while minimizing information losses.

- **Primary colonizers:** evaluation of the activity against three microalgae (*Micromonas pusilla*, *Pyramimonas amyliifera*, and *Dunaliella tertiolecta*), three marine bacteria (MB2, MB18, and MB32) 1 terrestrial bacteria (*Staphylococcus aureus*) and 1 marine fungus (*Dendryphellia salina*).
- **Secondary colonizers:** fixation rate of the four macroalgae (*Enteromorpha intestinalis*, *Sargassum muticum*, *Polysiphonia lanosa*, and *Ulva lactuca*).
- **Tertiary colonizers:** barnacles (*Balanus semibalanoides*) and the phenoloxydase activity of *Mytilus edulis*.
- **Toxicity:** Cell viability (3T3 cells) and evaluation of lysosomal activity.

By statistical treatment, the first result that we can note is that no extract was found to be active against all the biofoulers selected, which indicates specific action against particular epiphytes rather than global antifouling action. Second, as previously noticed (Steinberg, 2001), many chemically rich seaweed do not appear to use natural products such as antifoulants (no activity detected for any extracts). Indeed, it is well known instead of chemical methods, macroalgae can employ physical methods such as continuous shedding of the outer layer of cells and mucilaginous covering and the continuous erosion of the distal ends of

blades. Water turbulence (exposed shore) and abrasion also limit fouling (Armstrong *et al.*, 2001). Third, among those most active extracts, none was an aqueous one, which is in accordance with a part of the hypothesis formulated by (Steinberg, 2001) that natural antifoulants in seaweeds will most commonly be non-polar metabolites (Bergé *et al.* 2002).

Nowadays, crude chemical extracts of marine organisms or specific metabolites that deter or kill fouling organisms in laboratory assays have been obtained from a broad range of marine organisms. However, one major difficulty with interpreting the vast majority of these studies (including ours) is that rarely is any information provided on the concentration of metabolites at or near the surface of the host organisms. (De Nys *et al.*, 1998). Thus in accordance with these authors, we can say that although an extract or metabolite may be active at low concentrations, unless it can be demonstrated that those concentrations are present *in situ* or near the surface of the host, an antifouling function cannot be inferred. Due to such considerations, it seems hazardous to interpret our results through a biochemical point of view or through a chemical–ecology framework. However, in addition to the establishment of a global screening procedure for antifouling compounds, this work has given precious indications, notably by selecting seaweeds potentially rich in antifouling products.

Identification of the bioactive compounds is currently in progress. Afterwards, methods will be developed for quantifying them at or near the relevant surfaces of algae in order to verify if they are really antifoulants.

The main advantage of this new method is its representatives of the biofilm formation occurring on a temperate immersed area. Moreover, most of the biotests selected require only small amounts of products which are of great interest for screening natural compounds. In addition, due to the main use of *in vitro* tests, the screening can be realised all year long and with a high degree of sensibility. With such a procedure, one can choose to focus on globally antifouling products (those active on the 3 different types of biofoulers) or rather to look for specific actions, such as the inhibition of microfouler establishment or larval settlement. At least, this methodology would lead to the selection of non-toxic active molecules due to the high sensitivity of the toxicity biotest included.

In 2001, this team continued research with a red alga *Ceramium botryocarpum* (Ceramiales, Rhodophyta) which appeared spontaneously in the tanks of culture of *Chondrus crispus* (Gigartinales, Rhodophyta) - *Odonthella aurita* (Diatomophyceae, Heteroconta), produced by the SME Innovalg (France) (Figure 4.7).

During a first experimental cycle, it was observed that, contrary to the pilot tank, the walls and the propellers re-

mained clean of any exogenic algae colonization. The presence of *Ceramium* seems to limit the establishment of opportunist green algae (*Cladophora*, *Bryopsis* or *Ulva*) and the colonization of barnacles usually disturbing the cultures (Figure 4.7). This ecological phenomenon could reveal the release of allelopathically active compounds interfering with settlement and growth of competitors. From these ecological observations it seemed interesting to look for the substances responsible for these effects and to test their effectiveness against marine organisms (Bazes *et al.*, 2008). In order to demonstrate the allelopathic activity of substances released by *C. botryocarpum*, the activities of extracts with different polarity (ethanol, methanol, and dichloromethane) have been tested against representative marine species, such as marine bacteria (*Vibrio* sp., *Pseudovibrio denitrificans*, Rhodobacteraceae bacterium R11 A), phytoplankton (diatom species *Fragilaria pinnata* and *Cylindrotheca closterium*), and spores of macroalgae (*Ulva* sp.). Analysis of the seasonal activity of extracts shows no real variation during a year. The extracts from October strongly inhibit the development of marine bacteria, phytoplankton and spores of *Ulva* since 25 µg/ml.

The macroalgal compounds affected the growth and the pigment content of the diatoms. The mechanism by which the extracts act as antifouling agents relies on the alteration of the photosynthetic apparatus, as evidenced by pigment changes and PS II inhibition (Silkina *et al.*, 2009). The efficiency of the macroalgal extracts was lower than that of Diuron, but their influence reversible, hence they represent an environmentally acceptable alternative to toxic products for the control of fouling organisms (Bazes *et al.*, 2006, 2008; Silkina *et al.*, 2009).

The study of Silkina *et al.* (2009) was to determine their efficiency in comparison to booster biocides, by comparing the potential toxicity of some of the most frequently used alternative antifouling biocides (Diuron, Copper thiocyanate and Tolyfluanid). Bioassays were conducted with three diatom species: *F. pinnata* and *C. closterium*, which are well-known fouling species (Bellas, 2006; Silkina *et al.*, 2009), and *Thalassiosira pseudonana*, a non-target common species of marine phytoplankton, in order to provide relevant information on the different compounds regarding the evaluation of the risk to the marine environment. From the biological responses and the sensitivity of these diatoms exposed to the different biocides, the antifouling capacity of the tested compounds is discussed, as well as their potential impact to the aquatic environment. Three unialgal diatom cultures of *Fragilaria pinnata*, *Cylindrotheca closterium* and *Thalassiosira pseudonana* were exposed to several concentrations of the various biocides and natural extracts during a period of 72 h. Algal growth inhibition tests, and the irreversible growth effects were determined, and



Bassins de culture (cliché du 012/07/2002):  
 1403A: culture de *Monostroma* sp. - Bassin inoculé le 09/10/2001 (2 nettoyages)  
 1403B: culture de *Ceramium* sp. - Bassin inoculé le 16/10/2001 (aucun nettoyage)

**Figure 4.7** Culture of the red macroalga *Ceramium botryocarpum* in tanks (Innovalg).

the responses of the different diatoms exposed to the macroalgal extracts were compared to the booster biocides. This study shows that whatever the biological response chosen (growth rate, biomass or chlorophyll *a* content), all these parameters were inhibited by the booster biocides and the macroalgal extracts. The inhibition effect of the four extracts and three booster biocides, applied separately, was as follows, by increasing order of EC<sub>50</sub> values: Diuron > Tolyfluanid > Copper thiocyanate > A extract of *S. muticum* > A extract of *C. botryocarpum* > B extract of *S. muticum* > B extract of *C. botryocarpum*. Among the three diatom species tested, *F. pinnata* was the most sensitive, and *T. pseudonana* the less sensitive diatom, whatever the compounds. Results of the present work validate the use of natural macroalgal extracts as antifouling compounds, which thus represent valuable environment-friendly alternatives for new antifouling coatings.

**Conclusion:** These results confirm the presence of allelopathic substances in seaweeds and in particularly in *C. botryocarpum*. These extracts might be employed to fight bacterial and algal fouling organisms in the aquaculture industry. Compared with toxic biocides commonly used in antifouling paints, natural extracts of *Ceramium* present no cytotoxicity. Incorporated in antifouling coatings and immersed *in situ*, dichloromethane extracts from October samples of *C. botryocarpum* prevent the development of fouling organisms for two months.

#### *Antifouling molecules extracted from the invasive Grateloupia turuturu from France*

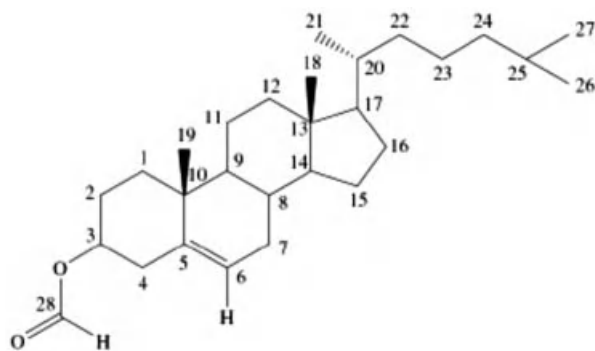
*Grateloupia turuturu* Yamada (Halymeniaceae, Rhodophyta) is a non-native alga that occurs along the coast of Brittany. This alga is rarely epiphyted, thus it represents an interesting model to study a potential antifouling defense. To valorise this large biomass, interesting antifouling biomolecules, polar and apolar metabolites were isolated.

Concerning the polar part of the algae, both isethionic acid and floridoside, extracted from *Grateloupia turuturu* present an antisettlement activity against the cyprid larvae of the barnacle *Balanus amphitrite* (Hellio *et al.*, 2004).

Following a screening carried out in *Grateloupia turuturu* (Plouguerné *et al.*, 2008) and the antifouling activity of a dichloromethane extract, a search for the antifouling molecule began.

We show here a procedure which permits the isolation of antimicrofouling compounds from *Grateloupia turuturu*.

Fresh material (17 kg) was ground and then extracted using CH<sub>2</sub>Cl<sub>2</sub> at room temperature. Solvent was removed *in vacuo*. Dried extract was washed using EtOAc/H<sub>2</sub>O (1/1). Three extractions were done using EtOAc, the resulting fraction was then evaporated. The extract (10.41 g dry weight) was separated by SiO<sub>2</sub> liquid chromatography (gradient elution Hexane/EtOAc) to yield 20 fractions each of 200 ml.



**Figure 4.8** Cholesteryl formate isolated from a dichloromethane extract from *Grateloupia turuturu* (Plouguerné *et al.*, 2006).

TLC examination of these fractions permitted us to select two fractions for further experiments.

The structure of the isolated compound (cholest-5-en-3-ol formate or cholesteryl formate) was elucidated by the analysis of high resolution proton and carbon nuclear magnetic resonance (NMR) data as well as by mass spectroscopic techniques (Figure 4.8). Cholesteryl formate was obtained as a white solid. Structural elucidation of compound 1 was achieved by the analysis of its NMR and MS spectral data. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound 1 were very similar to those of the cholesteryl acetate (Wilson *et al.*, 1996) except for the signals of the formate. Mass spectroscopy performed on a JEOL JMS DX-303 mass spectrometer indicated that compound 1 had the molecular formula  $\text{C}_{28}\text{H}_{46}\text{O}_2$ . The EIMS spectrum exhibited characteristic peaks at  $m/z$  414 (cholesteryl formate) and 368 (cholesterol). Detailed analysis of the NMR data demonstrated that apart from the formate signals, the rest of the signals correlated well with those of the cholesteryl group. From all the evidence mentioned above, the structure of compound 1 was elucidated as cholesteryl formate ester.

Sterol profiles have been shown to present specific features that can be used for the chemotaxonomic classification of algae. Cholesterol is usually the main sterol found in Rhodophyceae. In this study, cholesteryl formate was isolated for the first time in the red alga *Grateloupia turuturu*.

This pure molecule was tested at several concentrations on fouling bacteria and at a concentration of 0.75 g/l, cholesteryl formate inhibited the growth of *Vibrio aestuarianus* and *Polaribacter igensisii* (Plouguerné, 2006).

Previous work on lipids of *G. turuturu* is limited, and whether they too exhibit a bioactive function is unknown. Hotimchenko (2002) studied the variation of *G. turuturu* fatty acid components and highlighted a relation between such variation and an adaptative response of the alga to illumination. Moreover, cholesterol and demosterol have been identified as the major sterols in several Rhodophyta; more-

over, the brassicasterol in *Goniotrichum elegans*; it was the first time that a  $\text{C}_{28}$  sterol was identified as the major sterol present in a red alga (reviewed by Plouguerné *et al.*, 2006b).

#### Antifouling activities from brown seaweed compounds

Following a screening carried out in the invasive *Sargassum muticum* (Plouguerné *et al.*, 2008) and the finding of antimicrofouling activity of a chloroform extract, purification of the antifouling molecule(s) started with a control of the antifouling activity at each step of the purification process, using a concentration of 0.75 g/l for each fraction.

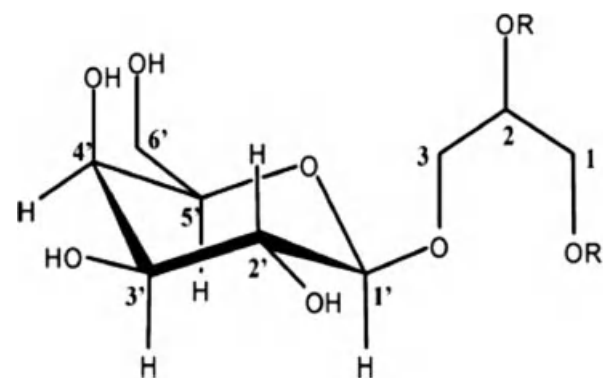
The fouling organisms tested were the following:

- bacteria: *Halomonas marina* (ATCC 25374), *Pseudoalteromonas elyakovii* (ATCC 25374), *Shewanella putrefaciens* (ATCC 8071), *Vibrio pomeroyi* (CAIM 578).
- microalgae: *Exanthemachrysis gayraliae* (AC 15), *Pleurochrysis roscoffensis* (AC 32), *Cylindrotheca closterium* (AC 170), *Navicula jeffreijii* (AC 181).

The purification of the chloroform extract from the brown invasive macroalga *Sargassum muticum*, through a series of chromatographic separations, yielded 12 fractions that were tested against strains of bacteria, microalgae and fungi involved in marine biofilm formation.

The chemical composition of four out of the six fractions that exhibited anti-microfouling activity was investigated. Fraction a contained saturated and unsaturated linear hydrocarbons (C12-C27).

Arachidonic acid was identified as the major metabolite in fraction c whereas fraction g contained mainly palmitic, linolenic and palmitoleic acids. Fraction k was submitted to further purification yielding fraction kAcaF1e that was composed of galactoglycerolipids (Figure 4.9), active against the growth of the two bacterial strains



**Figure 4.9** Galactoglycerolipids isolated from a chloroform extract from *Sargassum muticum* and active against two bacterial strains. R and R': fatty acid esters on the glycerol (Plouguerné *et al.*, 2010)

(*Shewanella putrefaciens* and *Polaribacter irgensii*) and all tested fungi.

These promising results show the potential of the huge biomass of *S. muticum* as a source of new environmentally friendly antifouling compounds. Alien species produce a full range of bioactive compounds or compounds with a wide range of activity that could be expressed in a context of colonisation processes as was shown by Maréchal and Hellio (2011) who studied the antifouling activities from extracts of numerous native and invasive macroalgal species from Brittany.

## 4.7 The use of metabolites as chemomarkers for taxonomy

In this paragraph, we discuss only the chemotaxonomy of brown macroalgae, since examples from French teams were only published for this group of seaweeds.

The taxonomy of the Phaeophyta still has some controversial points. The morphological species concept is still used as a basis for species-level and intraspecific studies in macroalgae (Wattier and Maggs, 2001). However, classification of algae exclusively by morphological criteria can be problematic because of morphological plasticity in response to environmental conditions (de Senerpont Domis *et al.*, 2003; Yotsukura *et al.*, 1999), such as light intensity, wave action, together to the position of the algae in the water column. Investigations into the chemistry of a number of taxa have given some credence to these concerns. In particular, the isolation of a series of terpenes has been used to argue for the reassignment of some species to closely related genus (Amico, 1995; Valls and Piovetti, 1995; Vallim *et al.*, 2005). Authors gave an ongoing effort to gain a more complete understanding of the chemistry of genera and tried to use the chemical information to distinguish individual species and establish an overall biochemical picture from which an evolutionary lineage in the genus may be inferred.

Among brown seaweeds, difficulties have been reported for the genera *Fucus* (Kalvas and Kautsky, 1998; Sideman and Mathieson, 1985), *Dictyota* (Teixeira *et al.*, 1990; Vallim *et al.*, 2005), *Turbinaria* (Rohfritsch *et al.*, 2007; Le Lann *et al.*, 2008) and *Cystoseira* (Draisma *et al.*, 2010; Jegou *et al.*, 2010). Chemotaxonomic approaches used from French teams to differentiate species within some genera were carried out, at the species level on the three following genera *Bifurcaria*, *Cystoseira* and *Turbinaria*.

The genus *Cystoseira* is composed of 47 validated species (Guiry and Guiry, 2010). Despite the recent and growing number of scientific publications focusing on some specific taxa, their classification remains unclear. Most of the chemical studies on *Cystoseira* species have been led on exclusively

Mediterranean taxa and/or in Mediterranean sites (Amico 1995; Valls *et al.*, 1995).

In Brittany, 5 species co-occurred along the coast: *Cystoseira baccata*, *C. foeniculacea*, *C. humilis*, *C. nodicaulis* and *C. tamariscifolia*, for which many taxonomical changes have occurred since their first description. As a consequence, numerous synonymous samples are recorded for each taxon (Guiry and Guiry, 2010; Dizerbo and Herpé, 2007). Misclassification due to the high morphological variability of these taxa, observed throughout space and time, and linked with multiple environmental conditions as described for the genus *Sargassum* (Mattio *et al.*, 2008) could explain these taxonomic ambiguities. An original procedure was carried out to distinguish and classify species of *Cystoseira* encountered in Brittany. For this, two innovative analytical techniques were used: liquid chromatography–electrospray ionization multiple-stage mass spectrometry (LC/ESI-MSn) to analyze lipophilic extracts, and *in vivo*  $^1\text{H}$  HR-MAS NMR to observe the global chemical profile of each taxon. In parallel, a molecular procedure using sequences of the ITS2 (nuclear DNA) commonly informative in phylogenetic studies within the Fucales, and especially in Sargassaceae species (Yoshida *et al.*, 2000; Stiger *et al.*, 2000, 2003) was obtained for each species of *Cystoseira* and used as a reference step to validate and discuss the results of both analytical methods (Jegou *et al.*, 2010). The power of both techniques was evaluated following two major criteria: their ability to be discriminant at the species level, and to establish relevant taxonomic boundaries, in comparison to the phylogenetic study data. LC/ESI-MSn and phylogenetic analyses converged into the discrimination of two taxonomical groups among the five species as follows: *C. baccata*/*C. nodicaulis*/*C. tamariscifolia* and *C. foeniculacea*/*C. humilis* (Jegou *et al.*, 2010). LC/ESI-MSn and  $^1\text{H}$  HR-MAS NMR appeared as two relevant and innovative techniques to discriminate taxonomically this complex genus (Jegou *et al.*, 2010).

Among the genus *Cystoseira*, on the basis of existing phytochemical data, species were classified into three chemical groups (I–III) based on the metabolites produced (Table IV, Reddy and Urban, 2008). Category I comprises those species that do not contain diterpenes, category II includes species that produce linear diterpenes, and species in category III contain meroditerpenoids. The meroditerpenes have been further subdivided into three classifications, which include linear, cyclic, and rearranged terpenoids (Table 4.4).

Dictyotales is the order that has been the most thoroughly studied by phytochemists.

Previous investigations suggested separation of this order into at least three groups, according to the nature of their chemical components: the groups “*Taonia*”, “*Dictyopteris*” and “*Dictyota*” (Teixeira *et al.*, 1990). The group “*Dictyota*” includes six genera that produce diterpenes, most of which possess skeletons found exclusively in marine organisms.

**Table 4.4** Examples of metabolites used to discriminate some genera within brown macroalgae: *Bifurcaria* (Daoudi *et al.*, 2001), *Cystoseira* (Kornprobst, 2005; Reddy and Urban, 2008; Mokrini *et al.*, 2008) and *Turbinaria* (Le Lann, 2009)

Genus	Chemical groups	Secondary metabolites	Species
<i>Bifurcaria</i>	I	No diterpenoids	<i>B. brassicaeformis</i>
	II	Linear diterpenes	<i>B. bifurcata</i>
	IIIB	Cyclic meroditerpenes	<i>B. galapagensis</i>
<i>Cystoseira</i>	I	No diterpenoids	<i>C. humilis</i> , <i>C. sedoides</i>
	II	Linear diterpenes	<i>C. balearica</i> , <i>C. brachycarpa</i>
	IIIA	Linear meroditerpenes	<i>C. adriatica</i> , <i>C. elegans</i>
	IIIB	Cyclic meroditerpenes	<i>C. algeriensis</i> , <i>C. baccata</i>
	IIIC	Transposed meroditerpenes	<i>C. mediterranea</i> , <i>C. stricta</i>
<i>Turbinaria</i>	Turbinaric acid	Presence	<i>T. conoides</i>
		Absence	<i>T. ornata</i>

In this group, the genus *Dictyota* Lamouroux has been the most widely investigated, and has yielded over 110 diterpenes from 17 species collected in all the oceans (in Teixeira *et al.*, 1990). The genus *Dictyota* presents problems in correctly establishing the separation limits between several of its species, and diterpenes have been frequently suggested as taxonomic markers (Blunt *et al.*, 2009; Teixeira *et al.*, 2001). Accordingly, further investigations have confirmed the effectiveness of these metabolites as taxonomic and phylogenetic markers of the genus.

From research carried out by French teams, we can cite Viano *et al.* (2009) who isolated new cyclised diterpenes from a Mediterranean specimen of *Dictyota*. These diterpenes possessed an antifouling activity against a *Pseudomonas* strain.

The same authors also isolated a *bis*-diterpene from a Mediterranean specimen of *Dictyota* and proposed a possible biogenetic pathway of this novel diterpene that they called dictyotadimer A (Viano *et al.*, 2011).

*Turbinaria* is a widespread tropical genus within the Phaeophyceae. It is relatively species-poor, so far only 17 species have been described and assigned to the genus (Wynne, 2002). Despite this limited number of species, the genus is anatomically complex and a wide variety of forms are recognized. Among them, *Turbinaria ornata* and *T. conoides* were chosen because of their similar morphology but distinctive molecular genomes (Rohfritsch *et al.*, 2007). These two allied species are widely distributed in tropical and subtropical areas of the Pacific and Indian Oceans. The main morphological differences between the two species are summarized by Rohfritsch *et al.* (2007). Leaf morphology is one of the major taxonomical criteria used to separate *Turbinaria* species, but in fact, this may be a source of systematic confusion rather than a diagnostic tool.

One original procedure was carried out to discriminate Pacific species of *Turbinaria* using  $^1\text{H}$  NMR HR-MAS (Le Lann *et al.*, 2008). This technique, originally useful to obtain an overview of metabolites in a sample was used by authors to obtain a chemical fingerprinting of both *Turbinaria conoides* and *T. ornata*. Several samples were obtained from different parts from the Pacific Ocean and passed in  $^1\text{H}$  NMR HR-MAS. Following different spectra obtained from both species, authors isolated discriminant molecules after an extraction of lipidic metabolites (Le Lann, 2009). This procedure permitted the isolation of turbinaric acid only in *T. conoides* samples, which represent a chemomarker of this species (Le Lann, 2009).

## 4.8 Industrial uses of metabolites from marine red and brown algae

### 4.8.1 Algae for nutritional foods

#### Context

In Europe, some marine organisms are protected and their use in food processing is forbidden or others, such as seaweeds, have an authorization for human consumption limited to few species (Mabeau and Fleurence, 1993). In France, the use of algae for human consumption is subject to a specific regulation (Rouxel *et al.*, 2001; Cordero, 2003). Only 12 macroalgae and two microalgae, *Spirulina* sp., and *Onodhella* sp. are authorized for use by the food industry as sea vegetables or ingredients, whereas 300 red seaweeds have been identified in Brittany.

Among the macroalgae, four species, belonging to the Rhodophyceae or red seaweeds, are affected by this regulation. They are *Palmaria palmata*, *Chondrus crispus*, *Gracilaria verrucosa*, and *Porphyra umbilicalis*, well known under the names of Dulse, Pioca, Ogo-nori and Nori, respectively. Generally, the seaweeds used as sea vegetables in Europe or in Japan, are dried in mild conditions (from 45 to 50 °C for a long time in a hot-air chamber or sun dried) (Nisizawa *et al.*, 1987). These processes are moderate and the proteins are little denatured in comparison with other heat treatments. *C. crispus* and *G. verrucosa* are also exploited for the production of additives such as carrageenans and agar.

Some French brown algae can be used in food products: the most used are *Undaria pinnatifida*, and *Himantalia elongata*. These macroalgae are very interesting to consumers and the food industry due to their low content in calories and high content in vitamins, minerals and dietetic fiber. The algae, *H. elongata* (dehydrated and canned) and *U. pinnatifida* (dehydrated) have been studied by Sanchez-Machado *et al.* (2004). These authors showed the valuable protein content of these algae and the low percentage of lipids (about 1% in all cases). Interestingly, although these algae showed a low lipid content, they possessed a high level of polyunsaturated fatty acids (PUFAs) (Sanchez-Machado *et al.*, 2004). Thus, these algae seem to be an interesting source of some polyunsaturated  $\omega$ -3 fatty acids, such as the eicosapentaenoic acid (EPA). These  $\omega$ -3 fatty acids have demonstrated their effect on the reduction of coronary diseases (Simopoulos, 2004). According to Le Tutour *et al.* (1998), the use of an extract containing soluble lipids from *H. elongata* increased synergically the antioxidant effect of vitamin E. *H. elongata* also presented high levels of  $\alpha$ -tocopherol (Sanchez-Machado *et al.*, 2002). Other compounds that could be found in these algae were sterols (Sanchez-Machado *et al.*, 2004): *H. elongata* and *U. pinnatifida* contained ethylenecholesterol and fucosterol. Cholesterol, in general, was present at very low quantities. *U. pinnatifida* also contained high levels of sulphated polysaccharides (sulphated fucans or fucoidans) that present potential antiviral activity (Hemmingson *et al.*, 2006).

The brown alga *U. pinnatifida* could be used as a food supplement to help meet the recommended daily intake of some minerals, macroelements (Na, K, Ca, Mg), and trace elements (Fe, Zn, Mn, Cu) according to Ruperez (2002). Ikeda *et al.*, (2003) showed the effect of fiber from *U. pinnatifida* on cardiovascular diseases (hypertension and hypercholesterolemia) and also discussed its possible preventive effect on cerebrovascular diseases, due to its fucoxanthin content. Other authors have claimed that fucoxanthin from algae could increase metabolism, thus helping to control weight and reducing obesity in some animals (Maeda *et al.*, 2005).

### Identification of species

Determining the biochemical composition is the first step in assessing the nutritional value of seaweed used as a food product (Darcy-Vrillon, 1993). The identification of marine species, well known under the name of species diagnosis, is also an important approach to guarantee the quality of sea products. But, macroscopic observation and microscopic examination are not often enough, they did not reveal any morphological differences between samples collected. For species identification of edible seaweeds, some approaches based on the recognition of specific protein patterns by electrophoresis techniques (SDS-PAGE, Urea-IEF) have been successfully applied (Etienne *et al.*, 1999, 2000; Mackie *et al.*, 2000; Rouxel *et al.*, 2001).

However, some processing methods, such as cooking in the production of canned foods, denature the proteins and the patterns obtained from native proteins are not suitable for species diagnosis (Mackie *et al.*, 1999). Some reports describe the application of electrophoresis for isoenzyme purification (Mardsen *et al.*, 1981), genetic differentiation (Sosa and Lindstrom, 1999) or for the identification of seaweeds (Mardsen *et al.*, 1984; Fleurence and Guyader, 1995). SDS-PAGE was successfully applied, in some conditions, for the distinction between two *Ulva* species frequently consumed as sea vegetables in Europe (Fleurence *et al.*, 1995b). Rouxel *et al.* (2001) show that *Palmaria palmata*, *Chondrus crispus*, *Gracilaria verrucosa*, and *Porphyra umbilicalis* could be characterized by their protein patterns obtained in SDS-PAGE. The comparison between the profiles shows significant differences. SDS-PAGE appears to be a more economic and more practical methodology than the molecular biological one for rapid control of the red algae species used by the food industry.

But recently, molecular techniques, using the characterization of specific DNA sequences from marine species of economic interest, have been developed. The application of PCR, sequencing, coupled or not to restriction fragment length polymorphism (RFLP) analysis has also been successfully tested for the identification of red seaweeds (Goff *et al.*, 1994; Mizukami *et al.*, 1999; Antoine and Fleurence, 2003) authorized in France since 1990 as sea vegetables or food ingredients. Three of these species are also used by industries for the production of technological additives such as alginates (*Laminaria digitata*) carrageenans (*Chondrus crispus*) and agar (*Gracilaria verrucosa*) (Joubert *et al.*, 2009). These authors tested the use of ITS sequences from 5.8 srDNA for species discrimination between different red seaweeds used for agar production. The amplification of genomic ITS sequences and sequencing shows that the different samples collected can be identified by the PCR fragment of 1124 bp or by the presence of two fragments of 1124

and 983 bp. A BLAST investigation in Genbank can show the sequence similarities between the different fragments of species. The RFLP method after *EcoRI* application could complete a characteristic pattern and could also be used for the identification of red seaweed (Joubert *et al.*, 2009). The application of DNA markers could be also justified to remove the problems of seasonal variations reported with the protein pattern.

### Proteins from red seaweeds

In most cases, the seaweeds are used in human or animal foods for their mineral contents or for the functional properties of their polysaccharides. Seaweeds are rarely promoted for the nutritional value of their proteins. The protein content of seaweeds differs according to species. Generally, the protein fraction of brown seaweeds is low ( $3 \pm 15\%$  dry weight) compared with that of the green or red seaweeds ( $10 \pm 47\%$  dry weight) (Arasaki and Arasaki, 1983; Fleurence, 1999). Red macroalgae will be specified in the following paragraph.

Rhodophyceae could be a complementary source of food proteins for human and animal nutrition. Higher protein levels were recorded for *Porphyra tenera* (47% of dry mass) or *Palmaria palmata* (35% of dry mass) (Morgan *et al.*, 1980). These algae have protein levels higher than those found in high-protein pulses such as soy bean. The protein content of marine algae also depends on the seasonal period. An annual monitoring of protein level from *Palmaria palmata* collected on the French Atlantic coast showed that the protein content of this alga can vary between 9 and 25% (dry weight). Higher protein levels were observed during the end of the winter period and spring whereas lower amounts were recorded during the summer months (Fleurence, 1999; Galland-Irmouli *et al.*, 1999).

Their amino acid content is of nutritional interest but their protein digestibility *in vivo* is still poorly described. The amino acid composition of seaweeds has been frequently studied and compared to that of other food, such as eggs or soy beans. For most seaweed, aspartic and glutamic acids constitute together a large part of the amino acid fraction. However, the glutamic and aspartic acid levels seem to be lower in red seaweed species such as *Palmaria palmata* and *Porphyra tenera* (14 and 19% of the total amino acids, respectively) (Indegaard and Minsaas, 1991). With respect to the high protein algae (e.g., *Palmaria* sp., *Porphyra* sp.), the comparison of their amino acid composition with those of other food proteins (soybeans, eggs) allows us to obtain a first estimate of the nutritional value of seaweed proteins. For *Palmaria palmata*, significant variations in protein content were observed according to the season: The highest protein content ( $21.9 \pm 3.5\%$ ) was found in the winter-spring period and the lowest ( $11.9 \pm 2.0\%$ ) in the

summer-early autumn period. Most of the essential amino acids were present through out the year: leucine, valine and methionine are well represented in the essential amino acid fraction (Galland-Irmouli *et al.*, 1999). Except for histidine content, the essential amino acid profile of *Porphyra tenera* seems to be relatively close to those of leguminous plants.

A biotechnological treatment of seaweeds by enzymatic degradation of algal could be attempted to improve protein digestibility and, therefore, increase the nutritional value of these proteins. The main studies on the *in vitro* digestibility of algal proteins were performed from proteins extracted in strong alkaline conditions (Indegaard and Minsaas, 1991). The digestion is carried out by means of three enzymes, pepsin, pancreatin, and pronase. The relative digestibility of algal proteins is generally expressed as a percentage of casein digestibility bases (100%). In this context, the relative digestibility of alkali-soluble proteins from *Porphyra tenera* is higher than 70% in the presence of pronase and 56% with pepsin and pancreatin (Fleurence, 1999). Studies were performed from water or low-ionic buffer-soluble proteins extracted from the red seaweed *Palmaria palmata* (Galland-Irmouli *et al.*, 1999). The results obtained from *Palmaria palmata* showed that the algal proteins were more sensitive to the action of trypsin than to actions of human intestinal juice or chymotrypsin. The relative digestibility of water soluble proteins of *Palmaria palmata* to pepsin and pancreatin was estimated to be 56% (Galland-Irmouli *et al.*, 1999). This result is similar to that obtained from the alkali-protein fraction on *Porphyra tenera* with high protein content. These compounds that limit the digestibility of algal proteins are either phenolic molecules or polysaccharides. The algal polysaccharides behave like soluble or insoluble fibres. However, an enzymatic pretreatment of algae, which allows for the removal of polysaccharides, as already described for *Palmaria palmata* (Lahaye and Vigouroux, 1992) could be an alternative way to limit the influence of algal fiber as antinutritional factors. On the contrary, *P. palmata* contains a high level of fiber (at least 30% of the dry mass), in cell wall structural polysaccharides. The marine algae contains 33.2–33.5% total dietary fiber. All soluble fiber fractions consisted of linear  $\beta$ -1,4/ $\beta$ 1,3 mixed linked xylans containing similar amounts of 1,4 linkages (70.5–80.2%). The insoluble fiber contained essentially 1,4 linked xylans with some 1,3 linked xylose and a small amount of 1,4-linked glucose (cellulose). The presence of polysaccharides and their interaction with proteins may reduce the accessibility of proteins to proteolysis, which causes decreased digestibility. The influence of fiber on algal protein digestibility should be investigated. The use of this alga as a protein source for the human diet requires the definition of an adequate extraction procedure. The goal of this procedure would be to separate the protein extract from the fibers (Lahaye *et al.*, 1993; Bobin-Dubigeon, 1996).

With respect to their high protein level and their amino acid composition, the red seaweeds appear to be an interesting potential source of food proteins. In Europe, the development of novel foods such as functional foods could be a new possibility for the use of seaweeds, especially for the protein-rich species, in human nutrition.

#### 4.8.2 Algae for health and cosmetics

##### Interest in health

Natural marine products derived from marine algae have proved to be rich sources of novel biologically active compounds (reviewed by Ioannou and Roussis, 2009). Among them, antitumor products have been the subject of many investigations (Mayer, 2002).

In the search for anticancer drugs, marine molecules have led to promising results in trials at different phases of cancer diseases (Mayer and Gustafson, 2006). Numerous macroalgae have shown potent cytotoxic activities (see reviews in Mayer and Gustafson, 2006; Smit, 2004), and certain authors have suggested consuming algae as a chemopreventive agent against several cancers (Yuan and Walsh, 2006). Dehydrothyriferol and halomon extracted from *Laurencia viridis* sp. nov. (Pec *et al.*, 2003) and *Portieria hornemanii* (Egorin *et al.*, 1997) respectively, have been tested in the preclinical phase. Extracted from brown seaweeds, polysaccharides (Aisa *et al.*, 2005; Dias *et al.*, 2005; Kwon and Nam, 2007) and terpenoids (Culioli *et al.*, 2004; Duran *et al.*, 1997) are considered as promising bioactive molecules.

An interesting study was carried out by Zubia *et al.* (2009) in which a strong cytotoxicity of crude extracts from several brown algae (Fucales, Desmarestiales and Dictyotales) against the human cancer cell lines Daudi, Jurkat and K562. Among these species, *C. tamariscifolia* and *H. siliquosa* extracts presented high cytotoxic activities. The demonstration of cytotoxic activity in *B. bifurcata* extracts (Culioli *et al.*, 2004; Moreau *et al.*, 2006) permit the identification of an acyclic diterpene, bifurcariol, as the cytotoxic compound. Further to studies about cytotoxic activity by species of the *Cystoseira* genus, various diterpenes have been identified as the bioactive compounds in *Cystoseira crinita* (Fisch *et al.*, 2003), *C. myrica* (Ayyad *et al.*, 2003) and *C. usneoides* (Urones *et al.*, 1992). Diterpenes compounds could be responsible for the antitumoural activities measured in the Sargassaceae species studied by Zubia *et al.* (2009). Moreover, the finding, in extracts from these species, of cytotoxic effects together with strong antioxidant activity makes these species promising candidates for further investigations. As damage events are frequently correlated with oxidative stress, the prevalence of both properties in a

single compound could be beneficial in terms of rational, preventive or therapeutic purposes.

Moreover, *D. ligulata* and *D. dichotoma* also exhibited the strongest cytotoxic activities with all of the tested tumoural cell lines. This is in accordance with Duran *et al.* (1997) and Kolesnikova *et al.* (2006) who demonstrated, that in *D. dichotoma*, diterpenes were the cytotoxic compounds. In fact, the Dictyotaceae family has been extensively studied for its wide variety of bioactive diterpenes with marked biological activities (Kornprobst, 2005). Phenolic compounds such as chromenols have been identified as the cytotoxic molecules in *Desmarestia menziesii* (Davyt *et al.*, 1997).

On the other hand, *Fucus ceranoides* crude extract exhibited protumoral activity by increasing the cell viability of Daudi tumoral cell lines. The phenolic compounds of this extract likely involved in its radical-scavenging activity could also be implicated in this protumoral activity. The ability of phenols to protect cells from oxidative stress has been demonstrated, but these compounds have a contradictory behaviour characterised by anti- and protumoral activities according to their chemical structure, the system and conditions used in the study (Gomes *et al.*, 2003). Zubia *et al.* (2009) suggest that the action of the bioactive polar molecules extracted from *F. ceranoides* as either antioxidants or pro-oxidants depends on the experimental conditions and on the tumoral cell lines under test.

Another work was carried out by Deslandes *et al.* (2000) on tropical marine brown seaweed extracts. These authors highlighted an antiproliferative effect on asynchronous cells of a human non-small-cell bronchopulmonary carcinoma line (NSCLC-N6).

An aqueous extract (fucan-like extract) from the brown seaweed *T. ornata* was tested on the NSCLC-N6 line. This extract was composed of a higher content of mannose in the active *T. ornata* extract, which was rather similar to sargassans from *Sargassum ringgoldianum* (Mori and Nisizawa, 1982). Indeed, the presence of mannose seems to be a general property of Sargassaceae (Fucales). The authors have also noticed the presence of a proteoglycan-like constituent in *T. ornata* extract; this observation highlighted the similarities with the study on *Fucus vesiculosus* by Nishino *et al.* (1994). In this context, authors hypothesized that the active fraction studied could be a new type of fucan sulfate incorporating amino sugar in its structure.

To investigate the potential antitumoral activity of *T. ornata* extract, its antiproliferative effect was first studied by characterizing the kinetics of cell growth induced by a continuous treatment and observed the changes in the cell cycle using flow cytometry. A trimodal distribution of DNA content related to 2n, 4n and 8n peaks was noticed, similar to those described in previous studies carried out on a marine sponge (Zidane *et al.*, 1996) and seaweeds (Riou *et al.*, 1996; Bergé *et al.*, 1997). After a 72 h culture

in the presence of the algal extract (90 and 110  $\mu\text{g/mL}$ ), all the cells were blocked in G1-phase. By comparison with controls, the dose-dependent increase in the 4n and 8n peaks showed that these peaks corresponded to cells with 4n and 8n chromosomes in G1-phase.

The authors suggested that the *T. ornata* extract could trigger the terminal differentiation of cancerous cells *in vitro*.

### *Interest in the cosmetic industry*

Under the form of extracts, meals, flours, and ground algae, seaweeds are widely used in cosmetology and in thalassotherapy, even though the relevant literature focuses generally on their roles as excipients (De Roeck-Holtzhauer, 1991; Pérez, 1997). In particular, phycocolloids may increase the viscosity of the solutions and play the role of gelling agents and stabilizers. With that respect, agars, alginate and carrageenans may be used in various sticks, creams, soaps, shampoos, toothpastes, lotions, foams, and gels. The efficiency of algal molecules in cosmetic preparations has been often claimed with a large variety of biological activities: tonifying, cleaning, hydrating, revitalizing, and antioxidant. However, the effectiveness of such activities is not generally well established, since most preparations contain very low amounts of added algal extracts (Pérez, 1997). Concerning the uses of phlorotannins as bioactive compounds in cosmetics, literature is rather scarce. European and US patents may be found, related generally to the activity of phenols as antioxidant and radical scavenging agents. So, phlorotannins might be putative agents for the protection of skin cells against aging and exposure to harmful solar radiation.

In relation to this last point, the increased exposure to solar UV radiations (200–400 nm) for leisure reasons and the depletion of the ozone layer in the upper stratosphere in some areas of the earth has been related to increases of skin pathologies. The damage caused to the skin by UV radiation depends on the wavelength and exposure time. The most visible short-term response to UV solar radiation is erythema. About a 1% decrease in the ozone layer can increase skin cancer of basocellular cells by about 70% and non-melanoma skin cancer (NMSC) by about 2% (de Gruijil and van der Leun, 1993). In addition, UV radiation is the most important factor in the long-term pathogenesis, which includes DNA damage, NMSC, photoaging, cataracts and immunosuppression. The local antigenic system in the skin is highly affected by UV radiation. In animals, it has been demonstrated that UV radiation suppresses the immune reactions and consequently the animal can suffer bacterial and viral infections (Yoshikawa *et al.*, 1990; Norval *et al.*, 1994).

The increase in skin cancer requires integral actions including change in the non health customs and the use of an effective photoprotection system against UV radiation.

Sunscreens have been developed which scatter, reflect or absorb UV radiation. The sequence events between the initial interaction (i.e., light penetration in the skin and the photobiological event that takes place) is quite complex with a large number of involved molecules and biochemical steps. Rather than preventing the initial interaction of UV radiation in the skin, it may be possible to reduce at least some of the adverse effects of UV radiation with agents that inhibit some downstream phenomena. Thus, ideal photoprotectors must include not only a shield capacity towards both UV-B (280–315 nm) and UV-A (315–400 nm) radiations but also other properties, for example antioxidant activity. The first generation photoprotectors included UV-B absorbing substances, but recently UV-A absorbing substances have also been included since UV-A exposure has been related to skin cancer, photoaging and immunosuppression (Farage *et al.*, 2010; Schwarz and Schwarz, 2002). Rosenstein *et al.* (1999) reported that solar UV-A wavelengths, which are not fully blocked by the sun care products employed by sunscreen users, may play a significant role in basal cell carcinoma through induction of mutations in oncogenes and tumor suppression genes. Recently, some UV-A photoprotectors, as the example of FILTROSOL® Crema (including Metilbencilidene) have been incorporated in the solar creams.

Most of the photoprotectors are assayed against actinic erythema but not against other biological effects such as immunosuppression, skin cancer or photo-allergy (Elmets and Andersson, 1996). Therefore, it is necessary to investigate for new substances with a high capacity for screening UV-A radiations and a strong efficiency to prevent other negative effects than erythema. Presently, there is an effort to find substances not only with UV-screen capacity, but also with an antioxidant function (Black *et al.*, 1993; Steenvoorden and van Henegouwen, 1997) or with a protective effect against DNA damage (i.e. protection against the formation of photodimers or photorepair of the damaged DNA) (Damian *et al.*, 1999; Seité *et al.*, 2000; Schwarz and Schwarz, 2002).

In this context, cosmetic companies are interested in developing hydrating, antibacterial and photoprotective products derived from marine plants. The best described molecules playing those roles are osmolytes, antioxidant, antibacterial and antifungal compounds.

### *Osmolytes*

Nowadays, numerous studies throughout the world are focusing on cell volume regulation. In response to various stresses, cells accumulate osmotically active organic substances – osmolytes – distributed among a very limited number of chemical families; they are characterized by a very high hydrosolubility and null electrical charge at physiological pHs. They mainly consist of polyols and soluble

sugars (glycerol, mannitol, glucose, saccharose...), non-essential amino acids (glycine, proline, taurine, pipecolic acid), molecules carrying a quaternary ammonium ion – betaines (glycine betaine, homobetaine), choline-derived compounds (choline-*O*-sulfate), and molecules carrying a dimethyl sulfonium ion ( $\beta$ -dimethyl-sulfoniopropionate). The comparison of these osmolytes at the scale of the living kingdom from bacteria to mammals, going through terrestrial and marine plants, highlights striking convergences in the structure of the molecules involved in osmotic adaptation. These organic solutes are now known to act, not only as osmotic effectors, but also as stabilizers for the structure and functions of macromolecules and organites. They also protect enzymatic activities and cell membranes in high osmolarity media. Because of their overall properties these compounds are termed compatible solutes.

### Antioxidant compounds

Two main classes of compounds present antioxidant properties: phenolics and mycosporin-like aminoacids (MAAs).

- 1 *Phenolic compounds* are secondary metabolites present in various organisms, including higher plants (e.g., Rohner and Ward, 1997), lichens (Hyvarinen *et al.*, 2000) and algae (Ragan and Glombitza, 1986). In algae, brown seaweeds contain high levels of phenols, essentially under the form of phlorotannins, that is polymers of phloroglucinol (1,3,5-trihydroxybenzene) up to 650 kDa. In spite of some structural differences, phlorotannins exhibit chemical properties and physiological roles similar to those of the tannins synthesised by vascular plants. Phaeophyceae accumulate phenolic compounds at high levels, up to nearly 20% DW in Fucales and 30% DW in Dictyotales (Ragan and Glombitza, 1986; Targett *et al.*, 1995). Phenolic compounds play an important role in the protection of the thallus against grazers (Sotka *et al.*, 2002; Stiger *et al.*, 2004), pathogens (Ragan and Glombitza, 1986) and epiphytes (Steinberg *et al.*, 1998). They are also involved in photoprotection mechanisms and seem particularly efficient in counteracting the cytotoxic effects of UV radiations (Pavia *et al.*, 1997; Targett and Arnold, 1998).

Plant polyphenols exert a double protective action against UV radiations, and particularly against UVB, by the screening effect of the phenol rings and by the neutralization of reactive oxygen species and free radicals. Some phenols found in higher plants (caffeic acid, ferulic acid or *p*-coumaric acid) are more efficient *in vitro* than vitamins C and E and protect cells *in vivo* against both photo-oxidation and mutagenesis induced by UV radiation (Rice-Evans *et al.*, 1997). As the other photosynthetic plants, seaweeds are exposed to photo-oxidation which induces the production of active oxygen species and free

radicals. However, the absence of any drastic oxidative damage in the cells of Phaeophyceae suggests the occurrence of an efficient cytoprotective system (Matsukawa *et al.*, 1997). As in fruits (Landrault *et al.*, 2002) and tea (Benzie and Szeto, 1999), radical scavenging activity is correlated to phenol levels in seaweed extracts (Jimenez-Escrig *et al.*, 2001; Connan *et al.*, 2004). However, the antioxidant activity of seaweed phenols seems to depend on their structure and especially on the degree of polymerisation of phloroglucinol, small phlorotannins being generally more active than highly polymerised compounds (Nakamura *et al.*, 1996).

- 2 *Mycosporine-like aminoacids (MAAs)* are UV-screen substances found in red algae, which have a high potential application as photoprotectors for human skin (Shick *et al.*, 2000). These nitrogenous compounds are water-soluble, and mainly absorb in the near UV region at wavelengths 310–360 nm (Karentz *et al.*, 1991; Dunlap and Shick, 1998). The occurrence of MAAs in macroalgae has been correlated with UV exposure, as relative to different geographical locations having different natural levels of UV radiation (Karsten *et al.*, 1998a). Surveys of MAAs have been reported in macroalgae from polar, warm-temperate (Karsten *et al.*, 1998a) and tropical regions (Karsten *et al.*, 1998b), as well as from the Antarctic (Hoyer *et al.*, 2001). Thus, it is generally admitted that MAAs represent a defense system against UV radiation in red algae mainly, whereas in green and brown algae other protective compounds (e.g. phenols) or mechanisms are likely more important.

MAAs are very efficient photoprotectors with high energy dissipation without production of oxidant photoproduct and with high photostability *in vitro* (Conde *et al.*, 2000) and *in vivo* (Adams and Shick, 2001). MAAs have been suggested as natural sunscreens for human skin (Bandaranayake *et al.*, 1998). The accumulation of MAAs can be stimulated by both blue/UV-A radiation and by ammonium supply (Franklin *et al.*, 1999; Korbee *et al.*, 2005). The accumulation of MAAs throughout the year varies a great deal, due to the effect of solar irradiance. On the other hand, the content of MAAs can be increased in algae grown in nitrogen-enriched waters (i.e., fishpond effluents).

In addition to UV screen properties, MAAs can have antioxidant activity (Dunlap and Yamamoto, 1995; Suh *et al.*, 2003). Nakayama *et al.* (1999) reported that the usjileno isolated from the red alga *Porphyra yezoensis* also has a high antioxidant activity. This function can be partly related to the capacity to block the production of thymine dimers (Misonou *et al.*, 2003). MAAs could have a similar effect as has been demonstrated in aqueous/organic extracts of fresh and processed edible seaweeds (Jiménez-Escrig *et al.*,

2001). At the present time, antioxidant substances obtained from different macroalgae are mainly  $\beta$ -carotene and astaxanthin (Edge *et al.*, 1997). Oral antioxidants then scavenge reactive oxidants and modulate cellular redox status may be useful systemic photoprotectors, which overcomes some of the problems associated with the topical use of sunscreens. Preclinical studies illustrate the photoprotective properties of supplemented antioxidants as nutraceutical compounds, mainly  $\beta$ -carotene and L-ascorbate (Fuchs, 1998). The beneficial effect of carotenoids is found in the presence of vitamin C, demonstrating an interaction between several antioxidant substances.

#### *Antibacterial and antifungal compounds*

Marine algae and halophytes produce a large variety of chemically bioactive metabolites for their defense. These metabolites, also known as biogenic compounds, have antibacterial, antialgal, antimicrofouling, and antifungal properties (Paul and Puglisi, 2004; Bhadury and Wright, 2004) and allow the organism to regenerate after a pathogen attack. The meristematic region presents a high potential of synthesis of such compounds, where they protect this growth region from epiphytes, abrasion and grazers (Vlachos *et al.*, 1999).

A large number of publications reported the production of such metabolites in algae from temperate and tropical regions (Vlachos *et al.*, 1999; Freile-Pelegri and Morales, 2004).

Biotic parameters, including the reproduction stage (Robles-Centeno *et al.*, 1996) or the different parts of the thallus (Vlachos *et al.*, 1999), as well as abiotic factors like seasonality and the geographical localization (Robles-Centeno *et al.*, 1996; Maréchal *et al.*, 2004) can influence the level of extract bioactivity.

Antimicrobial metabolites are present in all the classes of macroalgae, including green (Ulvales and Codiales), brown (Dictyotales, Laminariales, and Fucales) and red algae (Gigartinales and Ceramiales) from either temperate or tropical areas (Pesando, 1990). The molecules from marine algae responsible for antimicrobial activities are partially identified and such activities are associated to secondary metabolites, which are different according to the algal species.

Robles-Centeno *et al.* (1996) and Robles-Centeno and Ballantine (1999) highlighted the complexity of chemical defense in red algae, which could produce a large variety of antimicrobial compounds in their environment, but also in culture media. A number of red algae produced halogenated terpenoids (Vairappan *et al.*, 2001; Xu *et al.*, 2004; Freile-Pelegri and Morales, 2004; Vairappan *et al.*, 2004), while among green algae, Ulvales and Acrosiphoniales are rich in acrylic acid and Caulerpa produce highly bioactive diter-

penoids (Pesando, 1990). Among Fucales and Dictyotales which belong to brown algae, the antimicrobial compounds found include acrylic acid, complex diterpenoids derivatives, phlorotannins or phenolic lipids (Sastri and Rao, 1994; Van Heemst *et al.*, 1996; Abourriche *et al.*, 1999; Bennamara *et al.*, 1999; Hellio *et al.*, 2001; Sandsalen *et al.*, 2003; Maréchal *et al.*, 2004; Zinedine *et al.*, 2004).

An interesting study on slimming molecules was carried out by a French team from La Rochelle (Rouillé *et al.*, 2009). Calcimine<sup>®</sup> is the name which was given to a natural product obtained by the SME PhosphoTech after enzymic hydrolysis of a marine algal extract. This marine natural ingredient could play a role in weight management since it exhibited lipolytic activity and reduced fat storage when tested on human immortalized adipocytes. Calcimine<sup>®</sup> also reduced lipid accumulation when it is added during the pre-adipocyte differentiation step. This marine natural product allowed for fat reduction in adipocytes mainly through the apoptotic way but not only: it also exhibited lipolytic activity like caffeine but probably without nervous stimulation.

#### **4.8.3 Algae against microorganisms**

There is a growing demand in European countries for an alternative strategy in supplying chemical products to protect our environment: in antifouling paints to reply to the novel European norms and in agriculture, to plant protection products in order to reduce tonnages of pesticides consumed.

We do not present the use of marine macroalgae in marine paints, since it has already been discussed in section 4.7.2.

#### **Interest of marine macroalgae in agriculture**

Marine algae are known to be used in agriculture mainly as fertilisers and animal feeding. For a long time, farmers from Asian and European coasts have used floating seaweeds to enrich soil with nutrients. Nowadays, numerous companies develop algal fertilisers as leaf spray or as liquid (Mabeau *et al.*, 1990). Thus, an extensive literature reports the interesting effects observed using algae as fertilisers, due to their abundance in minerals and oligo-elements, organic matter, vitamins, growth hormones, phenolic compounds and polysaccharides. Verkleij (1992) observed variability in the quality of algal extracts depending on their origin, the season of harvest and the extraction method. Zemke-White and Ohno (1999) reported the use of numerous algal species (red seaweeds, as well as green or brown seaweeds) as fertilisers. Since they are rich in minerals and oligo-elements, seaweeds are also used as animal wheat in numerous European countries.

Concerning antimicrobial properties of marine plants, few studies reported the use of seaweeds in agriculture. Accordingly, the brown seaweed *Cystoseira tamariscifolia* was found to possess antimicrobial compounds with activity against yeast and mould (Zinedine *et al.*, 2004). Other species were reported to be active against some tomato pathogens (Bennamara *et al.*, 1999).

#### *Interest of marine macroalgae in aquaculture*

Since they are rich in minerals and oligo-elements, algae are used as animal wheat in numerous European countries (Mabeau, 1989; Chopin *et al.*, 1999; Jensen, 1993). Some fish aquafarmers develop animal wheat with algal origin, especially in Japan (Mustafa and Nagakawa, 1995).

Another activity sector concerns the protection of cultivated species. Antibiotic treatment of infectious diseases has been applied in aquaculture for many years; however, the incidence of drug-resistant bacteria and the spreading of these resistances has become an important problem in fish culture (Aoki, 1992; Miranda and Zemelman, 2002; Zorrilla *et al.*, 2003). The prevention of infectious diseases appears as a convenient alternative. However, although a variety of pathogens affecting cultured fish have been identified, effective vaccines are available only against a few of them and the control of all fish diseases using vaccines is impossible. For this reason, the development of immunostimulant substances capable of activating the fish immune system and inducing a better response against the pathogens is of interest. Immunostimulants increase resistance to infectious diseases by enhancing non-specific defence mechanisms and increasing the disease resistance of fish (Sakai, 1999; Raa, 2000). Moreover, Chotigeat *et al.* (2004) showed that the ingestion of fucoidans of the brown alga *Sargassum polycystum* by shrimps from the genus *Penaeus* can reduce the impact of some viral illnesses.

During the last years, attention has been focused on marine organisms as a source of substances of therapeutic interest. Thus, the ability of marine plants to produce secondary metabolites with pharmaceutical application has been extensively documented, especially in seaweeds. Such compounds include antibiotics, antivirals, antitumorals and anti-inflammatories. Immunomodulatory properties have also been found in extracts from algae such as *Laminaria* or *Dunaliella* (Okai *et al.*, 1996; Supamattaya *et al.*, 2005). In the latter, the extract (Algro Natural<sup>®</sup>) was used as a shrimp feed supplement with immunomodulation effect. Moreover, Hou and Chen (2005) showed that hot-water extract of the red macroalga *Gracilaria tenuistipitata* increased the immune ability and resistance of white shrimp (*Litopenaeus vannamei*) to *Vibrio alginolyticus* infection.

Recently, Díaz-Rosales *et al.* (2005) reported preliminary potential immunostimulant activities of algal extracts and three purified substances, that is mycosporine-like aminoacids from red algae and trihydroxicopumarin obtained from the green alga *Dasycladus vermicularis* (Pérez-Rodríguez *et al.* 1998).

## 4.9 Conclusion

Seaweeds are known to produce inhibitory allelochemicals that interfere with epiphytes and other epibiont competitors like bacteria, fungi, diatoms, macroalgae, larvae of mussels or barnacles. These allelochemicals thus constitute a source of natural bioactive products although little has been done to define their ecological role and to better understand their biosynthesis. On this last point, Cardozo *et al.* (2007) gave an interesting figure with the biosynthesis hypotheses for some metabolites (Figure 4.10).

Today, algae have long been recognized as rich and valuable natural resources of bioactive compounds because of their various biological properties. Substances extracted from these organisms currently receive more attention from the pharmaceutical, cosmetic or drug development fields.

The French research laboratories constitute a real potential in terms of upgrading marine substances extracted from seaweeds with very diverse and promising applications, in the cosmetic and food fields (University of Nantes, European Institute for Marine Studies/Université de Bretagne occidentale, IFREMER), in antifouling paints or antiviral applications (University de Bretagne-Sud, University of the Sud Toulon-Var), in chemiotaxonomy or in genome sequencing fields (University of the Sud Toulon-Var, European Institute for Marine Studies/Université de Bretagne Occidentale, Station Biologique de Roscoff UPMC) with various research networks and European programs.

## Acknowledgments

We wish to dedicate this article to Professor Floc'h. Jean-Yves Floc'h was a Professor at the Université de Bretagne Occidentale and Emeritus Professor since 2005. He was an exceptional teacher (phycology, marine ecology, eco-physiology) and a famous talented international researcher. He published more than 50 papers, proceedings, and chapters, with always a beautiful passion for seaweeds that he promoted. He was co-author of a classic, the *Guide to the Seaweeds of the Seas of Europe* (Ed. Delachaux et Niestlé, 1992, 2006) and in 2010, of *les Secrets des Algues*.

The preparation of this review was supported by the EU Interreg IVB project BIOTECMAR.

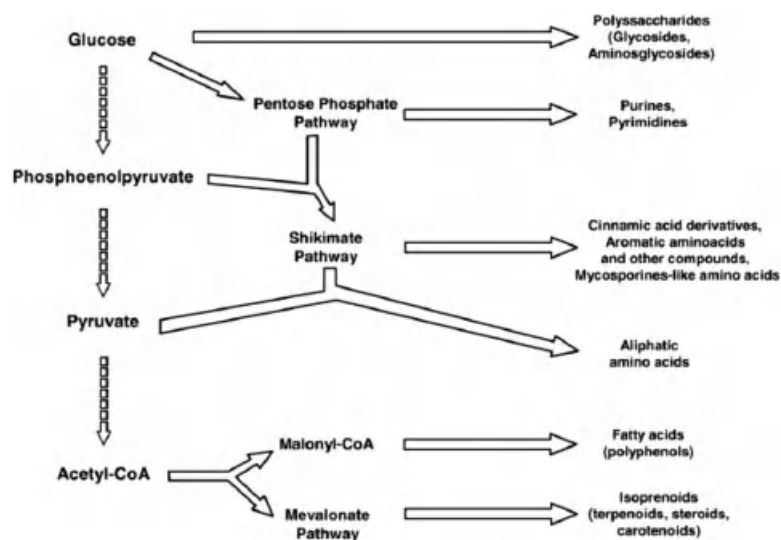


Figure 4.10 Main pathways of some metabolites biosynthesis (in Cardozo *et al.*, 2007)

## References

- Abarzua S. and Jakubowski S. (1995) Biotechnological investigation for the prevention of biofouling. I. Biological and biochemical principles for the prevention of biofouling. *Mar. Ecol. Progr. Series*, **20**, 111–123.
- Abdala-Díaz R.T., Cabello-Pasini A., Pérez-Rodríguez E., Conde Álvarez R.M. and Figueroa F.L. (2006) Daily and seasonal variations of optimum quantum yield and phenolic compounds in *Cystoseira tamariscifolia* (Phaeophyta). *Mar. Biol.*, **148**, 459–465.
- Abourriche A., Charrouf M., Berrada M., Bennamara A., Chaib N. and Francisco C. (1999) Antimicrobial activities and cytotoxicity of the brown algae *Cystoseira tamariscifolia*. *Fitoter.*, **70**, 611–614.
- Adams N.L. and Shick J.M. (1996) Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin, *Strongylocentrotus droebachiensis*. *Photochem. Photobiol.*, **64**, 149–158.
- Aisa Y., Miyakawa Y., Nakazato T., Shibata H., Saito K. and Ikeda Y. (2005) Fucoidan induces apoptosis of human HS-Sultan cells accompanied by activation of caspase-3 and down-regulation of ERK pathways. *Am. J. Hematol.*, **78**, 7–14.
- Alves de Sousa A.P., Torres M.R., Pessoa C., Odorico de Moraes M., Rocha Filho F.D. and Negreiros A.P. (2007) *In vivo* growth-inhibition of Sarcoma 180 tumor by alginates from brown seaweed *Sargassum vulgare*. *Carbohydrate Polymers*, **69**, 7–13.
- Alzieu C. (2000) Environmental impact of TBT: the French experience. *The science of Total Environment*, **258**, 99–102.
- Amico V. (1995) Marine brown algae of family Cystoseiraceae: chemistry and chemotaxonomy. *Phytochemistry*, **39**, 1257–1279.
- Amico V., Oriente G., Piattelli M., Ruberto G. and Tringali C. (1981) Novel acyclic diterpenes from the brown alga *Cystoseira crinita*. *Phytochemistry*, **20**, 1085–1088.
- Amsler C.D. and Fairhead V.A. (2006) Defensive and sensory chemical ecology of brown algae. *Adv. Bot. Res.*, **43**, 1–91.
- Andrade L.R., Salgado L.T., Farina M., Pereira M.S., Mourão P.A.S. and Amado Filho G.M. (2004) Ultrastructure of acidic polysaccharides from the cell walls of brown algae. *J. Struct. Biol.*, **145**, 216–225.
- Antoine E. and Fleurence J. (2003) Species identification of red and brown seaweeds using ITS ribosomal DNA amplification and RFLP patterns. *J. Sci. Food Agric.*, **83**, 709–773.
- Aoki T., Kitao T. and Kawano K. (1981) Changes in resistance of *Vibrio anguillarum* in cultured ayu, *Plecoglossus altivelis* Temminck and Schlegel, in Japan. *J. Fish Dis.*, **4**, 223–230.
- Arasaki A. and Arasaki T. (1983) *Low Calories, High Nutrition. Vegetables from the Sea to Help you Look and Feel Better*, pp. 39–42, Japan Publications Inc.
- Ar Gall E., Küpper F.C. and Kloareg B. (2004) A survey of iodine contents in *Laminaria digitata* suggests involvement of iodide with protection against oxidative stress. *Bot. Mar.*, **47**, 30–37.
- Armstrong E., Yan L., Boyd K.G., Wright P.C. and Burgess J.G. (2001) The symbiotic role of marine microbes on living surfaces. *Hydrobiologia*, **461**, 37–40.

- Arzel P. (1987) *Les goémoniers, Le Chasse-marée*, Editions de l'estran, page 102.
- Arzel P. (1998) *Les laminaires sur les côtes bretonnes, évolution de l'exploitation et de la flottille de pêche, état actuel et perspectives*. Edition de l'Ifremer, 139 p.
- Arzel P. (2000) *Sur la route des Algues, les goémoniers*. Patrimoine maritime de Bretagne, Ed. Uhel Izel, 65 p.
- Arzel P. and Barbaroux O. (2003) *Les Algues, produits, saveurs et santé de la mer*. Ed. Libris, 104 p.
- Audibert L., Fauchon M., Blanc N., Hauchard D. and Ar Gall E. (2010) Phenolic compounds in the brown seaweed *Ascophyllum nodosum*: distribution and radical-scavenging activities. *Phytochem. Anal.*, **21**, 399–405.
- Ayyad S.E.N., Abdel-Halim O.B., Shier W.T. and Hoyer T.R. (2003) Cytotoxic hydroazulene diterpenes from the brown alga *Cystoseira myrica*. *Zeitschrift für Naturforschung C – A Journal of Biosciences*, **58**, 33–38.
- Bakus G., Targett N. and Schulte B. (1986) Chemical ecology of marine organisms: an overview. *Journal of Chemical Ecology*, **12**, 951–987.
- Bandaranayake W.M. (1998) Mycosporines: are they nature's sunscreens? *Nat. Prod. Rep.*, **15**, 159–172.
- Bansemir A., Blume M., Schröder S. and Lindequist U. (2006) Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture*, **252**, 79–84.
- Barbosa J.P., Fleury B.G., da Gama B.A.P., Teixeira V.L. and Pereira R.C. (2007) Natural products as antifoulants in the Brazilian brown alga *Dictyota pfaffii* (Phaeophyta, Dictyotales) *Biochem. Syst. Ecol.*, **35**, 549–553.
- Barros M.P., Pinto E., Sigaud-Kutner T.C.S., Cardozo K.H.M. and Colepicolo P. (2005) Rhythmicity and oxidative/nitrosative stress in algae. *Biol. Rhythm. Res.* **36**, 67–82.
- Bazes A., Silkina A., Defer D., Quémener E., Braud, J.P. and Bourgougnon N. (2006) Allelopathic substances from *Ceramium botryocarpum* used as antifouling products. *Aquaculture*, **258**, 664–674.
- Bazes A., Silkina A., Douzenel P., Faÿ F., Kervarec N., Morin D., Berge J.P. and Bourgougnon N. (2008) Investigation of the antifouling constituents from the brown alga *Sargassum muticum* (Yendo) Fensholt. *J. Appl. Phycol.*, **21** (4), 395–403.
- Bellas J. (2006) Comparative toxicity of alternative antifouling biocides on embryos and larvae of marine invertebrates. *Science of The Total Environment*, **367**, 573–585.
- Bellas J. (2007) Toxicity of the booster biocide Sea-Nine to the early developmental stages of the sea urchin *Paracentrotus lividus*. *Aquatic Toxicol.*, **83**, 52–61.
- Bellas J. (2008) Prediction and assessment of mixture toxicity of compounds in antifouling paints using the sea-urchin embryo-larval bioassay. *Aquatic Toxicology* **88**, 308–315.
- Bellion C., Brigand G., Prome J.C., Welti D. and Bociek S. (1983) Identification et caractérisation des précurseurs biologiques des carraghénanes par spectroscopie RMN-13C. *Carbohydrate Res.*, **119**, 31–48.
- Bennamara A., Abourriche A., Berrada M., et al. (1999) methoxybifurcarenone: an antifungal and antibacterial meroditerpenoid from the brown alga *Cystoseira tamariscifolia*. *Phytochemistry*, **52**, 37–40.
- Benzie I.F.F., Szeto Y.T. (1999) Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *J. Agric. Food Chem.*, **47**, 633–636.
- Bergé J.P., Bourgougnon N., Carbonnelle D., et al. (1997) Antiproliferative effects of an organic extract from the marine diatom *Skeletonema costatum* against a non-small-cell bronchopulmonary carcinoma line (NSCLC-N6). *Anticancer Res.*, **17**, 2115–2120.
- Bergé J.-P., Courcoux O., Hellio C., Bourgougnon N. (2002) New global screening methods for potential antifouling compounds: application to macroalgae. IX<sup>th</sup> ESMB Meeting of marine biotechnologie, Nantes.
- Bermejo Román R., Álvarez-Pez J.M., Acien Fernández F.G. and Molina Grima E. (2002) Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum* J. *Biotechnol.*, **93** (1), 73–85.
- Berteau O. and Mulloy B. (2003) Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology*, **13**, 29–40.
- Bhadury P. and Wright P.C. (2004) Exploitation of marine algae: biogenic compounds for potential antifouling applications. *Planta*, **219**, 561–578.
- Biard J.F., Verbist J.F., Floch R. and Letourneux Y. (1980) Epoxyeleanolone et eleanediol, deux nouveaux diterpenes de *Bifurcaria bifurcata* Ross (Cystoseiracées). *Tetrahedron Lett.*, **21**, 1849–1852.
- Bisgrove S.R. and Kropf D.L. (2001) Asymmetric cell division in fucoid algae: A role for cortical adhesions in alignment of the mitotic apparatus. *J. Cell Sci.*, **114**, 4319–4328.
- Bjerke J.W., Gwynn-Jones D. and Callaghan T.V. (2005) Effects of enhanced UV-B radiation in the field on the concentration of phenolics and chlorophyll fluorescence in two boreal and arctic-alpine lichens. *Env. Exp. Bot.*, **53**, 139–149.
- Black H.S., Lenger W.A., Gerguis J. and Thornby J.I. (1993) Relation of antioxidants and level of dietary lipid to epidermal lipid peroxidation and ultraviolet carcinogenesis. *Cancer Res.*, **45**, 6254–6259.
- Blanc N., Hauchard D., Audibert L. and Ar Gall E. (2011) Radical-scavenging capacity of phenol fractions in the brown seaweed *Ascophyllum nodosum*: an electrochemical approach. *Talanta*, **84**, 513–518.

- Blunt J.W., Copp B.R., Munro M.H.G., Northcote P.T. and Prinsep M.R. (2006) Marine natural products. *Nat. Prod. Rep.*, **23**, 26–78.
- Blunt J.W., Copp B.R., Hu W.-P., Munro M.H.G., Northcote P.T. and Prinsep M.R. (2007) Marine natural products. *Nat. Prod. Rep.*, **24**, 31–86.
- Blunt J.W., Copp B.R., Hu W.-P., Munro M.H.G., Northcote P.T. and Prinsep M.R. (2009) Marine natural products. *Nat. Prod. Rep.*, **26**, 170–244.
- Bobin-Dubigeon C. (1996) Caractérisation chimique, physico-chimique et fermentaire de produits alimentaires à bases d'algues. PhD thesis, Nantes, France.
- Bondu S., Deslandes E., Fabre MF, Berthou C. and Guangli Y. (2010) Carrageenan from *Solieria chordalis* (Gigartinales): Structural analysis and immunological activities of the low molecular weight fractions. *Carbohydrate Polymers*, **81**, 448–460.
- Bouarab K., Adas, F., Gaquerel E., Kloareg B., Salaün J.-P. and Potin P. (2004) The innate immunity of a marine red alga involves oxylipins from both the eicosanoid and octadecanoid pathways. *Plant Physiol.*, **135**, 1838–1848.
- Bouhlal R., Riadi H., Lopez J.M. and Bourgougnon N. (2010a) The antibacterial potential of the Seaweeds (Rhodophyceae) of the Strait of Gibraltar and the Mediterranean Coast of Morocco. *Afr. J. Biotechnol.*, **9**, 6365–6372.
- Bouhlal R., Riadi H., Bourgougnon N. (2010b) Antiviral activities of Morocco seaweeds extracts. *Afr. J. Biotechnol.*, **9** (20), 7968–7975.
- Bourgougnon N. (2003) Anti-HIV compounds from red seaweeds. In: *Biomaterials and Bioprocessing. Volume 9. Recent Advances in Marine Biotechnology*, Science Publishers of Enfield, New Hampshire (USA), Plymouth (UK), pp. 165–206.
- Bourgougnon N., Lahaye M., Chermann J.C. and Kornprobst JM (1993) Composition and antiviral activities of sulfated polysaccharide from *Schizymenia dubyi* (Rhodophyta, Gigartinales), *Bioorg. Med. Chem. Lett.*, **3**, 1141–1146.
- Bourgougnon N., Roussakis C., Kornprobst J.-M. and Lahaye M. (1994) Effects *in vitro* of sulfated polysaccharide from *Schizymenia dubyi* (Rhodophyta, Gigartinales) on a non-small-cell-bronchopulmonary carcinoma line (NSCLC-N6), *Cancer Lett.*, **85**, 87–92.
- Bourgougnon N., Quemener B., Lahaye M., et al. (1996a) Annual variation in composition and *in vitro* anti-HIV-1 activity of the sulfated glucuronogalactan from *Schizymenia dubyi* (Rhodophyta, Gigartinales). *J. Appl. Phycol.*, **8**, 155–161.
- Bourgougnon N., Quemener B., Lahaye M., Cormaci M., Furnari G. and Kornprobst JM (1996b) Chemical structural approach of water-soluble sulfated glucuronogalactan from *Schizymenia dubyi* (Rhodophyta, Gigartinales). *J. Appl. Phycol.*, **8**, 147–153.
- Bourgougnon N., Chermann JC, Lahaye M., and Kornprobst JM (1996c) Anti-HIV-1 activity and mode of action *in vitro* of the sulfated polysaccharide from *Schizymenia dubyi* (Rhodophyta, Gigartinales). *AIDS Sci. Cell. Pharmacol.*, **3**, 104–108.
- Bourgougnon N., Quemener B., Lahaye M., Cormaci M., Furnari G. and Kornprobst JM (1996d) Chemical structural approach of water-soluble sulfated glucuronogalactan from *Schizymenia dubyi* (Rhodophyta, Gigartinales). *J. Appl. Phycol.*, **8**, 147–153.
- Brock E., Aberg P. and Pavia H. (2001) Phlorotannins as chemical defense against macroalgal epiphytes on *Ascophyllum nodosum*. *J. Phycol.*, **37**, 8–9.
- Brock E., Nylund G.M. and Pavia H. (2007) Chemical inhibition of barnacle larval settlement by the brown alga *Fucus vesiculosus*. *MEPS*, **337**, 165–174.
- Cardozo K.H.M Guaratini T., Barros M.P., Falcao V.R., Tonon A.P., Lopes N.P., Campos S., Torres M.A., Souza A.O., Colepicolo P. and Pinto E. (2007) Metabolites from algae with economical impact. *Comp. Biochem. Physiol.*, **C**, **146**, 60–78.
- Carlucci M.J., Pujol C.A., Cianca M., Nosedá M.D., Matulewicz M.C. and Damonte E.B. (1997) Anti herpetic and anticoagulant properties of carrageenans from the red seaweed *Gigartina skottsbergii* and their cyclized derivatives: Correlation between structure and biological activity. *Int. J. Biol. Macromolecules*, **20**, 97–105.
- Cerantola S., Breton F., Ar Gall E. and Deslandes E. (2006) Co-occurrence and antioxidant activity of fucol and fucophlorethol classes of polymeric phenols in *Fucus spiralis*. *Bot. Mar.*, **49**, 347–351.
- Chambers L.D., Stokes K.R., Walsh F.C. and Wood R.J.K. (2006) Modern approaches to marine antifouling coatings. *Surf. Coat. Tech.*, **201**, 3642–3652.
- Chevolot-Magueur A.M., Cave A., Potier P., Teste J., Chiaroni A. and Riche C. (1976) Composés bromés de *Rytiplea tinctoria* (Rhodophyceae). *Phytochemistry*, **15** (5), 767–771.
- Chopin T. and Sawhney M. (2009). Seaweeds and their mariculture. In: Steele, J.H., Thorpe, S.A., Turekian, K.K. (Eds.), *The Encyclopedia of Ocean Sciences*. Elsevier, Oxford, pp. 4477–4487.
- Chopin T., Yarish C., Wilkes R., Belyea E., Lu S. and Mathieson A. (1999) Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. *J. Appl. Phycol.*, **11**, 463–72.
- Chotigeat W., Tongsupa S., Supamataya K. and Phongdara A. (2004) Effect of fucoidan on disease resistance of black tiger shrimp. *Aquaculture*, **233**, 23–30.
- Clare A.S. (1998) Towards nontoxic antifouling. *J. Mar. Biotechnol.*, **6**, 3–6.

- Claude A., Bondu S., Michaud F., Bourgougnon N. and Deslandes E. (2009) X-ray structure of a sodium salt of digeneaside isolated from red alga *Ceramium botryocarpum*. *Carbohydrate Res.*, **344** (5), 707–710.
- Cock J. M., Sterck L., Rouze P., et al. (2010) The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature*, **b**, 617–621.
- Codomier L., Segot M. and Combaut G. (1981) Influence de composés organiques halogénés sur la croissance d'*Asparagopsis armata* (Rhodophycée, Bonnemaisoniales). *Bot. Mar.*, **24**, 509–513.
- Codomier L., Bruneau Y., Combaut G. and Teste J. (1977) Étude biologie et chimique d'*Asparagopsis armata* et de *Falkenbergia rufolanosa* (Rhodophycées bonnemaisoniales). *Compte Rendu Hebdomadaire des Séances de l'Académie des Sciences. Paris. Série D*, **284**, 1163–1165.
- Combaut G. and Piovetti L. (1983) A novel acyclic diterpene from the brown alga *Bifurcaria bifurcata*. *Phytochemistry* **22**, 1787–1789.
- Combaut G., Bruneau Y., Codomier L. and Teste J. (1979) Comparative sterols composition of the red alga *Asparagopsis armata* and its tetrasporophyte *Falkenbergia rufolanosa*. *J. Nat. Prod.*, **42**, 150–151.
- Combaut G., Bruneau Y., Teste J. and Codomier L. (1978) Composés halogénés d'une algue rouge, *Falkenbergia rufolanosa* tetrasporophyte d'*Asparagopsis armata*. *Phytochemistry*, **17**, 1661–1663.
- Conde F.R., Churio M.S. and Previtali C.M. (2000) The photoprotector mechanism of mycosporine-like amino acids. Excited-state properties and photostability of *Porphyra* 334 in aqueous solution. *J. Photochem. Photobiol. B.*, **56**, 139–144.
- Connan S. (2004) Etude de la diversité spécifique des macroalgues de la pointe de Bretagne et analyse des composés phénoliques des Phéophycées dominantes. PhD Thesis, University of Western Brittany, Brest (France).
- Connan S., Goulard F., Stiger V., Deslandes E. and Ar Gall E. (2004) Interspecific and temporal variation in phlorotannin levels in an assemblage of brown algae. *Bot. Mar.*, **47** (5), 410–416.
- Connan S., Delisle F., Deslandes E. and Ar Gall E. (2006) Intra-thallus phlorotannin content and antioxidant activities in Phaeophyceae of temperate waters. *Bot. Mar.*, **49**, 39–46.
- Cordero Jr P.A. (2003) Marine foods production and uses of marine algae. *Encyclopedia of Food Sciences and Nutrition*, Elsevier, Oxford pp. 3726–3728.
- Corre S. and Prieur D. (1990) Density and morphology of epiphytic bacteria on the kelp *Laminaria digitata*. *Bot. Mar.*, **33**, 515–523.
- Craigie J.S. (1990) Cells walls. In: *Biology of the Red Algae*, (K.M Cole and R.G Sheath, eds). Cambridge University Press, Cambridge, pp. 221–257.
- Craigie J. S. and Rivero-Carro H. (1992) Agarocolloids from carrageenophytes. *Proceedings of the XIVth International Seaweed Symposium, Abstracts and program*. Université de Bretagne Occidentale, France, p.71.
- Critchley A.T. and Ohno M.(eds) (1998) *Seaweed Resources of the World*. Japan International Cooperation Agency, Yokosuka.
- Cronin G. and Hay M.E. (1996) Induction of seaweed chemical defenses by amphipod grazing. *Ecology*, **77**, 2287–2301.
- Culioli G., Daoudi M., Mesguiche V., Valls R. and Piovetti L. (1999a) Geranylgeraniol-derived diterpenoids from the brown alga *Bifurcaria bifurcata*. *Phytochemistry*, **52**, 1447–1454.
- Culioli G., Mesguiche V., Piovetti L. and Valls R. (1999b) Geranylgeraniol and geranylgeraniol-derived diterpenes from the brown alga *Bifurcaria bifurcata* (Cystoseiraceae). *Biochem. Syst. Ecol.*, **27**, 665–668.
- Culioli G., Di Guardia S., Valls R. and Piovetti L. (2000) Geranylgeraniol-derived diterpenes from the brown alga *Bifurcaria bifurcata*: comparison with two other Cystoseiraceae species. *Biochem. Syst. Ecol.*, **28**, 185–187.
- Culioli G., Daoudi M., Ortalo-Magné A., Valls R. and Piovetti L. (2001) (S)-12-Hydroxygeranylgeraniol-derived diterpenes from the brown alga *Bifurcaria bifurcata*. *Phytochemistry*, **57**, 529–535.
- Culioli G., Ortalo-Magné A., Daoudi M., Thomas-Guyon H., Valls R. and Piovetti L. (2004) Trihydroxylated linear diterpenes from the brown alga *Bifurcaria bifurcata*. *Phytochemistry*, **65**, 2063–2069.
- Da Gama B.A.P., Pereira R.C., Carvalho A.G.V., Coutinho R. and Yoneshigue-Valentin Y. (2002) The effects of seaweed secondary metabolites on biofouling. *Biofouling*, **18**, 13–20.
- Damian D., Barnetsion R. and Halliday G.M. (1999) Measurement of *in vivo* sunscreen immune protection factor in humans. *Photochem. Photobiol.*, **70**, 910–915.
- Damonte E.B., Matulewicz M.C. and Cerezo A.S. (2004) Sulfated seaweed polysaccharides as antiviral agents. *Curr. Med. Chem.*, **11**, 2399–419.
- Daoudi M., Bakkas S., Culioli G., Ortalo-Magne A., Piovetti L. and Guiry M.D. (2001) Acyclic diterpenes and sterols from the genera *Bifurcaria* and *Bifurcariopsis* (Cystoseiraceae, Phaeophyceae). *Biochem. Syst. . Ecol.*, **29**, 973–978.
- Darcy-Vrillon B. (1993) Nutritional aspects of the developing use of marine macroalgae for the human food industry. *Int. J. Food Sci. Nutr.*, **44**, S23–S25.
- Davis A.R., Targett N.M., Mc Connel O.J. and Young C.M. (1989) Epibiosis of marine algae and benthic invertebrates : natural products chemistry and other mechanisms inhibiting settlement and overgrowth. *Bioorg. Mar. Chem.*, **3**, 85–114.

- Davyt D., Enz W., Manta E., Navarro G. and Norte N. (1997) New chromenols from the brown alga *Desmarestia menziesii*. *Nat. Prod. Lett.*, **9**, 305–312.
- Dawson E.Y. (1966) *Marine Botany – An Introduction*. Holt, Rinehart and Wilson, Inc., New-York, Chicago, San Francisco, Toronto, London.
- De Gruijl F.R., Sterenberg H.J.C.M., Forbes P.D., *et al.* (1993) Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. *Cancer Res.*, **53**, 53–60.
- Denis C., Moranc ais M., Li M., Deniaud E., *et al.* (2010) Study of the chemical composition of edible red macroalgae *Grateloupia turuturu* from Brittany (France). *Food Chem.*, **119**, 913–917.
- Denis C., Mass  A., Fleurence J. and Jaouen P. (2009) Concentration and pre-purification with ultrafiltration of a R-phycoerythrin solution extracted from macro-algae *Grateloupia turuturu*: Process definition and up-scaling. *Separation and Purification Technology*, **69**, 37–42.
- Denton A., Chapman A.R.O. and Markham J. (1990) Size-specific concentrations of phlorotannins (anti-herbivore compounds) in three species of *Fucus*. *Mar. Ecol. Prog. Ser.*, **65**, 103–104.
- De Nys R., Steinberg P.D., Willemsen P., Dworjanyn S.A., Gabelish C.L. and King R.J. (1995) Broad spectrum effects of secondary metabolites from the red algae *Delisea pulchra* in antifouling assays. *Biofouling*, **8**, 259–271.
- De Nys R., Dworjanyn S.A. and Steinberg P.D. (1998) A new method for determining surface concentrations of marine natural products on seaweeds. *Mar. Ecol. Prog. Ser.*, **162**, 79–87.
- De Senerpont Domis L.N., Fama P., Bartlett A.J., Prud'homme van reine W.F., Espinosa C.A. and Trono G.C. (2003) Defining taxon boundaries in members of the morphologically and genetically plastic genus *Caulerpa* (Caulerpales Chlorophyta). *J. Phycol.*, **39**, 1019–1037.
- Deslandes E. (1988) Etudes biochimiques des polysaccharides extraits de quelques algues carragh nophytes des c tes de Bretagne. Doctoral Thesis. University of Western Brittany, Brest, France, p.280.
- Deslandes E., Floc'h J.Y., Bodeau-Bellion C., Brault D. and Braud J.P. (1985) Evidence of  -carrageenanin *Solieria chordalis* (Solieriaceae) and *Calliblepharis jubata*, *Calliblepharis ciliata*, *Cystoclonium purpureum* (Rhodophyllidaceae). *Bot. Mar.*, **28**, 317–318.
- Deslandes E., Pondaven P., Auperin T., *et al.* (2000) Preliminary study of the *in vitro* antiproliferative effect of a hydroethanolic extract from the subtropical seaweed *Turbinaria ornata* (Turner) J. Agardh on a human non-small-cell bronchopulmonary carcinoma line (NSCLC-N6). *J. Appl. Phycol.*, **12**, 257–262.
- De Roeck-Holtzhauer Y. (1991) Uses of seaweeds in cosmetics. In: Guiry and Blunden, *Seaweed Resources in Europe: Uses and Potential*. John Wiley & Sons, pp. 83–94.
- Devi P., Solimabi W., D'Souza L., Sonak S., Kamat S.Y. and Singhal S.Y.S. (1997) Screening of some marine plants for activity against marine fouling bacteria. *Bot. Mar.*, **40**, 87–91.
- Dias P.F., Siqueira J.M., Vendruscolo L.F., Neiva T.J., Gagliardi A.R. and Maraschin M. (2005) Antiangiogenic and antitumoral properties of a polysaccharide isolated from the seaweed *Sargassum stenophyllum*. *Cancer Chemother. Pharmacol.*, **56**, 436–446.
- Diaz-Rosales P., Burmeister A., Aguilera J., *et al.* (2005) Screening of algal extracts as potential stimulants of chemotaxis and respiratory burst activity of phagocytes from sole (*Solea senegalensis*). *Bull. Eur. Assoc. Fish Pathologists*, **25**, 9–19.
- Di Guardia S., Valls R., Mesguiche V., Brunel J.-M. and Culioli G. (1999) Enantioselective syntheses of (-)-Bifurcadiol: a natural antitumor marine product. *Tetrahedron Lett.*, **40**, 8359–8360.
- Dizerbo A.H. and Herp  E. (2007) *Liste et r partition des algues marines des c tes fran aises de la Manche et de l'Atlantique, Iles Normandes incluses*.  ditions Anaximandre, Landernau, pp. 1–315, 92.
- Doty M.S. (1988) *Prodromusad systematica Eucheumatoideorum: A tribe of commercial seaweeds related to Eucheuma* (Solieriaceae, Gigartinales). In: Abbott, I.A. (ed.) *Taxonomy of Economic Seaweeds with Reference to some Pacific and Caribbean Species* California Sea Grant College Program, University of California, LaJolla, pp. 159–207.
- Draisma S.G.A., Ballesteros E., Rousseau F. and Thibaut T. (2010) DNA sequence data demonstrate the polyphyly of the genus *Cystoseira* and other sargassaceae genera (phaeophyceae). *J. Phycol.*, **46**, 1329–1345.
- Dubber D. and Harder T. (2008) Extracts of *Ceramium rubrum*, *Mastocarpus stellatus* and *Laminaria digitata* inhibit growth of marine and fish pathogenic bacteria at ecologically realistic concentrations. *Aquaculture*, **274**, 196–200.
- Dunlap W.C. and Shick J.M. (1998) Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective. *J. Phycol.*, **34**, 418–430.
- Dunlap W.C., Chalker B.E., Bandaranayake W.M. and Wuwon J.J. (1998) Nature's sunscreen from the Great Barrier Reef, Australia. *Int. J. Cosm. Sci.*, **20**, 41–51.
- Dunlap W.C. and Yamamoto Y. (1995) Small-molecule antioxidants in marine organisms: Antioxidant activity of mycosporine-glycine. *Comparative Biochemistry and*

- Physiology Part B: Biochemistry and Molecular Biology*, **112**, 105–114.
- Duran R., Zubia E., Ortega M.J. and Salva J. (1997) New diterpenoids from the alga *Dictyota dichotoma*. *Tetrahedron*, **53** (25), 8675–8688.
- Edge R., Mcgarvey D.J. and Truscott T.G. (1997) The carotenoids as anti-oxidants – a review. *J. Photochem. Photobiol.*, **41**, 189–200.
- Egorin M.J., Rosen D.M., Benjamin S.E., Callery P.S., Sentz D.L. and Eiseman J.L. (1997) *In vitro* metabolism by mouse and human liver preparations of halomon, an antitumor halogenated monoterpene. *Cancer Chemother. Pharmacol.*, **41**, 9–14.
- El Gamal Ali A. (2010) Biological importance of marine algae. *Saudi Pharm. J.*, **18** (1), 1–25.
- El Hattab M., Bouzidi N., Ortalo-Magne A., et al. (2009) Eicosapentaenoic acid: Possible precursor of the phloroglucinol derivatives isolated from the brown alga *Zonaria tournefortii* (J.V. Lamouroux) Montagne. *Biochem. Syst. Ecol.*, **37**, 55–58.
- El Hattab M., Ben Mesaoud M., Daoudi M., et al. (2008) Trihydroxylated linear diterpenes from the brown alga *Bifurcaria bifurcata* (Fucales, Phaeophyta). *Biochem. Syst. Ecol.*, **36**, 484–489.
- El Hattab M., Culioli G., Pioveti L., Chitour S.E. and Valls R. (2007) Comparison of various extraction methods for identification and determination of volatile metabolites from the brown alga *Dictyopteris membranacea*. *Journal of Chromatography A*, **1143**, 1–7.
- Elmets C.A. and Anderson C. (1996) Sunscreens and photocarcinogenesis: an objective assessment. *Photochem. Photobiol.*, **63**, 435–440.
- Engel S. and Pawlik J.R. (2000) Allelopathic activities of sponge extracts. *Mar. Ecol. Progr. Ser.*, **207**, 273–281.
- Enoki N., Tsuzuki K., Omura S., Ishida R. and Matsumoto T. (1983) New antimicrobial diterpenes, dictyol F and epidictyol F, from the brown alga *Dictyota dichotoma*. *Chem. Lett.*, **12**, 1627–1630.
- Etienne, M., Jerome, M., Fleurence, J., et al. (2000) Identification of fish species after cooking by SDS-PAGE and urea IEF: a collaborative study. *J. Agric. Food Chem.*, **48**, 2653–2658.
- Etienne M., Jérôme M., Fleurence J., Rehbein H., Kündiger R. and Malmheden-Yman I. (1999) A standardised method for identification of raw and heat processed fish by urea isoelectric focusing: A collaborative study. *Electrophoresis*, **20**, 1923–1933.
- Evans S.M., Birchenough A.C. and Brancata M.S. (2000) The TBT ban: out of the frying pan into the fire? *Mar. Poll. Bull.*, **40** (3), 204–211.
- Fairhead V.A., Amsler C.D., McClintock J.B. and Baker B.J. (2006) Lack of defense of phlorotannins induction by UV radiation or mesograzers in *Desmarestia anceps* and *D. meniesii* (Phaeophyceae). *J. Phycol.*, **42**, 1174–1183.
- FAO (2004) The State of the World Fisheries and Aquaculture 2004 (SOFIA), FAO, Rome, <http://www.fao.org/sof/sofia/index'en.htm> (accessed 31 March 2011).
- Farage M.A., Miller K.W. and Maibach O.I. (eds) (2010) *Textbook of Aging Skin*. Springer-Verlag Berlin Heidelberg.
- Fisch K.M., Böhm V., Wright A.D. and König G.M. (2003) Antioxidative meroterpenoids from the brown alga *Cystoseira crinita*. *J. Nat. Prod.*, **66**, 968–975.
- Fleurence J. (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.*, **10**, 25–28.
- Fleurence J. and Guyader O. (1995) Apport de l'électrophorèse à l'identification d'algues rouges (*Gracilaria* sp.) à usage alimentaire. *Sciences des Aliments*, **15**, 43–48.
- Fleurence J., Massiani L., Guyader O. and Mabeau S. (1995a) Use of enzymatic cell wall degradation for improvement of protein extraction from *Chondrus crispus*, *Gracilaria verrucosa* and *Palmaria palmata*. *J. Appl. Phycol.*, **7**, 393–395.
- Fleurence J., Le Cœur C., Mabeau S., Maurice M. and Landrein A. (1995b) Comparison of different extractive procedures for proteins from the edible seaweeds *Ulva rigida* and *Ulva rotundata*. *J. Appl. Phycol.*, **7**, 577–582.
- Fournet I., Zinoun M., Deslandes E., Diouris M. and Floc'h J.Y. (1999) Floridean starch and carrageenan contents as responses of the red alga *Solieria chordalis* to culture conditions. *Eur. J. Phycol.*, **34**, 125–130.
- Franklin L.A., Yakovleva I., Karsten U. and Lüning K. (1999) Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae) and the consequences for sensitivity to ultraviolet B radiation. *J. Phycol.*, **35**, 682–693.
- Freile-Pelegrín Y. and Morales J.L. (2004) Antibacterial activity in marine algae from the coast of Yucatan, Mexico. *Bot. Mar.*, **47** (2), 140–146.
- Fuchs J. (1998) Potentials and limitations of the natural antioxidants RRR- $\alpha$ -tocopherol, L-ascorbic acid and  $\beta$ -carotene in cutaneous photoprotection. *Free Rad. Biol. Med.*, **25**, 848–873.
- Fusetani N. (2004) Biofouling and antifouling. *Nat. Prod. Rep.*, **21**, 94–104.
- Gabrielson P.W. and Hommersand M.H. (1982) The morphology of *Agardhiella subulata* representing the Agardhielleae, a new tribe in the Solieriaceae (Gigartinales, Rhodophyta). *J. Phycol.*, **18**, 46–58.
- Galland-Irmouli A.V., Fleurence J., Lamghari R., et al. (1999) Nutritional value of proteins from edible

- seaweed *Palmaria palmata* (Dulse). *J. Nutr. Biochem.*, **10** (6), 353–359.
- Gantt, E. and 18 intermediate authors & S. H. Brawley. 2010. Porphyra: Complex life histories in a harsh environment. P. umbilicalis, the genomics' project. In: J. Seckbach & D. Chapman (eds), *Red Algae in the Genomic Age* (v. 13, Cellular Origins, Life in Extreme Habitats and Astrobiology), Springer
- Gaquerel E., Hervé C., Labrière C., Boyen C., Potin P. and Salaün JP (2007) Evidence for oxylipin synthesis and induction of a new polyunsaturated fatty acid hydroxylase activity in *Chondrus crispus* in response to methyl jasmonate. *Biochim. Biophys. Acta*, **1771**, 565–575.
- Glazer A.N. (1989) Light guides. *J. Biol. Chem.*, **264**, 1–4.
- Goff L.J., Moon D.A. and Coleman W. (1994) Molecular delineation of species and Species relationships in the red algal agarophytes *Gracilariopsis* and *Gracilaria* (Gracilariales). *J. Phycol.*, **30**, 521–537.
- Gomes C.A., Girão da Cruz T., Andrade J.L., Milhazes N., Borges F. and Marques M.P.M. (2003) Anticancer activity of phenolic acids of natural or synthetic origin: A structure-activity study. *J. Med. Chem.*, **46**, 5395–5401.
- Gonzales M.E., Alarcon B. and Carrasco L. (1987) Polysaccharides as antiviral agents: Antiviral activity of carrageenan. *Antimicrob. Agents Chemother.*, **31**, 1388–1393.
- Gonzales M.E., Crance J.M., VanCuyck-Gandre H., Renaudet J. and Deloince R. (1991) Antiviral activity of carrageenan on hepatitis A virus replication in cell culture. *Res. Virol.*, **142**, 261–270.
- Goulard F., Diouris M., Quere E., Deslandes E. and Floc'h J.Y. (2001) Salinity effects on NDP-sugars, floridoside, starch, and carrageenan yield, and UDP-glucose- pyrophosphorylase and-epimerase activities of cultivated *Solieria chordalis*. *J. Plant Physiol.*, **158**, 1387–1394.
- Goultiquet S., Ritter A., Thomas F., Ferec C., Salaün J.-P. and Potin P. (2009) Release of volatile aldehydes by the brown algal kelp *Laminaria digitata* in response to both biotic and abiotic stress. *Chem. Bio Chem.*, **10**, 977–82.
- Guiry M.D., Guiry G.M. (2010) *AlgaeBase*, World-wide electronic publication, National University of Ireland, Galway, <http://www.algaebase.org> (accessed 31 March 2011).
- Harlin M. 1996. Allelochemistry in marine algae. *CRC Crit. Rev. Plant Sci.*, **5** (3), 237–249.
- Haslin C. and Pellegrini M. (2001) Culture medium composition for optimal thallus regeneration in the red alga *Asparagopsis armata* Harvey (Rhodophyta, Bonnemaisoniaceae). *Bot. Mar.*, **44**, 23–30.
- Haslin C., Lahaye M. and Pellegrini M. (2000) Chemical composition and structure of sulphated water-soluble cell-wall polysaccharides from gentic, carposporic and tetrasporic stages of *Asparagopsis armata* Harvey (Rhodophyta, Bonnemaisoniaceae). *Bot. Mar.*, **43**, 475–482.
- Haslin C., Lahaye M., Pellegrini M. and Chermann J.-C. (2001) *In vitro* anti-HIV activity of sulfated cell-wall polysaccharides from gentic, carposporic and tetrasporic stages of the Mediterranean red alga *Asparagopsis armata*. *Planta Med.*, **67**, 301–305.
- Haug A., Larsen B., Smidsrod O. and Painter T. (1969) Development of compositional heterogeneity in alginate degraded in homogeneous solution. *Acta Chem. Scand.*, **23**, 2955–2962.
- Hay M.E. (1996) Marine chemical ecology: what's known and what's next? *J. Exp. Mar. Biol. Ecol.*, **200**, 103–134.
- Hay M.E. and Steinberg P.D. (1992) The chemical ecology of plant–herbivore interactions in marine versus terrestrial communities. In: Rosenthal, L., Berenbaum, M. (Eds.), *Herbivores: Their Interaction with Secondary Plant Metabolites*. Academic Press, New York, pp. 371–413.
- Hellio C., De La Broise D., Dufosse L., Le Gal Y. and Bourgougnon N. (2000a) Inhibition of marine bacteria by extracts of macroalgae: potential use for environmentally friendly antifouling paints. *Mar. Env. Res.*, **52** (3), 231–247.
- Hellio C., Bremer G., Pons A.-M., Le Gal Y. and Bourgougnon N. (2000b) Antibacterial and antifungal activities of extracts of marine algae from Brittany (France): the potential for use as antifouling compounds. *Appl. Microbiol. Biotechnol.*, **54**, 543–549.
- Hellio C., Bourgougnon N. and Le Gal Y. (2000c) Phenoloxidase (E.C. 1.14.18.1) from *Mytilus edulis* byssus gland: purification, partial characterization and application to a new test for screening products with potential antifouling activities. *Biofouling*, **16**, 235–244.
- Hellio C., Thomas-Guyon H., Culioli G., Piovetti L., Bourgougnon N. and Le Gal Y. (2001) Marine antifoulants from *Bifurcaria bifurcata* (Phaeophyceae, Cystoseiraceae) and other brown macroalgae. *Biofouling*, **17**, 189–201.
- Hellio C., Pons A.-M., Beaupoil C., Le Gal Y. and Bourgougnon N. (2002a) Antimicrobial activities of extracts from fishes epiderm and epidermal mucus. *Int. J. Antimicrob. Agents*, **20** (3), 220–225.
- Hellio C., Bergé J.-P., Beaupoil C., Le Gal Y. and Bourgougnon N. (2002b) Screening of Marine Algal extracts for anti-settlement activities against microalgae and spores of macroalgae. *Biofouling*, **18** (3), 205–215.
- Hellio C., Maréchal J.P., Véron B., Bremer G., Clare A.S. and Le Gal Y. (2004) Seasonal variation of antifouling activities of marine algae from the Brittany Coast (France). *Mar. Biotechnol.*, **6**, 67–82.
- Hemmi A., Makinen A., Jormalainen V. and Honkanen T. (2005) Responses of growth and phlorotannins in *Fucus vesiculosus* to nutrient enrichment and herbivory. *Aquat. Ecol.*, **39**, 201–211.

- Hemmingson J.A., Falshaw R., Furneaux R.H. and Thompson K. (2006) Structure and antiviral activity of the galactofucan sulfates extracted from *Undaria pinnatifida* (Phaeophyta). *J. Appl. Phycol.*, **18**, 185–193.
- Henry B.E. and Van Alstyne K.L. (2004) Effects of UV radiations on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. *J. Phycol.*, **40**, 527–533.
- Herrero M., Cifuentes A. and Ibanez E. (2006) Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. *Food Chem.*, **98**, 136–148.
- Hotimchenko S.V. (2002) Fatty acid composition of algae from habitats with varying amounts of illumination. *Russ. J. Mar. Biol.*, **28** (3), 218.
- Hou W.Y. and Chen J. C. (2005) The immunostimulatory effect of hot-water extract of *Gracilaria tenuistipitata* on the white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. *Fish Shellfish Immunol.*, **19**, 127–138.
- Hougaard L., Anthoni U., Christophersen C. and Nielsen P.H. (1991) Eleanolone derived diterpenes from *Bifurcaria bifurcata*. *Phytochemistry*, **30**, 3049–3051.
- Hoyer K., Karsten U., Sawall T. and Wiencke C. (2001) Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages. *Mar. Ecol. Prog. Ser.*, **211**, 117–129.
- Hyvärinen M., Koopmann R., Hormi O. and Tuomi J. (2000) Phenols in reproductive and somatic structures of lichens: a case of optimal defence? *Oikos*, **91**, 371–375.
- Ikedo K., Kitamura A., Machida H., Watanabe M., Negishi H. and Hiraoka J. (2003) Effect of *Undaria pinnatifida* (Wakame) on the development of cerebrovascular diseases in stroke-prone spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.*, **30**, 44–48.
- Indegaard M. and Minsaas J. (1991) *Animal and Human Nutrition. Seaweed Resources in Europe. Uses and Potential* (Guiry M.D. and Blunden G., eds), John Wiley & Sons, Chichester, pp. 21–64.
- Ioannou E. and Roussis V. (2009) *Natural Products From Seaweeds*. A.E. Osbourn and V. Lanzotti (eds.), Plant-derived Natural Products, Springer Science, Berlin, pp. 51–81.
- Jegou C., Culioli G., Kervarec N., Simon G. and Stiger-Pouvreau V. (2010) LC/ESI-MSn and 1H HR-MAS NMR analytical methods as useful taxonomical tools within the genus *Cystoseira* C. Agardh (Fucales; Phaeophyceae). *Talanta*, **83**, 613–622.
- Jensen K.R. (1993) Morphological adaptations and plasticity of radular teeth of the Sacoglossa (=Ascoglossa) (Mollusca: Opisthobranchia) in relation to their food plants. *Biol. J. Linn. Soc.*, **48**, 135–155.
- Jimenez-Escrig A., Jimenez-Jimenez I., Pulido R. and Saura-Calixto F. (2001) Antioxidant activity of fresh and processed edible seaweeds. *J. Sci. Food Agric.*, **81**, 530–534.
- Jothisarawathi S., Babu B. and Rengasamy R. (2006) Seasonal studies on alginate and its composition. II: *Turbina-ria conoides* (J.Ag.) Kütz. (Fucales, Phaeophyceae). *J. Appl. Phycol.*, **18**, 161–166.
- Joubert Y., Ben Abdeladhim L., Ksouri J. and Fleurence J. (2009) Development of a molecular method for the rapid discrimination of red seaweeds used for agar production. *Food Chem.*, **113** (4), 1384–1386.
- Kalvas A. and Kautsky L. (1998) Morphological variation in *Fucus vesiculosus* populations along temperature and salinity gradients in Iceland. *J. Mar. Biol. Assoc. UK*, **78**, 985–1001.
- Karabay-Yavasoglu N.U., Sukatar A., Ozdemir G. and Horzum Z. (2007) Antimicrobial activity of volatile components and various extracts of the red alga *Jania rubens*. *Phytother. Res.*, **21**, 153–156.
- Karentz D. (1994) Ultraviolet tolerance mechanisms in Antarctic marine organisms. In: *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*. Antarctic Research Series, **62**, 93–110.
- Karsten U., Sawall T. and Wiencke C. (1998a) A survey of the distribution of UV-absorbing substances in tropical macroalgae. *Phycol. Res.*, **46**, 271–279.
- Karsten U., Franklin L.A., Lüning K. and Wiencke C. (1998b) Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like aminoacids in the marine macroalga *Chondrus crispus* (Rhodophyta). *Planta*, **205**, 257–262.
- Keyrouz R., Abasq M.L., Le Bourvellec C., et al. (2011) Total phenolic contents, radical scavenging and cyclic voltammetry of seaweeds from Brittany. *Food Chem.*, **126** (3), 831–836.
- Kladi M., Vagias C. and Roussis V. (2004) Volatile halogenated secondary metabolites from marine red algae. *Phytochem. Rev.*, **3**, 337–366.
- Kloareg B. and Quatrano R. (1988) Structure of the cell walls of marine algae and ecophysiological function of the matrix polysaccharides. *Annu. Rev. Ocean Marine Biol.*, **26**, 259–315.
- Kloareg B., Demarty M. and Mabeau S. (1986) Polyanionic characteristics of purified sulfated homofucans from brown algae. *Int. J. Biol. Macromol.*, **8**, 380–386.
- Knutsen S.H., Myslabodski D.E., Larsen B. and Usov A.I. (1994) A modified system of nomenclature for red algal galactans. *Bot. Mar.*, **37**, 163–169.
- Koivikko R., Eränen J., Loponen J. and Jormalainen V. (2008) Variation of phlorotannins among three populations of *Fucus vesiculosus* as revealed by HPLC and colorimetric quantification. *J. Chem. Ecol.*, **34**, 57–64.

- Koivikko R., Lopenen J., Pihlaja J.K. and Jormalainen V. (2007) High performance liquid chromatography analysis of phlorotannins from the brown alga *Fucus vesiculosus*. *Phytochem. Anal.*, **18**, 326–332.
- Kolesnikova S.A., Kalinovsky A.I., Fedorov S.N., Shubina L.K. and Stonik V.A. (2006) Diterpenes from the Far-eastern brown alga *Dictyota dichotoma*. *Phytochemistry*, **67**, 2115–2119.
- Konstantinou I.K. and Albanis T.A. (2004) Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review. *Environ. Int.* **30**, 235–248.
- Korbee N., Huovinen P., Figueroa F.L., Aguilera J. and Karsten U. (2005) Availability of ammonium influences photosynthesis and the accumulation of mycosporine-like aminoacids in two *Porphyra* species (Bangiales, Rhodophyta). *Mar. Biol.*, **146**, 645–654.
- Kornprobst J.M. (2005) *Substances naturelles d'origine marine, Tome 1 : Généralités, micro-organismes, algues*. Editions Tec and Doc, Paris.
- Kubanek J., Jensen P.R., Keifer P.A., Sullards M.C., Collins D.O. and Fenical W. (2003) Seaweed resistance to microbial attack: a targeted chemical defense against marine fungi. *Proc. Natl Acad. Sci. USA*, **100**, 6916–6921.
- Kuda T., Kunii T., Goto H., Suzuki T. and Yano T. (2007) Varieties of antioxidant and antibacterial properties of *Ecklonia stolonifera* and *Ecklonia kurome* products harvested and processed in the Noto peninsula, Japan. *Food Chem.*, **103**, 900–905.
- Kumar Chandini S., Ganesan P. and Bhaskar N. (2008) *In vitro* antioxidant activities of three selected brown seaweeds of India. *Food Chem.*, **107**, 707–713.
- Küpper F.C., Kloareg B., Guern J. and Potin P. (2001) Oligoguluronates elicit an oxidative burst in the brown algal kelp *Laminaria digitata*. *Plant Physiol.*, **125**, 278–291.
- Küpper F.C., Müller D.G., Peters A.F., Kloareg B. and Potin P. (2002) Oligoalginat recognition and oxidative burst play a key role in natural and induced resistance of the sporophytes of Laminariales. *J. Chem. Ecol.*, **28**, 2057–2081.
- Küpper F.C., Carpenter L.J., McFiggans G.B., Palmer C.J. and Waite T.J. (2008) Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. *Proc. Natl Acad. Sci. USA*, **105**, 6954–6958.
- Küpper F.C., Gaquerel E., Cosse A., et al. (2009) Free fatty acids and methyl jasmonate trigger defense reactions in *Laminaria digitata*. *Plant Cell Physiol.*, **50** (4), 789–800.
- Kurata K., Taniguchi K., Suzuki M. (1996) Cyclozonarone, a sesquiterpene-substituted benzoquinone derivative from the brown alga *Dyctiopteris undulata*. *Phytochemistry*, **41**, 749–752.
- Kwon M.J. and Nam T.J. (2007) A polysaccharide of the marine alga *Capsosiphon fulvescens* induces apoptosis in AGS gastric cancer cells via an IGF-IR-mediated PI3K/Akt pathway. *Cell Biol. Int.*, **31**, 768–775.
- La Barre S. and Haras D. (2007) Encounters with marine bacteria. *J. Soc. Biol.*, **201** (3), 281–289.
- La Barre S., Weinberger F., Kervarec N. and Potin P. (2004) Monitoring defensive responses in macroalgae: limitations and perspectives. *Phytochem. Rev.*, **3**, 371–379.
- Lahaye M. (1995) Algal polysaccharides. Workshop on Carbohydrates from algae: occurrence, structure and Properties. University of Qatar, 50p.
- Lahaye M. and Vigouroux J. (1992) Liquefaction of dulce (*Palmaria palmata* (L.) Kuntze) by a commercial enzyme preparation and purified endo- $\beta$ -1,4-d-xylanase'. *J. Appl. Phycol.*, **4**, 329–337.
- Lahaye M., Michel C. and Barry J.L. (1993) Chemical, physicochemical and *in-vitro* fermentation characteristics of dietary fibres from *Palmaria palmata* (L.) Kuntze. *Food Chem.*, **47** (1), 29–36.
- Larsen B., Salem D.M.S.A., Sallam M.A.E., Mishrikey M.M. and Beltagy A.I. (2003) Characterization of the alginates from algae harvested at the Egyptian Red Sea coast. *Carbohydrate Res.*, **338**, 2325–2336.
- Le Gal Y. (1988) *Biochimie marine*. Masson, Paris.
- Le Lann K. (2009) Etude de la biodiversité des Sargassaceae (Fuciales, Phaeophyceae) en milieux tempérés et tropicaux : écologie, chimiotaxonomie et source de composés bioactifs. PhD Thesis of the University of Western Brittany (UBO) under the seal of the European University of Brittany (UEB)
- Le Lann K. and Stiger-Pouvreau V. (2009) Spatio-temporal phenologies of temperate Sargassaceae: coexistence of invasive and native species. *Phycologia*, **48** (4 suppl), 74.
- Le Lann K., Kervarec N., Payri C.E., Deslandes E. and Stiger-Pouvreau V. (2008a) Discrimination of allied species within the genus *Turbinaria* (Fuciales, Phaeophyceae) using HRMAS NMR spectroscopy. *Talanta*, **74**, 1079–1083.
- Le Lann K., Jegou C. and Stiger-Pouvreau V. (2008b) Impact of different conditioning treatments on total phenolic content and antioxidant activities in two Sargassacean species: comparison of the frondose *Sargassum muticum* (Yendo) Fensholt and the cylindrical *Bifurcaria bifurcata* R. Ross. *Phycol. Res.*, **56**, 238–245.
- Le Tutour B., Benslimane F., Gouleau M.P., Gouygou J.P., Saadan B. and Quemeneur F. (1998) Antioxidant and pro-oxidant activities of the brown algae, *Laminaria digitata*, *Himanthalia elongata*, *Fucus vesiculosus*, *Fucus serratus* and *Ascophyllum nodosum*. *J. Appl. Phycol.*, **10**, 121–129.
- Lüder U. and Clayton M. (2004) Induction of phlorotannins in the brown macroalga *Ecklonia radiata* (Laminariales, Phaeophyta) in response to simulated herbivory – the first microscopic study. *Planta* **218**, 928–937.

- Mabeau S. and Fleurence J. (1993) Seaweed in food products: Biochemical and nutritional aspects. *Trends Food Sci. Technol.*, **4**, 103–107.
- Mabeau S., Kloareg B. and Joseleau J.P. (1990) Fractionations and analysis of fucans from brown algae. *Phytochemistry*, **29**, 2441–2445.
- Mackie I., Craig A., Etienne M., Jérôme M., Fleurence J. and Jessen F. (2000) Species identification of smoked and gravad fish products by sodium dodecyl sulphate polyacrylamide gel electrophoresis, urea isoelectric focusing and native isoelectric focusing: A collaborative study. *Food Chem.*, **71**, 1–7.
- Mackie I.M., Pryde S.E., Gonzales-Sotelo C., Medina I., Pérez-Martin R. and Quinteiro J. (1999) Challenges in identification of species of canned fish. *Trends Food Sci. Technol.*, **10**, 9–14.
- Maeda H., Hosokawa M., Sashima T., Funayama K. and Miyashita K. (2005) Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commun.*, **332** (2), 392–397.
- Mardsen W.J.N., Callow J.A. and Evans L.V. (1981) A novel and comprehensive approach to the extraction of enzymes from brown algae and their separation by polyacrylamide gel electrophoresis. *Mar. Biol. Lett.*, **2**, 353–362.
- Mardsen W.J.N., Evans L.V., Callow J.A. and Keen J.N. (1984) A preliminary electrophoretic comparison of *Fucus serratus* and *Fucus vesiculosus*. *Bot. Mar.*, **22**, 79–83.
- Maréchal J.P., Hellio C. (2011) Antifouling activity against barnacle cypris larvae: Do target species matter (*Amphibalanus amphitrite* versus *Semibalanus balanoides*)? *Int. Biodeter. Biodegrad.*, **65** (1), 92–101.
- Maréchal J.P. and Hellio C. (2009) Challenges for the development of new non-toxic antifouling solutions. *Int. J. Mol. Sci.*, **10**, 4623–4637.
- Maréchal J.P., Culioli G., Hellio C., *et al.*, (2004) Seasonal variation in antifouling activity of crude extracts of the brown alga *Bifurcaria bifurcata* (Cystoseiraceae) against cyprids of *Balanus amphitrite* and the marine bacteria *Cobetia marina* and *Pseudoalteromonas haloplanktis*. *J. Exp. Mar. Biol. Ecol.*, **313**, 47–62.
- Matsukawa R., Dubinsky Z., Kishimoto E., *et al.* (1997) A comparison of screening methods for antioxidant activity in seaweeds. *J. Appl. Phycol.*, **9**, 29–35.
- Mattio L., Payri C.E. and Stiger-Pouvreau V. (2008) Taxonomic revision of the genus *Sargassum* (Fucales, Phaeophyceae) from French Polynesia based on morphological and molecular analyses. *J. Phycol.*, **44**, 1541–1555.
- Mayer A.M.S. (2002) Current marine pharmacology contributions to new drug development in the biopharmaceutical industry. *Pharm. New.*, **9**, 479–482.
- Mayer A.M.S. and Gustafson K.R. (2006) Marine pharmacology in 2003–2004: Antitumour and cytotoxic compounds. *Eur. J. Cancer*, **42**, 2241–2270.
- Mayer A.M.S. and Hamann M.T. (2004) Marine pharmacology in 2000: marine compounds with antibacterial, anticoagulant, antifungal, antiinflammatory, antimalarial, antiplatelet, antituberculosis, and antiviral activities; affecting the cardiovascular, immune, and nervous systems and other miscellaneous mechanisms of action. *Mar. Biotechnol. (NY)*, **6**, 37–52.
- Mayer A.M.S. and Hamann M.T. (2005) Marine pharmacology in 2001–2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.*, **140**, 265–286.
- Mayer A.M.S., Rodríguez A.D., Berlinck R.G.S. and Hamann M.T. (2007) Marine pharmacology in 2003–4: Marine compounds with anthelmintic antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.*, **145**, 553–581.
- Mayer A.M.S., Rodríguez A.D., Roberto B.G.S., Hamann, M.T. 2009. Marine pharmacology in 2005–6: Marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. *Biochimica and Biophysica Acta (BBA) - General Subjects*, 1790: 283–308.
- Mazumder S., Ghosal P.F., Pujol C.A., Carlucci M.J., Dammonte E.B. and Ray B. (2002) Isolation, chemical investigation and antiviral activity of polysaccharides from *Gracilaria corticata* (Gracilariaceae, Rhodophyta). *Int. J. Biol. Macromolecules*, **31**, 87–95.
- Michel G., Tonon T., Scornet D., Cock J.M. and Kloareg B. (2010) The cell wall polysaccharide metabolism of the brown alga *Ectocarpus siliculosus*. Insights into the evolution of extracellular matrix polysaccharides in Eukaryotes. *New Phytol. Special Issue* **188** (1), 82–97.
- Miller I.J. (1996) Alginate composition of some New Zealand brown Seaweeds. *Phytochemistry*, **41**, 1315–1317.
- Miranda C.D. and Zemelman R. (2002) Antimicrobial multiresistance in bacteria isolated from freshwater Chilean

- salmon farms. *Sciences of the Total Environment*, **293**, 207–218.
- Misonou T., Saitoh J. and Oshiba S. (2003) UV-absorbing substance in the red alga *Porphyra yezoensis* (Bangiales, Rhodophyta) block thymine photodimer production. *Mar. Biotechnol.*, **5**, 194–200.
- Mizukami Y., Kito H., Kaminishi Y., Murase N. and Kunitomo M. (1999) Nucleotide sequence variation in the ribosomal internal transcribed spacer regions of cultivated (cultivars) and field-collected thalli of *Porphyra yezoensis*. *Fish Sci.*, **65**, 788–789.
- Mokrini R., Mesaoud M., Daoudi M., *et al.* (2008) Meroditerpenoids and derivatives from the brown alga *Cystoseira baccata* and their antifouling properties. *J. Nat. Prod.*, **71**, 1806–1811.
- Monthana J.A., Bourgougnon N., Boustie J. and Amoros M. (2009) Antiviral Activity of Carrageenans from Marine Red Algae. *Lat. Am. J. Pharm.*, **28** (3), 443–8.
- Moreau D., Thomas-Guyon H., Jacquot C., Jugé M., Culioli G. and Ortalo-Magné A. (2006) An extract from the brown alga *Bifurcaria bifurcata* induces irreversible arrest of cell proliferation in a non-small-cell bronchopulmonary carcinoma line. *J. Appl. Phycol.*, **18**, 87–93.
- Morgan K.C., Wright J.L.C. and Simpson F.J. (1980) Review of chemical constituents of the red alga *Palmaria palmata* (Dulse). *Econ. Bot.*, **34**, 27–50.
- Mori H. and Nisizawa K. (1982) Sugar constituents of sulfated polysaccharides from the fronds of *Sargassum ringgoldianum*. *Bull. J. Soc. Sci. Fish.*, **48**, 981–986.
- Murano E., Toffanin R., Cecere E., Rizzo R. and Knutsen S.H. (1997) Investigation of the carrageenans extracted from *Solieria filiformis* and *Agardhiella subulata* from Mar Piccolo, Taranto. *Mar. Chem.*, **58**, 319–325.
- Mustafa M.G. and Nagakawa H. (1995) A review: Dietary benefits of algae as an additive in fish feed. *Israeli J. Aquaculture – Bamindgeh*, **47**, 155–162.
- Nakai M., Kagayama N., Nakahara K. and Miki W. (2006) Phlorotannins as radical scavengers from the extract of *Sargassum ringgoldianum*. *Mar. Biotechnol.*, **8**, 409–414.
- Nakamura T., Nagayama K., Uchida K. and Tanaka R. (1996) Antioxidant activity of phlorotannins isolated from the brown alga *Eisenia bicyclis*. *Fish. Sci.*, **62**, 923–926.
- Nakayama R., Tamura Y., Kikuzaki H. and Nakatani N. (1999) Antioxidant effect of the Constituents of Susabinori (*Porphyra yezoensis*). *IAOCS*, **76**, 649–653.
- Nishino T., Nishioka C., Ura H. and Nagumo (1994) Isolation and partial characterisation of a novel aminosugar-containing fucan sulphate from commercial *Fucus vesiculosus* fucoidan. *Carbohydr. Res.*, **255**, 213–224.
- Nisizawa K., Noda H., Kikuchi R. and Watanabe T. (1987) The main seaweed foods in Japan. *Hydrobiologia*, **151/251**, 92–5.
- Norval M., El-Ghorr A., Garssen J. and Van Loveren H. (1994) The effects of ultraviolet light irradiation on viral infections. *Br. J. Dermatol.*, **130**, 693–700.
- Okai Y., Ishizaka S., Higashi-Okai K. and Yamashita U. (1996) Detection of immunomodulating activities in an extract of Japanese edible seaweed *Laminaria japonica* (Makonbu). *J. Sci. Food Agric.*, **72**, 455–460.
- Onofrejová L., Vasícková J., Klejdusa B., *et al.* (2010) Bioactive phenols in algae: The application of pressurized-liquid and solid-phase extraction techniques. *J. Pharm. Biomed. Anal.*, **51**, 464–470.
- Opoku G., Qiu X. and Doctor V. (2006) Effect of over sulfatation on the chemical and biological properties of kappa carrageenan. *Carbohydr. Polym.*, **65**, 134–138.
- Ortalo-Magné A., Culioli G., Valls R., Pucci B. and Piovetti L. (2005) Polar acyclic diterpenoids from *Bifurcaria bifurcata* (Fucales, Phaeophyta). *Phytochemistry*, **66**, 2316–2323.
- Paul V.J. and Puglisi M.P. (2004) Chemical mediation of interactions among marine organisms. *Nat. Prod. Rep.*, **21**, 189–209.
- Paul V.J., Cruz-Rivera E. and Thacker R.W. (2001) Chemical mediation of macroalgal–herbivore interactions: ecological and evolutionary perspectives. In: McClintock, J.B. and Baker, B.J. (eds), *Marine Chemical Ecology*. CRC Press, Boca Raton, pp. 227–265.
- Pavia H. and Toth G. (2000) Influence of light and nitrogen on the phlorotannin content of the brown seaweed *Ascophyllum nodosum* and *Fucus vesiculosus*. *Hydrobiologia*, **440**, 299–305.
- Pavia H., Cervin G., Lindgren A. and Aberg P. (1997) Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. *Mar. Ecol. Prog. Ser.*, **157**, 139–146.
- Pec M.K., Aguirre A., Moser-Their K., Fernandez J.J., Souto M.L. and Dorta J. (2003) Induction of apoptosis in estrogen dependent and independent breast cancer cells by the marine terpenoid dehydrothysiferol. *Biochem. Pharmacol.*, **65**, 1451–1461.
- Pedersen A. (1984) Studies on phenol content and heavy metal uptake in fucoids. *Hydrobiologia*, **116/117**, 498–504.
- Pereira H.S., Leão-Ferreira L.R., Moussatché N., *et al.* (2004) Antiviral activity of diterpenes isolated from the Brazilian marine alga *Dictyota menstrualis* against human immunodeficiency virus type 1 (HIV-1). *Antivir. Res.*, **64**, 69–76.
- Perez M., Blustein G., García M., del Amo B. and Stupak M. (2006) Cupric tannate: A low copper content antifouling pigment. *Prog. Org. Coat.*, **55**, 311–315.
- Pérez R. (1997) *Ces algues qui nous entourent. Conception actuelle, rôle dans la biosphère, utilisation, culture*. Ifremer, Brest.

- Perez R., Kaas R., Campello F., Arbault S. and Barbaroux O. (1992) *La culture des algues marines dans le monde*. Ifremer, France
- Pérez-Rodríguez E., Gómez I., Karsten U. and Figueroa F.L. (1998) Effects of UV radiation on photosynthesis and excretion of UV-absorbing compounds of *Dasycladus vermicularis* (Dasycladales, Chlorophyta) from southern Spain. *Phycologia*, **37**, 379–387.
- Pesando D. (1990) Antibacterial and antifungal activities of marine algae. In: *Introduction to Applied Phycology* (I. Akatsuka, ed.). SPB Academic Publishing bv, The Hague. pp. 3–27.
- Plouguerné E. (2006) Etude écologique et chimique de deux algues récemment introduites sur les côtes bretonnes, *Grateloupia turuturu* Yamada et *Sargassum muticum* (Yendo) Fensholt: nouvelles ressources biologiques de composés à activité antifouling. PhD dissertation. Université de Bretagne Occidentale, Brest
- Plouguerné E., Le Lann K., Connan S., Jechoux G., Deslandes E. and Stiger-Pouvreau V. (2006a) Spatial and seasonal variations in density, maturity, length and phenolic content of the invasive brown macroalga *Sargassum muticum* along the coast of Western Brittany (France). *Aquat. Bot.*, **85**, 337–344.
- Plouguerné E., Oshima Y., Deslandes E. and Stiger-Pouvreau V. (2006b) Isolation and characterization of Cholesteryl formate in *Grateloupia turuturu*. *Biochem. Syst. Ecol.*, **34**, 714–717.
- Plouguerné E., Hellio C., Deslandes E., Véron B., Bremer G. and Stiger-Pouvreau V. (2008) Anti micro-fouling activities of extracts of two of invasive algae: *Grateloupia turuturu* and *Sargassum muticum*. *Bot. Mar.*, **51**, 202–208.
- Plouguerné E., Georgantea P., Ioannou E., Georgantea P., Vagias C., Roussis V., Hellio C., Kraffe E. and Stiger-Pouvreau V. (2010) Anti-microfouling activity of lipidic metabolites from the invasive brown alga *Sargassum muticum* (Yendo) Fensholt. *Mar. Biotechnol.*, **12** (1), 52–61.
- Potin P. and Leblanc C. (2006) Phenolic-based adhesives of marine brown algae. *Biological Adhesives*, pp 105–124.
- Potin P., Bouarab K., Salaun J.P., Pohnert G. and Kloareg B. (2002) Biotic interactions of marine algae. *Curr. Opin. Plant Biol.*, **5**, 308–317.
- Potin P., Bouarab K., Küpper E.C. and Kloareg B. (1999) Oligosaccharide recognition signals and defence reactions in marine plant-microbe interactions. *Curr. Opin. Microbiol.*, **2**, 276–283.
- Pruja S. (1986) Contribution à l'étude de la composition chimique de *Sargassum muticum*. Thèse de Doctorat en Pharmacie, Université de Nantes, France.
- Pujol C.A., Scolaro L.A., Cianca M., Matulewicz M.C., Cerezo A.S. and Damonte E.B. (2006) Antiviral activity of a carrageenan from *Gigartina skottsbergii* against intraperitoneal murine herpes simplex virus infection. *Planta Med.*, **72**, 121–125.
- Raa J. (1996) The use of immunostimulatory substances in fish and shellfish farming. *Rev. Fish. Sci.*, **4**, 229–288.
- Ragan M.A. and Glombitza K.-W. (1986) Phlorotannins, brown algal polyphenols. *Progr. Phycol. Res.*, **4**, 129–241.
- Ragan M.A. and Jensen A. (1978) Quantitative studies on brown algal phenols. II. Seasonal variation in polyphenol content of *Ascophyllum nodosum* (L.) Le Jol. and *Fucus vesiculosus* (L.). *J. Exp. Mar. Biol. Ecol.*, **34**, 245–258.
- Reddy P. and Urban S. (2008) Linear and cyclic C18 terpenoids from the southern Australian marine brown alga *Cystophora moniliformis*. *J. Nat. Prod.*, **71**, 1441–1446.
- Rice-Evans C.A., Miller N.J. and Paganga G. (1997) Antioxidant properties of phenolic compounds. *Trends Plant Sci.*, **2**, 152–159.
- Riou D., Collic-Jouault S., Pinczon Du Sel D., et al. (1996) Antitumor and antiproliferative effects of a fucan extracted from *Ascophyllum nodosum* against a non-small-cell bronchopulmonary carcinoma line. *Anticancer Res.*, **16**, 1213–1218.
- Robles-Centeno P.O., Ballantine D.L. and Gerwick W.H. (1996) Dynamics of antibacterial activity in three species of Caribbean marine algae as a function of habitat and life history. *Hydrobiologia*, **326/327**, 457–462.
- Rohfritsch A., Payri C., Stiger V. and Bonhomme F. (2007) Molecular and morphological relationships between two closely related species, *Turbinaria ornata* and *T. conoides* (Sargassaceae, Phaeophyceae). *Biochem. Syst. Ecol.*, **35**, 91–98.
- Rohner C. and Ward D. (1997) Chemical and mechanical defense against herbivores in two sympatric species of desert. *Acacia J. Veg. Sci.*, **8**, 717–726.
- Rosenstein B.S., Phelps R., Weinstock M.A., et al. (1999) P53 mutations in basal cell carcinomas arising in routine users of sunscreens. *Photochem. Photobiol.*, **70**, 798–806.
- Rouillé T., Hammé V., Roy P. and Bordenave-Juchereau S. (2009) Algae hydrolysate Calcimine®: The sea contribution to slimming. *Journal des Sciences Halieutique et Aquatique*, **2**, 77.
- Rouxel C., Daniel A., Jerome M., Etienne M. and Fleurence J. (2001) Species identification by SDS-PAGE of red algae used as sea food or a food ingredient. *Food Chem.*, **74**, 349–353.
- Rowan K.S. (1989) Photosynthetic pigments of algae. Cambridge University Press, Cambridge.
- Ruperez P. (2002) Mineral content of edible marine seaweeds. *Food Chem.*, **79** (1), 23–26.
- Saito R.M. and De Oliveira E.C. (1990) Chemical screening of Brazilian marine algae producing carrageenans. *Hydrobiologia*, **204–205**, 585–588.

- Sakai M. (1999) Current research status of fish immunostimulants. *Aquaculture*, **172**, 63–92.
- Salvador N., Garreta A.G., Lavelli L. and Ribera M.A. (2007) Antimicrobial activity of Iberian macroalgae. *Sci. Mar.*, **71**, 101–113.
- Sanchez-Machado D.I., Lopez-Hernandez J. and Paseiro-Losada P. (2002) High-performance liquid chromatographic determination of  $\alpha$ -tocopherol in macroalgae. *J. Chromatogr. A*, **976**, 277–284.
- Sanchez-Machado D.I., Lopez-Cervantes J., Lopez-Hernandez J., Paseiro-Losada P. and Simal-Lozano J. (2004) Determination of the uronic acid composition of seaweed dietary fibre by HPLC. *Biomed. Chromatogr.*, **18**, 90–97.
- Sandsdalen E., Haug T., Stensvag K. and Styrvold OB. (2003) The antibacterial effect of a polyhydroxylated fucophlorethol from the marine brown alga, *Fucus vesiculosus*. *World J. Microbiol. Biot.*, **19**, 777–782.
- Sangiardi A. (2010) Utilisation contemporaine des algues en Bretagne. La filière algue en Bretagne. Rapport Master 2 Gestion et Valorisation des ressources biologiques. Université de Bretagne-Sud. 91p.
- Sastry V.M.V.S. and Rao G.R.K.. (1994) Antibacterial substances from marine algae: successive extraction using benzene, chloroform and methanol. *Bot. Mar.*, **37**, 357–360.
- Shick J.M., Dunlap W.C. and Buettner G.R. (2000) Ultraviolet (UV) protection in marine organisms II. Biosynthesis, accumulation, and suncreening function of mycosporine-like amino acids. In: *Free Radicals in Chemistry, Biology and Medicine* (Yoshikawa S., Toyokuni S., Yamamoto Y. and Naito Y., eds) OICA International, London, pp. 215–228.
- Schmitt T. M., Lindquist N. and Hay M. E. (1998) Seaweed secondary metabolites as antifoulants: effects of *Dictyota* spp. diterpenes on survivorship, settlement, and development of marine invertebrate larvae. *Chemoecology*, **8**, 125–131.
- Schoenwaelder M.E.A. (2002) The occurrence and cellular significance of physodes in brown algae. *Phycologia*, **41**, 125–139.
- Schoenwaelder M.E.A. and Clayton M.N. (1999a) The presence of phenolic compounds in isolated cell walls of brown algae. *Phycologia*, **38**, 161–166.
- Schoenwaelder M.E.A. and Clayton M.N. (1999b) The role of the cytoskeleton in brown algal physode movement. *Eur. J. Phycol.*, **34**, 223–229.
- Schwarz A. and Schwarz T. (2002) Molecular determinants of UV-induced immunosuppression. *Exp. Dermatol.*, **11**, 9–12.
- Seité S., Moyal D., Verdier M.P., Horseau C. and Fourtanier A. (2000) Accumulated p53 protein and UVA protection level of sunscreens. *Photodermatol., Photoimmunol. Photomed.*, **16**, 3–9.
- Sekar S. and Chandramohan M. (2008) Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. *J. Appl. Phycol.*, **20**, 113–136.
- Semmak L., Zerzouf A., Valls R., Banaigs B., Jeanty G. and Francisco C. (1988) Acyclic diterpenes from *Bifurcaria bifurcata*. *Phytochemistry*, **27**, 2347–2349.
- Shibata T., Hama Y., Miyasaki T., Ito M. and Nakamura T. (2006) Extracellular secretion of phenolic substances from living brown algae. *J. Appl. Phycol.*, **18**, 787–794.
- Sideman E.J. and Mathieson A.C. (1985) Morphological variation within and between natural populations of non-tide pool *Fucus distichus* (Phaeophyceae) in New England. *J. Phycol.*, **21**, 250–257.
- Silkina A., Bazes A., Vouve F., et al. (2009) Antifouling activity of macroalgal extracts on *Fragilaria pinnata* (Bacillariophyceae): a comparison with Diuron. *Aquat. Toxicol.*, **94**, 245–254.
- Simopoulos A.P. (2004) Omega-3 essential fatty acid ratio and chronic diseases. *Food Rev. Int.*, **20**, 77–90.
- Singh I.P. and Bharate S.B. (2006) Phloroglucinol compounds of natural origin. *Nat. Prod. Rep.*, **23**, 558–591.
- Smit A.J. (2004) Medicinal and pharmaceutical uses of seaweed natural products: a review. *J. Appl. Phycol.*, **16**, 245–262.
- Solimabi, Das B. (1980) Antispasmodic and anti inflammatory activity of carrageenan from *Hypnea musciformis* wulfen. *India J. Pharmacol.*, **12**, 259–261.
- Sosa P.A. and Lindstrom S.C. (1999) Isoenzymes in macroalgae (seaweeds): genetic differentiation, genetic variability and applications in systematics. *Eur. J. Phycol.*, **34**, 427–442.
- Sotka E.E., Taylor R.B. and Hay M.E. (2002) Tissue-specific induction of resistance to herbivores in a brown seaweed: the importance of direct grazing versus waterborne signals from grazed neighbors. *J. Exp. Mar. Biol. Ecol.*, **277**, 1–12.
- Steenvoorden D-P.T. and Beijersbergen van Henegouwen G.M.J. (1997) The use of endogenous antioxidants to improve photoprotection. *J. Photochem. Photobiol.*, **41**, 1–10.
- Steinberg P.D. (2001) Chemical mediation of interactions at seaweed surfaces. *J. Phycol.*, **37**, 46–47.
- Steinberg P.D., de Nys R. and Kjelleberg S. (1998) Chemical inhibition of epibiota by Australian seaweeds. *Biofouling*, **12**, 217–224.
- Stiger V., Payri C.E. (1999) Spatial and temporal patterns of settlement of the brown macroalgae, *Turbinaria ornata* and *Sargassum mangarevense* in a coral reef on Tahiti. *Mar. Ecol. Progr. Ser.*, **191**, 91–100.

- Stiger V., Payri C.E. (2005) Natural settlement of a young population of *Turbinaria ornata* and phenological comparisons with older populations. *Aquat. Bot.*, **81**, 225–243.
- Stiger V., Horiguchi T., Yoshida T., Coleman A.W. and Masuda M. (2000) Phylogenetic relationships of *Sargassum* (Sargassaceae, Phaeophyceae) with reference to a taxonomic revision of the section *Phyllocystae* based on ITS-2 nrDNA sequences. *Phycol. Res.*, **48**, 251–260.
- Stiger V., Horiguchi T., Yoshida T., Coleman A.W., Masuda M. (2003) Phylogenetic relationships inferred from ITS-2 nrDNA comparisons within the genus *Sargassum* (Fucales, Phaeophyceae) from the Pacific basin, with an emphasis on the taxonomic subdivision of the genus. *Phycol. Res.*, **51**, 1–10.
- Stiger V., Deslandes E. and Payri C.E. (2004) Phenolic contents of two brown algae, *Turbinaria ornata* and *Sargassum mangarevense* on Tahiti (French Polynesia): inter-specific, ontogenic and spatio-temporal variations. *Bot. Mar.* **47** (5), 402–409.
- Suh H.J., Lee H.W. and Jung J (2003). Mycosporine glycine protects biological systems against photodynamic damage by quenching singlet oxygen with a high efficiency. *Photochem. Photobiol.*, **78** (2), 109–113.
- Supamattaya K., Bundit O., Boonyaratpatin M., Schatzmayr G. and Chittiwat V. (2005) Effects of ochratoxin A and deoxynivalenol on growth performance and immunophysiological parameters in black tiger shrimp (*Penaeus monodon*). *Songklanakarin J. Sci. Technol.*, **2b7** (suppl 1), 91–99.
- Svensson C.J., Pavia H. and Toth G.B. (2007) Do plant density, nutrient availability, and herbivore grazing interact to affect phlorotannin plasticity in the brown seaweed *Ascophyllum nodosum*. *Mar. Biol.*, **151**, 2177–2181.
- Swanson A.K. and Druehl L.D. (2002) Induction, exudation and the UV protective role of kelp phlorotannins. *Aquat. Bot.*, **73**, 241–253.
- Talarico L.B., Zibetti R.G.M., Faria P.C.S., Scolaro L.A., Duarte M.E.R. and Nosedá M.D. (2004) Anti-herpes simplex virus activity of sulphated galactans from the red seaweeds *Gymnogongrus griffithsia* and *Cryptonemia crenulata*. *Int. J. Biol. Macromolecules*, **34**, 63–71.
- Talarico L.B., Pujol C.A., Zibetti R.G.M., Faria P.C.S., Nosedá M.D. and Duarte M.E.R. (2005) The antiviral activity of sulphated polysaccharides against dengue virus is dependant on virus serotype and host cell. *Antivir. Res.*, **66**, 103–110.
- Targett N.M. and Arnold T.M. (1998) Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. *J. Phycol.*, **34**, 195–205.
- Targett N., Boettcher A., Targett T. and Vrolijk N. (1995) Tropical marine herbivore assimilation of phenolic-rich plants. *Oecologia*, **103**, 170–179.
- Teixeira V.L., Almeida S.A.S. and Kelecom A. (1990) Chemosystematic and biogeographic studies of the diterpenes from the marine brown alga *Dictyota dichotoma*. *Biochem. Syst. Ecol.*, **18**, 87–92.
- Teixeira V.L., Cavalcanti D.N. and Pereira R.C. (2001) Chemotaxonomic study of the diterpenes from the brown alga *Dictyota menstrualis*. *Biochem. Syst. Ecol.*, **29**, 313–316.
- Thomas K.V., McHugh M. and Waldock M. (2002) Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate. *The Science of the Total Environment*, **293**, 117–127.
- Toth G.B. and Pavia H. (2002) Intraplant habitat and feeding preference of two gastropod herbivores inhabiting the kelp *Laminaria hyperborea*. *J. Mar. Biol. Assoc. U.K.*, **82**, 243–247.
- Tringali C., Oriente G., Piattelli M. and Nicolosi G. (1985) Two minor dolabellane diterpenoid constituents from a *Dictyota* species. *J. Nat. Prod.*, **48**, 484–485.
- Tseng C.K. (2001) Algal biotechnology industries and research activities in China. *J. Appl. Phycol.*, **13**, 375–380.
- Urones J.G., Araujo M.E.M., Brito Palma F.M.S., Basabe P., Marcis I.S. and Moro R.F. (1992) Meroterpenes from *Cystoseira usneoides* II. *Phytochemistry*, **31**, 2105–2109.
- Usov A. I. (1992) Sulfated polysaccharides of red seaweeds. *Foods Hydrocolloids*, **6**, 9–23.
- Vairappan C.S., Suzuki M., Abe T. and Masuda M. (2001) Halogenated metabolites with antibacterial activity from the Okinawan *Laurencia* species. *Phytochemistry*, **58**, 517–523.
- Vallim M.A., De Paula J.C., Pereira R.C. and Teixeira V.L. (2005) The diterpenes from Dictyotacean marine brown algae in the Tropical Atlantic American region. *Biochem. Syst. Ecol.*, **33**, 1–16.
- Valls R. and Piovetti L. (1995) The chemistry of the Cystoseiraceae (Fucales: phaeophyceae): chemotaxonomic relationships. *Biochem. Syst. Ecol.*, **23**, 723–745.
- Valls R., Banaigs B., Francisco C., Codomier L. and Cave A. (1986) An acyclic diterpene from the brown alga *Bifurcaria bifurcata*. *Phytochemistry*, **25**, 751–752.
- Valls R., Piovetti L., Banaigs B., Archavlis A. and Pellegrini M. (1995) (S)-13-Hydroxygeranylgeraniol-derived furanoditerpenes from *Bifurcaria bifurcata*. *Phytochemistry*, **39**, 145–149.
- Valls R., Banaigs B., Piovetti L., Archavilis A. and Artaud J. (1993) Linear diterpenes with antimitotic activity from the brown alga *Bifurcaria bifurcata*. *Phytochemistry*, **34**, 1585–1588.

- Van Alstyne K., Whitman S. and Ehlig J. (2001) Differences in herbivore preferences, phlorotannin production, and nutritional quality between juvenile and adult tissues from marine brown algae. *Mar. Biol.*, **139**, 201–210.
- Van Alstyne K.L., McCarthy J.J., Hustead C.L. and Duggins D.O. (1999b) Geographic variation in polyphenolic levels of Northeastern Pacific kelps and rockweeds. *Mar. Biol.*, **133** (2), 371–379.
- Van Heemst J.D.H., Peulve S. and De Leeuw J.W. (1996) Novel algal polyphenolic biomacromolecules as significant contributors to resistant fractions of marine dissolved and particulate organic matter. *Org. Geochem.*, **24** (6–7), 629–640.
- Verkleij F.N. (1992) Seaweed extracts in agriculture and horticulture – a review. *Biol. Agric. Hort.*, **8**, 309–324.
- Viano Y., Bonhomme D., Camps M., et al. (2009) Diterpenoids from the Mediterranean brown alga *Dictyota* sp. evaluated as antifouling substances against a marine bacterial biofilm. *J. Nat. Prod.*, **72**, 1299–1304.
- Viano Y., Bonhomme D., Ortalo-Magne A., Thomas O.P., El Hattab M., Piovetti L., Blache Y., and Culioli G. (2011) Dictyotadimer A, a novel dissymmetric bis-diterpene from a brown alga of the genus *Dictyota*. *Tetrahedron Lett.*, **52**, 1031–1035.
- Vlachos V., Critchley A.T. and von Holy A. (1999) Differential antibacterial activity of extracts from selected Southern African macroalgal thalli. *Bot. Mar.*, **42** (2), 165–173.
- Voulvoulis N., Scrimshaw M.D. and Lester J. N. (2002) Partitioning of selected antifouling biocides in the aquatic environment. *Mar. Env. Res.*, **53**, 1–16.
- Wahl M. (1989) Marine epibiosis: I. Fouling and antifouling: some basic aspects. *Mar. Ecol. Prog. Ser.*, **58**, 175–189.
- Wattier R. and Maggs C.A. (2001) Intraspecific variation in seaweeds: The application of new tools and approaches. *Adv. Bot. Res.*, **35**, 171–212.
- Weinberger F. and Friedlander M. (2000a) Endogenous and exogenous elicitors of a hypersensitive response in *Gracilaria conferta* (Rhodophyta). *J. Appl. Phycol.*, **12**, 139–145.
- Weinberger F. and Friedlander M. (2000b) Response of *Gracilaria conferta* (Rhodophyta) to oligogars results in defense against agar-degrading epiphytes. *J. Phycol.*, **36**, 1079–1086.
- Weinberger F., Friedlander M. and Hoppe H.G. (1999) Oligogars elicit a physiological response in *Gracilaria conferta* (Rhodophyta). *J. Phycol.*, **35**, 747–755.
- Wikström S.A. and Pavia H. (2004) Chemical settlement inhibition versus post-settlement mortality as an explanation for differential fouling of two congeneric seaweeds. *Oecologia*, **138**, 223–230.
- Wilson W.K., Sumpter R.M., Warren J.J., Rogers P.S., Ruan B. and Schroepfer Jr. G.J. (1996) Analysis of unsaturated C27 sterols by nuclear magnetic resonance spectroscopy. *J. Lipid Res.*, **37**, 1529–1555.
- Womersley H.B.S. (1994) The marine benthic flora of Southern Australia. Rhodophyta. IIIA. Bangiophyceae and Florideophyceae (Acrochaetiales, Nemaliales, Gelidiales, Hildenbrandiales and Gigartinales sensu lato). Australian Biological Resources Study, Canberra.
- Wynne M.J. (2002) *Turbinaria foliosa* sp. nov. (Fucales, Phaeophyceae) from the Sultanate of Oman, with a census of currently recognized species in the genus *Turbinaria*. *Phycol. Res.*, **50**, 283–293.
- Xu N., Fan X., Yan X. and Tseng C. (2004) Screening marine algae from China for their antitumor activities. *J. Appl. Phycol.*, **16**, 451–456.
- Xuelian T., Jing L., Xianliang X. and Meiyu G. (2006) A new marine-derived sulphated polysaccharide from brown alga suppresses tumor metastasis both *in vitro* and *in vivo*. *Cancer Biol. Ther.*, **5**, 474–480.
- Yates J.L. and Peckol P. (1993) Effects of nutrient availability and herbivory on polyphenolics in the seaweed *Fucus vesiculosus*. *Ecology*, **74**, 1757–1766.
- Yebra D.M., Kiil S. and Dam-Johansen K. (2004) Antifouling technology-past, present and future step towards efficient and environmentally friendly antifouling coatings. *Prog. Org. Coat.*, **50**, 75–104.
- Yoshida T., Stiger V. and Horiguchi T. (2000) *Sargassum boreale* sp. nov. (Fucales, Phaeophyceae) from Hokkaido, Japan. *Phycol. Res.*, **48**, 125–131.
- Yoshikawa T., Rae V., Bruins-Slot W., van den Berg J.W., Taylor J.R. and Streilein J.W. (1990) Susceptibility to effects of UVB radiation on induction of contact hypersensitivity as a risk factor for skin cancer in humans. *J. Invest. Dermatol.*, **95**, 530–536.
- Yotsukura N., Denboh T., Motomura T., Horiguchi T., Coleman A.W., Ichimura T. (1999) Little divergence in ribosomal DNA internal transcribed spacer -1 and -2 sequences among non-digitate species of *Laminaria* (Phaeophyceae) from Hokkaido, Japan. *Phycol. Res.* **47**, 71–80.
- Yuan Y.V. and Walsh N.A. (2006) Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food Chem. Toxicol.*, **44**, 1144–1150.
- Zaragoza M.C., Lopez D., Saiz M.P., et al. (2008) Toxicity and antioxidant activity *in vitro* and *in vivo* of two *Fucus vesiculosus* extracts. *Agric. Food Chem.*, **56** (17), 7773–7780.
- Zemke-White W.L. and Ohno M. (1999) World seaweed utilization: an end-of-century summary. *J. Appl. Phycol.* **11**, 369–376.
- Zidane M., Pondaven P., Roussakis C. and More M.-T. (1996) Effects *in vitro* of Pachymatissmin, a glycoprotein from the marine sponge *Pachymatisma johnstonii*,

- on a non-small-cell bronchopulmonary carcinoma line (NSCLC-N6). *Anticancer Res.*, **16**, 280–2812.
- Zinedine A., Elakhdari S., Faid M. and Benlemlih M. (2004) Antifungal and anti-aflatoxinogenic activity of the brown algae *Cystoseira tamariscifolia*. *Journal de mycologie médicale*, **14**, 201–205.
- Zorrilla I., Chabrilón M., Arijo S., *et al.* (2003) Bacteria recovered from diseased cultured gilthead seabream (*Sparus aurata*, L.) in southwestern Spain. *Aquaculture*, **218**, 11–20.
- Zubia M., Payri C., Deslandes E. and Guezennec J. (2003) Chemical Composition of Attached and Drift Specimens of *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales) from Tahiti, French Polynesia1. *Bot. Mar.*, **46**, 562–571.
- Zubia M., Payri C. and Deslandes E. (2008) Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales), from Tahiti (French Polynesia). *J. Appl. Phycol.*, **20**, 1033–1043.
- Zubia M., Fabre M.S., Kerjean V., Le Lann K., Stiger-Pouvreau V., Fauchon M. and Deslandes E. (2009) Antioxidant and antitumoral activities of some Phaeophyta from Brittany coasts. *Food Chem.*, **116**, 693–701.

# 5

## Physiological Basis for the use of Seaweeds as Indicators of Anthropogenic Pressures: The Case of Green Tides

Jesús M. Mercado

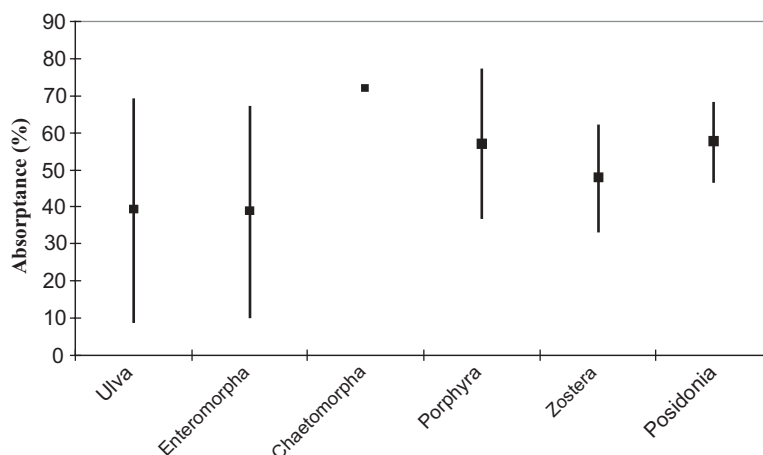
*Centro Oceanográfico de Málaga, Instituto Español de Oceanografía, Fuengirola, Spain*

### 5.1 Introduction

Seaweeds and seagrasses respond directly to the abiotic and biotic aquatic environment and thus could represent suitable indicator of its changes. Concordantly, seaweeds have been proposed as a quality element for classification of marine coastal areas (Schiel *et al.*, 2006). Specific indices to assess the ecological status of the macroalgal communities are being developed (Orfanidis *et al.*, 2003; Ballesteros *et al.*, 2007; Juanes *et al.*, 2008). These indices are based on the taxonomic composition of the communities and assume that sensitivity to anthropogenic disturbances differs among species (Orfanidis *et al.*, 2001). Normally, a distinction between perennial and opportunistic species is adopted. Changes in abundance of opportunistic versus perennial algae are interpreted as a deterioration of the environmental quality putatively caused by anthropogenic pressures.

Several examples of the pollution impact on seaweed communities in different intertidal systems can be found in the literature (Done, 1992; Hughes, 1994; Baharthan 2010). However, the phenomenon most widely described is the occurrence of the so-called green-tides; massive proliferations of ephemeral macroalgae that replace the

pre-existing macrophyte communities (Fletcher, 1996; Morand and Briand, 1996; Hernández *et al.*, 1997; Valiela *et al.*, 1997; Raffaelli *et al.*, 1998; Hiraoka *et al.*, 2004; Morand and Merceron, 2005; Merceron *et al.*, 2007; Liu *et al.*, 2009). The particular physiological performance of the algae forming green tides is often invoked to explain its explosive proliferation and capacity for replacing the native macrophyte communities (Lavery and McComb, 1991; Fong *et al.*, 1996). In particular, it is normally accepted that the fast growth macroalga have higher capacity for nutrient assimilation than perennial species, consequently green-tides are associated to nutrient pollution (Morand and Briand, 1996; Morand and Merceron, 2005). However, Lotze and Schramm (2000) did not find a clear relationship between nutrient uptake capacity and dominance patterns of two species of opportunistic macroalgae. More recently, Liu *et al.* (2009) found that the proliferation of *Enteromorpha prolifera* (one of the green alga species that forms green tides) off the China coast was due to a combination of favorable oceanic conditions related to temperature, wind, and currents. According to these authors, the occurrence of green tides in the China coast cannot be explained uniquely from the nutrient pollution that suffers this



**Figure 5.1** Range of variability of absorbance (%) published for different macroalgae and seagrasses (for *Chaetomorpha* a single value is reported). Sources of the data: San-Jensen, 1988; Enríquez *et al.*, 1992; Figueroa *et al.*, 1995; Mercado *et al.*, 1996; Vergara *et al.*, 1998; Cumming and Zimmerman, 2003; Beer and Axelsson, 2004; Gómez *et al.*, 2004; Durako, 2007.

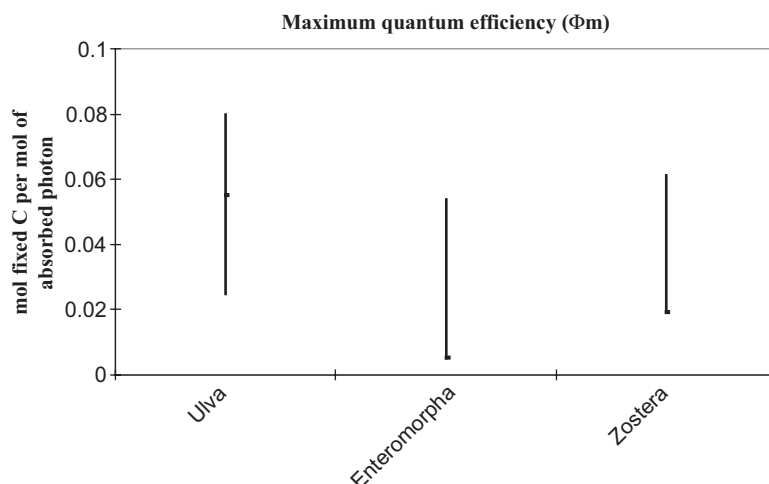
marine area. In the same sense, Yabe *et al.* (2009) described green tides off the Japan coast that were not related with nutrient pollution. In turn, it has been proposed that light attenuation by other macro- or microalgae or cyanobacteria could be the reason for explaining the non-occurrence of excessive proliferation of ephemeral algae in some systems with relatively high nutrient levels (Lavery and McComb, 1991; Sfriso and Pavoni, 1994). These works demonstrate that other physiological features, in addition to nutrient uptake capacity, have to be considered for explaining the capacity of the ephemeral algae for massive growth and displacement of perennial benthic communities.

In the present work, published information about the photosynthetic performance of the algae forming green-tides is assessed in order to determine its possible role in the formation of the blooms. For this objective, the variability range of the photosynthetic parameters of these species is described. The main green proliferating algae are considered: *Ulva*, *Chaetomorpha*, *Cladophora*, and *Enteromorpha* (Fletcher, 1996; Morand and Briand, 1996; Hernández *et al.*, 1997; Valiela *et al.*, 1997; Raffaelli *et al.*, 1998; Hiraoka *et al.*, 2004; Morand and Merceron, 2005; Merceron *et al.*, 2007; Liu *et al.* 2009). This physiological information is compared with available information about *Zostera* and *Posidonia*, two temperate seagrass species that grow in the intertidal fringe and are often replaced by opportunistic algae.

## 5.2 Light absorption

Photosynthetic features are normally described by analysing the response of photosynthesis to increasing doses of light

(P-I curves). The ascending slope at irradiance limiting of P-I curves ( $\alpha$ ) is the parameter used to compare the photosynthetic efficiency of macrophytes acclimated to low irradiance (Henley, 1993). This parameter is a function of both light-harvesting efficiency and photosynthetic energy conversion efficiency. Determination of light absorption features by sheetlike macroalgae is relatively easy. Consequently, absorbance (i.e., percentage of incident light that is effectively absorbed by the thallus) has been routinely determined in studies on the photosynthetic performance of *Ulva* sp. (Sand-Jensen, 1988; Mercado *et al.*, 1996; Vergara *et al.*, 1998; Gómez *et al.*, 2004; Beer and Axelsson, 2004). According to these works, absorbance by *Ulva* ranges from 10% to 50% depending on the growth light conditions (Figure 5.1). These highest values have been reported for thalli grown at very low irradiance (lower than 50  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ; Sand-Jensen, 1988) while growth at light saturation produces a reduction of absorbance by almost fivefold. This variability in absorbance is due to *Ulva* modifies drastically its pigment content in response to changes in light intensity. In fact, a linear relationship between pigment content and absorbance of *U. rigida* has been described (Mercado *et al.*, 1996). These results indicate that both light absorption by non-pigmented cell structures and pigment packaging have a limited effect on light absorption efficiency per chlorophyll *a* unit in *Ulva*. The scarce published data of light absorption by *Enteromorpha* show a variation range of absorbance similar to that described for *Ulva* (Frost-Christensen and Sand-Jensen, 1992). Absorbance data for *Chaetomorpha* and *Cladophora* are not available. Therefore, the data summarized in Figure 5.1 indicate that the fast growth algae have a high capacity for adjusting



**Figure 5.2** Range of variability of the maximum quantum efficiency ( $\Phi_m$ ; mol fixed C per mol of absorbed photon) for the genera *Ulva* and *Enteromorpha* (Henley *et al.*, 1992; Beach *et al.*, 1995; Sand-Jensen, 1998). The data are compared with those one available for the seagrass *Zostera* (Lee *et al.*, 2007).

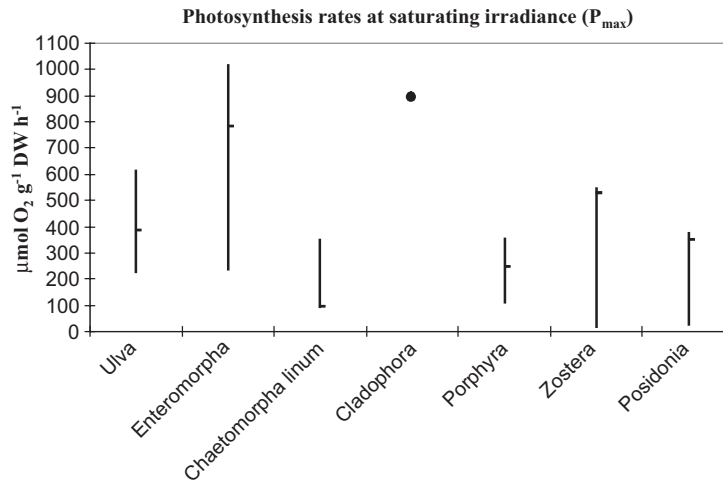
their light absorption properties depending on the growth irradiance. However, it has to be note that this capacity is not exclusive of the fast growth algae. For instance, absorptance by *Porphyra* varies from 37% to 77% depending on the light conditions of growth (Figueroa *et al.*, 1995).

Light absorption by macrophytes depends strongly on the structural complexity of their photosynthetic tissues (Markager and Sand-Jensen, 1996). Light harvesting in angiosperms is essentially restricted to the external cell layers, which implies a reduction of the light absorption efficiency per chlorophyll *a* unit, due to self-shading of the chloroplasts. The pigment packing effect in angiosperms is higher than in fast growth algae (Enríquez *et al.*, 1992; Cumming and Zimmerman, 2003; Durako, 2007). The main consequence of this structural constrain is that the capacity of angiosperms for reducing their light absorption at increasing irradiance is limited (Dennison and Alberte, 1982; Abal *et al.*, 1994; Major and Dunton, 2002). For instance, reduction of chlorophyll *a* by a factor of five results in absorptance decrease by less than 15% (Figure 5.1; Cummings and Zimmerman, 2003). Consequently, the lowest values of absorptance published for *Zostera* and *Posidonia* grown at high irradiance are considerably higher than the values published for *Ulva* and *Enteromorpha* (Figure 5.1). Therefore, the fast growth macroalgae have a comparatively higher capacity for regulating their light absorption capacity. Theoretically, this plasticity could reduce the risks of photoinhibition and the costs of investment and repair of pigments (Major and Dunton 2002). Consequently, light absorption could be a factor favouring the growth of ephemeral algae under high solar irradiance conditions.

### 5.3 Photosynthesis at sub- and saturating irradiance

As commented above, the photosynthesis rates at subsaturating irradiance depend on the photosynthetic energy conversion efficiency that is normally expressed as maximum quantum efficiency ( $\Phi_m$ ; mol fixed C per mol of absorbed photons; Henley, 1993). The calculation of  $\Phi_m$  from P-I curves implies to know the light-harvesting efficiency (Figure 5.2). Sand-Jensen (1988) reported values of  $\Phi_m$  ranging from 0.03 to 0.06 mol C per mol photon absorbed for *Ulva lactuca* acclimated to very low-irradiance. The variation range of  $\Phi_m$  published for *U. rotundata* is similar (Henley *et al.*, 1992). Values ranging from 0.049 to 0.054 have been published for *Enteromorpha* (Beach *et al.*, 1995). Unfortunately, data for  $\Phi_m$  expressed on an adequate basis for comparison with *Ulva* and *Enteromorpha* have been only published for *Zostera* (see the review by Lee *et al.*, 2007). This seagrass exhibits values of  $\Phi_m$  similar to those reported for these macroalgae.

Photosynthesis at saturating irradiance expressed on dry weight basis ( $P_{max}$ ) is highly variable within the same genus of algae. For instance,  $P_{max}$  varies by threefold in *Ulva* and by fivefold in *Enteromorpha* (Figure 5.3; Sand-Jensen, 1988; Henley *et al.*, 1992; Plus *et al.*, 2005; Han *et al.*, 2007). The highest values of  $P_{max}$  among the algae that produce green-tides have been published for *Enteromorpha* and *Cladophora* (Beach *et al.*, 1995; Choo *et al.*, 2005). On the contrary, the lowest  $P_{max}$  have been published for *Chaetomorpha* (Beach *et al.*, 1995). Comparatively, *Zostera* and *Posidonia* present a high variation range of  $P_{max}$  (Figure 5.3). However, the

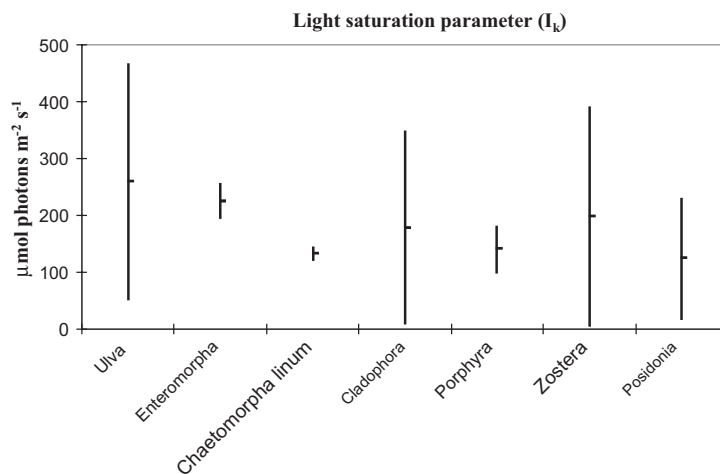


**Figure 5.3** Range of variability of photosynthesis rates at saturating irradiance ( $P_{max}$ ) published for different macroalgae and seagrasses. For *Cladophora*, a single point is presented. Sources of the data: Sand-Jensen, 1988; Beach *et al.*, 1995; Henley *et al.*, 2002; Mercado *et al.*, 2002; Choo *et al.*, 2005; Plus *et al.*, 2005; Han *et al.*, 2007; Lee *et al.*, 2007.

highest  $P_{max}$  values published for these two genera are lower than those for *Ulva* and *Enteromorpha*. Interestingly, the lower limit of  $P_{max}$  for these seagrasses is notably lower than the one for the fast growth macroalgae (Figure 5.3).

The so-called light saturation parameter ( $I_k$ ) is normally used to study photoacclimation.  $I_k$  is calculated by dividing  $P_{max}$  by the initial slope of P-I curves ( $\alpha$ ). This parameter is independent on the units in which photosynthesis rates are expressed therefore abundant data can be found in the literature for multiple macroalga species. Plus *et al.* (2005) published values of  $I_k$  for a number of macrophytes during

different phases of the seasonal cycle (i.e., plants acclimated to a wide range of irradiances). According to these data and some other available in the literature (Han *et al.*, 2007; Figure 5.4),  $I_k$  for *Ulva* can vary by almost fivefold. The range of variability of  $I_k$  values published for *Enteromorpha* and *Chaetomorpha* is lower (Beach *et al.*, 1995; McGlathery and Pedersen, 1999; Choo *et al.*, 2005). The highest  $I_k$  values have been published for *Ulva* and *Cladophora*. The variability range of  $I_k$  for the seagrasses *Zostera* and *Posidonia* is relatively wide and comparable with the one obtained for those two green macroalgae. However, the minimum  $I_k$



**Figure 5.4** Range of variability of the light saturation parameter ( $I_k$ ) published for different macroalgae and seagrasses. Sources of the data: Dunton, 1994; Beach *et al.*, 1995; Zimmerman *et al.*, 1995; McGlathery and Pedersen, 1999; Choo *et al.*, 2005; Han *et al.*, 2007; Lee *et al.*, 2007.

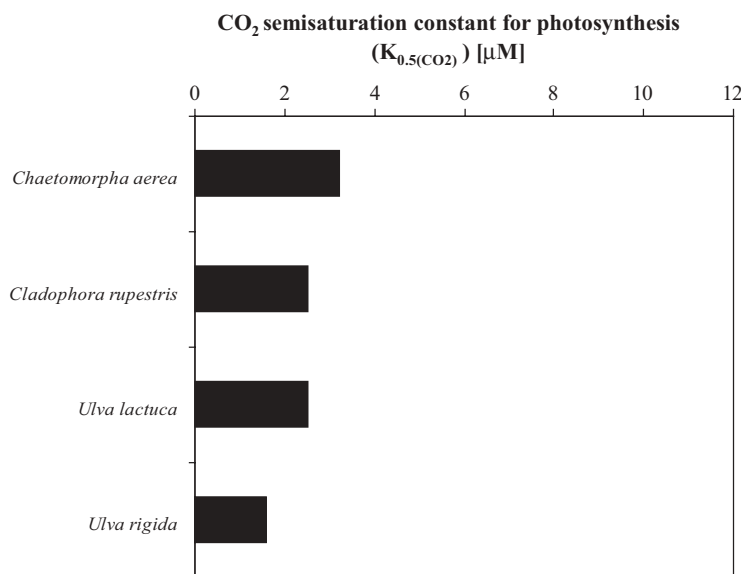
values published for these two seagrasses are lower than the corresponding values reported for the fast growth macroalgae with the exception of *Cladophora* (Figure 5.4; Dunton, 1994; Zimmerman *et al.*, 1995; Lee *et al.*, 2007). According to Henley (1993), low values of  $I_k$  could indicate efficient use of low irradiance and/or inefficient use of high irradiance. As commented above, the minimum values of  $P_{max}$  for *Zostera* and *Posidonia* are also lower than for fast growth algae. Consequently, the differences in the variability range of  $I_k$  values between fast growth macroalgae and seagrasses could indicate that the algae have the potential to maintain a higher photosynthetic capacity at saturating irradiance under a more varied range of environmental conditions.

## 5.4 Inorganic carbon acquisition

According to Sukenik *et al.* (1987), higher photosynthetic capacity is related to differences in the turnover time of the photosynthetic apparatus that can be due to alteration in (1) electron flow between PSII and PSI and/or (2) rate of carbon fixation. The key enzyme implied in  $CO_2$  fixation is the ribulose-biphosphate carboxylase oxygenase (Rubisco), which uses  $CO_2$  as substrate for its carboxylation reaction. Two forms of Rubisco have been described in algae: green-like (present in green algae and higher plants) and red-like (of non-green algae). Both forms differ in their affinity to  $CO_2$  and  $O_2$  (see Badger *et al.*, 1998, for a review about the molecular and kinetic properties of the different algal

Rubisco families) with semi-saturation constants for  $CO_2$  ranging from 30 to 250  $\mu M$ . Note that these semisaturation constants are higher than the  $CO_2$  concentration available in air-equilibrated seawater (at pH 8.1,  $CO_2$  concentration is about 10–15  $\mu M$ ). Consequently, it could be expected that the  $CO_2$  concentration in seawater is not enough to saturate the macroalgal photosynthesis at saturating irradiance. However, about 70% out of the macroalgal species researched in the laboratory have photosynthesis rates fully saturated by  $CO_2$  in air-equilibrated seawater (Axelsson and Uusitalo 1988; Axelsson *et al.*, 1991, 2000; Surif and Raven, 1989; Maberly 1990; Haglund *et al.*, 1992; Börjk *et al.*, 1992; Beer and Koch, 1996; Mercado *et al.*, 1998; Zou and Gao, 2002; Zou *et al.*, 2003; Middelboe and Hansen, 2007). Figure 5.5 shows the available data of semisaturation  $CO_2$  concentration for photosynthesis ( $K_{0.5(CO_2)}$ ) published for four opportunistic species.  $K_{0.5(CO_2)}$  for these species is lower than 5  $\mu M$ . It indicates that their photosynthesis rates are fully saturated by  $CO_2$  in air-equilibrated seawater. In fact, and in comparison to other macroalgae, these four species exhibit the lowest  $K_{0.5(CO_2)}$  values (Mercado and Gordillo, 2011).

The physiological reason for this relatively high affinity to  $CO_2$  in seawater at pH 8.1 is that these species depict mechanisms to increase the  $CO_2$  concentration around Rubisco (the so-called carbon concentrating mechanism, CCM). The presence of CCM is relatively frequent among the macroalgae (Figure 5.5). All the CCMs are based on the use of bicarbonate as a source of inorganic carbon for



**Figure 5.5**  $CO_2$  semisaturation constant for photosynthesis ( $K_{0.5(CO_2)}$ ) for four species of green macroalgae belonging to genus that form green tides. Note that the  $CO_2$  in air-equilibrated seawater is around 10–15  $\mu M$ . The sources of data are specified in the text.

photosynthesis (note that the bicarbonate concentration in seawater at pH 8.1 is about 100 times higher than the  $\text{CO}_2$  concentration). The CCMs in *Ulva* and *Enteromorpha* have been extensively studied (Beer and Israel, 1986; Axelsson *et al.*, 1995; Larsson *et al.*, 1997). In both species, the presence of an active transport mechanism of high affinity for bicarbonate at plasmalemma level has been inferred. Besides, Axelsson *et al.* (1995) demonstrated that this mechanism is up-regulated when the external  $\text{CO}_2$  availability is reduced. Bicarbonate transport in *Cladophora* has been also suggested by Choo *et al.* (2002).

The presence of CCMs has been reported in seagrasses (see Beer *et al.*, 2002 for a review). However, the photosynthetic affinity for  $\text{CO}_2$  of these macrophytes is low in comparison with the high affinity of the fast growth algae (Figure 5.5; as an example, see the values of  $K_{0.5(\text{CO}_2)}$  published by Mercado *et al.*, 2003 for *Zostera*). In fact, it is normally accepted that photosynthesis by seagrasses is not fully saturated at the  $\text{CO}_2$  concentration in air-equilibrated seawater (Beer *et al.*, 2002). For instance, the degree of photosynthetic saturation in *Zostera* ranges from 86% (Mercado *et al.*, 2003) to about 60% (Hellblom *et al.*, 2001). These differences in affinity between green macroalgae and seagrasses are probably a consequence of different functioning of their respective CCMs.

The higher capacity for using bicarbonate of the fast growth algae (Figure 5.5.) in comparison to seagrasses could have important implications for their photosynthesis and growth rates. First, it could contribute to improve the photosynthetic capacity of the macroalgae under conditions of irradiance saturation (which could explain the highest  $P_{\text{max}}$  values commented above). Second, it confers a higher growth potential when other resources (i.e. nutrients) are in excess.

## 5.5 Does the high capacity for using bicarbonate favor the development of green tides?

Nowadays it is accepted that massive proliferation of opportunistic macroalgae is due to a combination of factors that include high nutrient concentration, appropriate light and high temperatures (Schramm, 1999; Nelson *et al.*, 2008; Liu *et al.*, 2009; Gao *et al.*, 2010). Furthermore, Monrad and Merceron (2005) and Liu *et al.* (2009) pointed out that the episodes of green tides normally occur under hydrological conditions of calm. From a physiological viewpoint, these environmental conditions should hamper the photosynthesis since they slow down the diffusion of  $\text{CO}_2$  in seawater. In addition, calm conditions (i.e., absence of turbulence)

combined with high photosynthetic activity can produce a substantial increase of the pH that contributes to reduce the free  $\text{CO}_2$  concentration (the decrease of bicarbonate concentration following increase of pH is comparatively lower). The combination of high photosynthetic activity and calm conditions are not infrequent in coastal habitats. In particular, they occur during the dinoflagellate blooms that produce increase of pH by 0.5–1.0 units (Hinga, 1992; Spilling, 2007). In some shallow and semienclosed water bodies the pH keeps high during several months due to the photosynthetic activity of the macroalgal communities. Thus, Macedo *et al.* (2001) reported pH values above 8.7 for part of the annual cycle in Santo André coastal Lagoon (Portugal). Similarly, pH above 9 (with peaks of 9.75) has been described from May to August in Mariager Fjord in Denmark (Hansen, 2002). In these systems, the diurnal variability of the pH could become more relevant than the seasonal cycle. For instance, Yates *et al.* (2007) documented that the range of diurnal variation of pH linked to photosynthetic activity in Tampa Bay (a shallow tidal estuary on the west coast of Florida) was 93% of the seasonal variability range. Similarly, Middelboe and Hansen (2007) described peaks of pH above 9 at midday in shallow water sites located at the Danish shore. The most extreme pH values in coastal areas have been described in the rock-pools of the upper inter-tidal fringe (Larsson *et al.*, 1997). In these habitats the pH can become higher than 10.5 and the  $\text{CO}_2$  concentration lower than 1  $\mu\text{M}$ . The tidal pools are often dominated by green macroalgae, mainly of the genera *Ulva* and *Enteromorpha* (Axelsson *et al.*, 1995; Bjork *et al.*, 2004). It is obvious that the relatively high capacity for using bicarbonate of the green macroalgae must confer a competitive advantage in environments with high pH since these species are able to keep relatively high photosynthesis rates at a given high pH (Bjork *et al.*, 2004) while other less efficient bicarbonate-users do not. This hypothesis was proposed by Larsson *et al.* (1997) and Bjork *et al.* (2004) for explaining the dominance of *Enteromorpha* in tidal pools.

There are no available data about the pH values reached during the development of green-tides. However, it could be speculated that the high photosynthetic activity associated with this phenomenon and the hydrological conditions under which it occurs, must deal with substantial increases of pH as described for other equivalent phenomenon (for instance, for red tides; Hinga, 1992; Spilling, 2007).

## 5.6 Conclusions

The comparison of the photosynthetic performance of fast growth algae and other intertidal macrophytes reveals that

the macroalgae have a higher photosynthetic potential under conditions of light saturation. Thus, the green macroalgae are able to reduce more efficiently their light absorption in response to increasing irradiance. Consequently, the risk of photoinhibition and the costs of investment and repair of pigments are minimized. The capacity of the opportunistic macroalgae to use bicarbonate for photosynthesis is also higher. Furthermore, they are able to modify the expression of their bicarbonate transport systems depending on the external pH conditions. Consequently, they are able to fix inorganic carbon under a wide range of pH values and conditions that hamper the CO<sub>2</sub> diffusion in seawater (for instance, low renewal of the water or high temperature).

Most of the studies devoted to research the formation of green tides from a physiological viewpoint have focused on the capacity of the opportunistic algae for assimilation of nitrate and phosphate. Little attention has been paid to other aspects.

The analysis showed in the present work indicates that photosynthetic performance can play an important role in favouring the growth of these species under conditions that hamper the growth of other inter-tidal macrophytes. It is reasonable to think that these particular characteristics are implied in the development of the green-tides. This hypothesis is in agreement with the suggestion that green-tides are due to combination of favourable hydrological factors, high temperature and excess of nutrients. It has to be noted that this finding implies that other anthropogenic impacts (in addition to eutrophication) should be taken into account in prevention of this phenomenon. For instance, the climate change is producing alterations in the oceanic circulation patterns that affect the coastal currents. Consequently, it could be speculated that frequency of green-tides is being modulated by the climate change. Ocean acidification, which is increasing the CO<sub>2</sub> availability in seawater, could decrease the competitive advantage of the fast growth algae in comparison to other macrophytes. Consequently, it could affect the occurrence of green tides. In any case, much more information about the physical and chemical conditions occurring within the green-tides is necessary to test these hypotheses.

## Acknowledgments

This study has been supported by the projects TROFOALB-ORAN (CTM2009-07776/MAR) of the Spanish National Programme in Marine Science and Technology from the Ministerio Español de Innovación y Ciencia (co-funded by EU) and 3-ESMAREU (Ministerio Español de Medio Ambiente, Medio Rural y Marino).

## References

- Abal, E., Loneragan, N., Bowen, P., Perry, C. and Udy, J. (1994) Physiological and morphological responses of the seagrass *Zostera capricorni* aschers to light intensity. *J. Exp. Mar. Biol. Ecol.*, **178**, 113–129.
- Axelsson, L. and Uusitalo, J. (1988) Carbon acquisition strategies for marine macroalgae. I. Utilization of proton exchanges visualized during photosynthesis in a closed system. *Mar. Biol.*, **97**, 295–300.
- Axelsson, L., Uusitalo, J. and Ryberg, H. (1991) Mechanisms for concentrating and storage of inorganic carbon in marine macroalgae. In: *Seaweed Cellular Biotechnology* (eds G. García-Reina and M. Pédersen). Universidad de las Palmas de Gran Canaria, pp. 185–198.
- Axelsson, L., Ryberg, H. and Beer, S. (1995) Two modes of bicarbonate utilization in the marine green macroalga *Ulva lactuca*. *Plant Cell Env.*, **18**, 439–445.
- Axelsson, L., Mercado, J.M. and Figueroa, F.L. (2000) Utilization of HCO<sub>3</sub><sup>−</sup> at high pH by the brown macroalga *Laminaria saccharina*. *Eur. J. Phycol.*, **35**, 53–59.
- Badger, M.R., Andrews, T.J., Whitney, S.M., *et al.* (1998) The diversity and co-evolution of Rubisco, plastids, pyrenoids and chloroplast-based CCMs in the algae. *Can. J. Bot.*, **76**, 1052–1071.
- Bahartan, K., Zibdah, M., Ahmed, Y., Israel, A. and Brickner, I. (2010) Macroalgae in the coral reefs of Eilat (Gulf of Aqaba, Red Sea) as a possible indicator of reef degradation. *Mar. Poll. Bull.*, **60**, 759–764.
- Ballesteros, E., Torras, X., Pinedo, S., García, M., Mangialajo, L. and Torres, M. (2007). A new methodology based on littoral community cartography dominated by macroalgae for the implementation of the European water Framework Directive. *Mar. Poll. Bull.*, **55**, 172–180.
- Beach, K.S., Smith, C.M., Michael, T. and Shin, H.-W. (1995) Photosynthesis in reproductive unicells of *Ulva fasciata* and *Enteromorpha flexuosa* : implications for ecological success. *Mar. Ecol. Progr. Ser.*, **125**, 229–237.
- Beer, S. and Axelsson, L. (2004) Limitations in the use of PAM fluorometry for measuring photosynthetic rates of macroalgae at high irradiances. *Eur. J. Phycol.*, **39**, 1–7.
- Beer, S. and Israel, A. (1986) Photosynthesis of *Ulva* sp.: III. O<sub>2</sub> effects, carboxylase activities, and the CO<sub>2</sub> incorporation pattern. *Plant Physiol.*, **81**, 937–938.
- Beer, S. and Koch, E. (1996) Photosynthesis of marine macroalgae and seagrasses in globally changing CO<sub>2</sub> environments. *Mar. Ecol. Progr. Ser.*, **41**, 199–204.
- Beer, S., Bjork, M., Hellblom, F. and Axelsson, L. (2002) Inorganic carbon utilization in marine angiosperms (seagrasses). *Funct. Plant Biol.*, **29**, 349–354.

- Björk, M., Haglund, K., Ramazanov, Z., García-Reina, G. and Pedersen, M. (1992) Inorganic-carbon assimilation in the green seaweed *Ulva rigida* C. Ag. (Chlorophyta). *Planta*, **187**, 15–46.
- Björk, M., Axelsson, L. and Beer, S. (2004) Why is *Ulva intestinalis* the only macroalga inhabiting isolated rock-pools along the Swedish Atlantic coast? *Mar. Ecol. Progr. Ser.*, **284**, 109–116.
- Choo, K.S., Snoeijs, P. and Pedersen, M. (2002) Uptake of inorganic carbon by *Cladophora glomerata* (Chlorophyta) from the Baltic Sea. *J. Phycol.*, **38**, 493–502.
- Choo, K.S., Nilsson, J., Pedersen, M. and Snoeijs, P. (2005) Photosynthesis, carbon uptake and antioxidant defence in two coexisting filamentous green algae under different stress conditions. *Mar. Ecol. Progr. Ser.*, **292**, 127–138.
- Cummings, M.E. and Zimmerman, R.C. (2003) Light harvesting and the package effect in the seagrasses *Thalassia testudinum* Bank ex König and *Zostera marina* L.: optical constraints on photoacclimation. *Aquat. Bot.*, **75**, 261–274.
- Dennison, W.C. and Alberte, R.S. (1982) Photosynthetic response of *Zostera marina* L. (eelgrass) to in situ manipulations of light intensity. *Oecologia*, **55**, 137–144.
- Done, T.J. (1992) Phase shifts in coral reef communities and their ecological significance. *Hydrobiologia*, **247**, 121–132.
- Dunton, K.H. (1994) Seasonal growth and biomass of the subtropical seagrass *Halodule wrightii* in relation to continuous measurements of underwater irradiance. *Mar. Biol.*, **120**, 479–489.
- Durako, M.J. (2007) Leaf optical properties and photosynthetic leaf absorptances in several Australian seagrasses. *Aquat. Bot.*, **87**, 83–89.
- Enríquez, S., Agustí S. and Duarte, C.M. (1992) Light absorption by seagrass *Posidonia oceanica* leaves. *Mar. Ecol. Progr. Ser.*, **86**, 201–204.
- Figueroa, F.L., Aguilera, J. and Niell, F.X. (1995). Red and blue light regulation of growth and photosynthetic metabolism in *Porphyra umbilicalis* (Bangiales, Rhodophyta). *Eur. J. Phycol.*, **30**: 11–18.
- Fletcher, R.T. (1996) The occurrence of 'green-tide'. In: *Marine Benthic Vegetation—Recent Changes and the Effects of Eutrophication* (eds W. Schramm and P.H. Nienhuis). Springer Verlag, Berlin, pp. 7–43.
- Fong, P., Boyer, K.E., Desmond, J.S. and Zedler, J.B. (1996) Salinity stress, nitrogen competition, and facilitation: what controls seasonal succession of two opportunistic green macroalgae? *Journal of Exp. Mar. Biol. and Ecol.*, **206**, 203–221.
- Frost-Christensen, H. and Sand-Jensen, K. (1992) The quantum efficiency of photosynthesis in macroalgae and submerged angiosperms. *Oecologia*, **91**, 377–384.
- Gao, S., Chen, X., Yi, Q., *et al.* (2010) A strategy for the proliferation of *Ulva prolifera*, Main Causative Species of Green Tides, with Formation of Sporangia by Fragmentation. *PLoS ONE*, **5**(1), e8571. doi:10.1371/journal.pone.0008571.
- Gómez, I., López-Figueroa, F., Ulloa, N., *et al.* (2004) Patterns of photosynthesis in 18 species of intertidal macroalgae from southern Chile. *Mar. Ecol. Progr. Ser.*, **270**, 103–116.
- Haglund, K., Björk, M., Ramazanov, Z., García-Reina, G. and Pedersen, M. (1992) Role of carbonic anhydrase in photosynthesis and inorganic-carbon assimilation in the red alga *Gracilaria tenuistipitata*. *Planta*, **187**, 275–281.
- Han, Y.-S., Kang, S.H. and Han, T. (2007) Photosynthesis and photoinhibition of two green macroalgae with contrasting habitats. *J. Plant Biol.*, **50**, 10–16.
- Hansen, P.J. (2002) Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession. *Aquat. Microb. Ecol.*, **28**, 279–288.
- Hellblom, F., Beer, S., Björk, M. and Axelsson, L. (2001) A buffer sensitive inorganic carbon utilisation system in *Zostera marina*. *Aquat. Bot.*, **69**, 55–62.
- Henley, W.J. (1993) Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *J. Phycol.*, **29**, 729–739.
- Henley, W.J., Lindley, S.T., Levavasseur, G., Osmond, C.B. and Ramus, J. (1992) Photosynthetic response of *Ulva rotundata* to light and temperature during emersion on an intertidal sand flat. *Oecologia*, **89**, 516–523.
- Hernández, I., Peralta, G., Pérez-Lloréns, J. L., Vergara, J.J. and Niell, F.X. (1997) Biomass and dynamics of growth of *Ulva* species in Palmones River Estuary. *J. Phycol.*, **33**, 764–772.
- Hinga, K.R. (1992) Co-occurrence of dinoflagellate blooms and high pH in marine enclosures. *Mar. Ecol. Progr. Ser.*, **86**, 181–187.
- Hiraoka, M., Ohno, M., Kawaguchi, S. and Yoshida, G. (2004) Crossing test among floating *Ulva* thalli forming 'green-tide' in Japan. *Hydrobiologia*, **512**, 239–245.
- Hughes, T., Szmant, A.M., Stenebeck, R., Carpenter, R. and Miller, S. (1994) Catastrophes, phase shifts, and large scale degradation of a Caribbean coral reef. *Science*, **265**, 1547–1551.
- Juanes, J.A., Guinda, X., Puente, A. and Revilla, J.A. (2008) Macroalgae, a suitable indicator of the ecological status of coastal rocky communities in the NE Atlantic. *Ecological Indicators*, **8**, 351–359.
- Larsson, C., Axelsson, L., Ryberg, H. and Beer, S. (1997) Photosynthetic carbon utilization by *Enteromorpha intestinalis* (Chlorophyta) from a Swedish rockpool. *Eur. J. Phycol.*, **32**, 49–54.

- Lavery, P.S. and McComb, A.J. (1991). The nutritional eco-physiology of *Chaetomorpha linum* and *Ulva rigida* in Peel Inlet, Western Australia. *Bot. Mar.*, **34**, 251–260.
- Lee, K.-S., Park, S. R. and Kim, Y. K. (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *J. Exp. Mar. Biol. Ecol.*, **350**, 144–175.
- Liu, D., Keesing, J.K., Xing, Q. and Shi, P. (2009) World's largest macroalgal bloom caused by expansion of seaweed aquaculture in China. *Mar. Poll. Bull.*, **58**, 888–895.
- Lotze, H.K. and Schramm, W. (2000) Ecophysiological traits explain species dominance patterns in macroalgal blooms. *J. Phycol.*, **36**, 287–295.
- Maberly, S.C. (1990) Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. *J. Phycol.*, **26**, 439–449.
- Macedo, M.F., Duarte, P., Mendes, P. and Ferreira, J.G. (2001) Annual variation of environmental variables, phytoplankton species composition and photosynthetic parameters in a coastal lagoon. *J. Plankton Res.*, **23**, 719–732.
- Major, K.M. and Dunton, K.H. (2002) Variations in light-harvesting characteristics of the seagrass *Thalassia testudinum*: evidence for photoacclimation. *J. Exp. Mar. Biol. Ecol.*, **275**, 173–189.
- Markager, S. and Sand-Jensen, K. (1996) Implications of thallus thickness for growth-irradiance relationships of marine macroalgae. *Eur. J. Phycol.*, **31**, 79–87.
- McGlathery, K. and Pedersen, M.F. (1999) The effect of growth irradiance on the coupling of carbons and nitrogen metabolism in *Chaetomorpha linum* (Chlorophyta). *J. of Phycol.*, **35**, 721–731.
- Mercado, J.M., Gordillo, F.J.L. (2011). Inorganic carbon acquisition in algal communities: are the laboratory data relevant to the natural ecosystems? *Photos. Res.*, doi: 10.1007/s11120-011-9646-0.
- Mercado, J.M., Jiménez, C., Niell, F.X. and Figueroa, F.L. (1996). Comparison of methods for measuring light absorption by algae and their application to the estimation of the package effect. *Sci. Mar.*, **60**, 39–45.
- Mercado, J.M., Gordillo, F.J.L., Figueroa, F.L. and Niell, F.X. (1998) External carbonic anhydrase and affinity for inorganic carbon in intertidal macroalgae. *J. Exp. Mar. Biol. Ecol.*, **221**, 209–220.
- Mercado, J.M., Niell, F.X., Silva, J. and Santos, R. (2003) Use of light and inorganic carbon acquisition by two morphotypes of *Zostera noltii* Hornem. *J. Exp. Mar. Biol. Ecol.*, **297**, 71–84.
- Merceron, M., Antoine, V., Auby, I. and Morand, P. (2007) In situ growth potential of the subtidal part of green tide forming *Ulva* spp. Stocks. *Science of Total Environment*, **384**, 293–305.
- Middelboe, A.L. and Hansen, P.J. (2007) High pH in shallow-water macroalgal habitats. *Mar. Ecol. Progr. Ser.*, **338**, 107–117.
- Morand, P. and Briand, X. (1996) Excessive growth of macroalgae: a symptom of environmental disturbance. *Bot. Mar.*, **39**, 491–516.
- Morand, P. and Merceron, M. (2005) Macroalgal population and sustainability. *J. Coastal Res.*, **21**, 1009–1020.
- Nelson, T.A., Haberlin, K., Nelson, A.V., et al. (2008) Ecological and physiological controls of species composition in green macroalgal blooms. *Ecology*, **89**, 1287–1298.
- Orfanidis, S., Panayotidis, P. and Stamatis, N. (2001). Ecological evaluation of transitional and coastal waters: A marine benthic macrophytes-based model. *Mediterr. Mar. Sci.*, **2/2**, 45–65.
- Orfanidis, S., Panayotidis, P. and Stamatis, N. (2003). An insight to the ecological evaluation index (EEI). *Ecol. Indic.*, **3**, 37–33.
- Plus, M., Auby, I., Verlaque, M. and Levavasseur, G. (2005) Seasonal variations in photosynthetic irradiance response curves of macrophytes from a Mediterranean coastal lagoon. *Aquat. Bot.*, **81**, 157–173.
- Raffaelli, D.G., Raven J.A., and Poole L.J. (1998) Ecological impact of green macroalgal blooms, *Oceanogr. Mar. Biol.*, **36**, 97–125.
- Sand-Jensen, K. (1988) Minimum light requirements for growth in *Ulva lactuca*. *Mar. Ecol. Progr. Ser.*, **50**, 187–193.
- Sand-Jensen, K. (1998) Photosynthetic responses of *Ulva lactuca* at very low light. *Mar. Ecol. Progr. Ser.*, **50**, 195–201.
- Schiel, D.R., Wood, S.A., Dunmore, R.A. and Taylor, D.I. (2006) Sediment on rocky intertidal reefs: effects on early postsettlement stages of habitat-forming seaweeds. *J. Exp. Mar. Biol. Ecol.*, **331**, 158–172.
- Schramm, W. (1999) Factors influencing seaweed responses to eutrophication: some results from EU- project EU-MAC. *J. Appl. Phycol.*, **11**, 69–78.
- Sfriso, A. and Pavoni, B. (1994) Macroalgae and phytoplankton competition in the central Venice Lagoon. *Env. Technol.*, **15**, 1–14.
- Spilling, K. (2007) Dense sub-ice bloom of dinoflagellates in the Baltic Sea, potentially limited by high pH. *J. Plankton Res.*, **29**, 895–901.
- Sukenik, A., Bennett, J. and Falkowski, P. (1987) Light-saturated photosynthesis – limitation by electron transport or carbon fixation? *Biochim. Biophys. Acta*, **891**, 205–215.
- Surif, M.B. and Raven, J.A. (1989) Exogenous inorganic carbon sources for photosynthesis in seawater by members of the Fucales and the Laminariales (Phaeophyta): Ecological and taxonomic implications. *Oecologia*, **78**, 97–105.

- Valiela I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D. and Foreman, K. (1997) Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnol. Oceanogr.*, **42**, 1105–1118.
- Vergara, J.J., Sebastián, M., Pérez-Llórens, J.L. and Hernández, I. (1998) Photoacclimation of *Ulva rigida* and *U. rotundata* (Chlorophyta) arranged in canopies. *Mar. Ecol. Progr. Ser.*, **165**, 283–292.
- Yabe, T., Ishii, Y., Amano, Y., *et al.* (2009) Green tide formed by free-floating *Ulva* spp. at Yatsu tidal flat, Japan. *Limnologia*, **10**, 239–245.
- Yates, K.K., Dufore, C., Smiley, N., Jackson, C. and Halley, R.B. (2007). Diurnal variation of oxygen and carbonate system in Tampa Bay and Florida Bay. *Mar. Chem.*, **104**, 110–124.
- Zimmerman, R.C., Reguzzoni, J.L. and Alberte, R.S. (1995) Eelgrass (*Zostera marina* L.) transplants in San Francisco Bay: role of light availability on metabolism, growth and survival. *Aquat. Bot.*, **51**, 67–86.
- Zou, D.H. and Gao, K.S. (2002) Photosynthetic utilization in *Porphyra haitanensis* (Bangiales, Rhodophyta). *Chinese Sci. Bull.*, **47**, 1629–1633.
- Zou, D., Gao, K. and Xia, J. (2003) Photosynthetic utilization of inorganic carbon in the economic brown alga, *Hizikia fusiforme* (Sargassaceae) from the South China Sea. *J. Phycol.*, **39**, 1095–1100.

# 6

## Significance of the Presence of Trace and Ultratrace Elements in Seaweeds

**Antonio Moreda-Piñeiro, Elena Peña-Vázquez and Pilar Bermejo-Barrera**

*Department of Analytical Chemistry, Nutrition and Bromatology, University of Santiago de Compostela, Santiago de Compostela, Spain*

### 6.1 Introduction

Seaweeds (or marine macroalgae) are one of the most ecologically and economically important resources of the oceans (Dhargalkar and Pereira, 2005). Marine macroalgae concentrate minerals from seawater, and they are consequently rich in macroelements and trace and ultratrace elements. The World Health Organization (WHO) arbitrarily applies the term “trace” to an element not exceeding 250 µg/g per matrix. A trace element is nutritionally significant if it is either essential or potentially toxic for an organism when present at low concentrations in tissues, food or drinking water. An element is considered to be essential to an organism when reduction of its exposure under certain limits results in a reduction in a physiological important function, or when the element is an integral part of an organic structure performing a vital function for the organism (WHO Report: Trace elements in human nutrition and health, 1996). Minerals such as Ca, Fe, Mg, F, Se, Cu, Cr, Mo or I are examples of essential elements, whereas arsenic and heavy metals such as Cd, Pb and Hg are of great concern because of their excessive concentration in certain foods. The essentiality of an element depends on the intake level, which must be adequate to minimize risk of nutrient deficit or excess (WHO Report: Vitamin and mineral requirements in human nutrition, 2004). For example, large amounts of iodine in the diet can be damaging to neonates

or infants, and aggravate thyroid problems. However, the benefits of correcting iodine deficiency seem to exceed the risks posed by supplementation (WHO Report: Vitamin and mineral requirements in human nutrition, 2004).

Seaweeds have many applications (Zemke-White and Ohno, 1999; Dhargalkar and Pereira, 2005), and many of their applications are related to their mineral content (as foods and nutritional supplements, fertilizers, in medicine, in industry, etc.). Seaweeds are a good source of iodine; thus, brown algae such as *Fucus* and *Laminaria* sp. have been used to treat thyroid goiter. They are also an important vegetable source of other essential elements such as iron and calcium, but their absorption could be affected by the linkage of the elements with phycocolloids (Burtin, 2003).

The various atomic spectroscopy options have been the most commonly used techniques to determine trace and ultratrace elements in seaweeds, usually after a microwave sample digestion or calcination (Maher, 1986; Sharp *et al.*, 1988; Muse *et al.*, 1995, 1999; Malea *et al.*, 1995; Ostapezuk *et al.*, 1997; Riget *et al.*, 1997; Struck *et al.*, 1997; Afzal-Rizvi and Shameel, 2001; Barreiro *et al.*, 2002; Rupérez, 2002; Fariás *et al.*, 2002; Olivares *et al.*, 2004; Ladra-Ramos *et al.*, 2005; Shiraishi, 2005, 2006; Santoso *et al.*, 2006; Fernández-Fernández *et al.*, 2007; Tuzen *et al.*, 2009; Rodríguez-Figueroa *et al.*, 2009; Besada *et al.*, 2009; Conti *et al.*, 2010; Romarís-Hortas *et al.*, 2010; Taboada *et al.*,

2010). Nowadays, inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Sharp *et al.*, 1988; Sakao *et al.*, 1997; Struck *et al.*, 1997; Peña-Farfal *et al.*, 2005; Domínguez-González *et al.*, 2005; Santoso *et al.*, 2006; Moreda-Piñero *et al.*, 2007; Subba-Rao *et al.*, 2007; Grotti *et al.*, 2008; Astorga-España *et al.*, 2008; Choi *et al.*, 2009; Romarís-Hortas *et al.*, 2010; Taboada *et al.*, 2010) and inductively coupled plasma-mass spectrometry (ICP-MS) (Sakao *et al.*, 1997; Ostapezuk *et al.*, 1997; Van Netten *et al.*, 2000; Shiraishi, 2005, 2006; Ródenas de la Rocha *et al.*, 2009; Conti *et al.*, 2010; Romarís-Hortas *et al.*, 2010; Domínguez-González *et al.*, 2010) are the main determination techniques due to their ability to perform simultaneous multielemental analysis. ICP-MS provides the lowest detection limits and also makes possible the determination of radionuclides. Multi-element analysis was also performed by non-destructive instrumental neutron activation analysis (INAA) (Jayasekera and Rossbach, 1996; Hou and Yan, 1998; Serforh-Armah *et al.*, 2001; Truus *et al.*, 2004; Rodríguez-Figueroa *et al.*, 2009).

## 6.2 Mineral content in seaweed

Seaweeds contain 10 to 20 times the minerals of land plants (Philpott and Bradford, 2006); minerals constitute approximately 36% of their dry matter (Burtin, 2003). McDermid and Stuercke (2003) evaluated the nutritional composition of 22 species of macroalgae consumed in Hawaii (6 green algae or Chlorophyta, 4 brown algae or Phaeophyta, 12 red algae or Rhodophyta). They reported ash values ranging from 22.4% to 64.2% d.w. (dry weight), even though none of those macroalgae were calcareous. Shiraishi (2005, 2006) observed that the highest element concentrations were found in four types of foods (nuts and seeds, bean products, seaweeds, and fish and shellfish) among 18 food categories in the Japanese diet. These authors found that seaweeds had an important contribution to the daily intake of some of the minerals: Na (5.3%), K (2.5%), Ca (2.8%), Mg (5.5%), Li (6.9%), Ba (5.1%), Fe (2.5%), Co (2.5%) and Cd (8.1%). Seaweeds also contributed 47.7% percentage of the total daily intake of strontium.

The presence of a balanced content of Na and K in seaweed is common, and this is nutritionally important because diets with a high Na/K ratio have been linked to the incidence of hypertension. For example, *Ulva rigida* showed a ratio near to one for Na/K (15.9 g/kg and 15.6 g/kg respectively) (Taboada *et al.*, 2009). Dawczynski *et al.* (2007) reported that many of the Rhodophytes have low Na/K ratios while *Undaria pinnatifida* had the highest concentration of Na, with a Na/K ratio of approximately 21.

Concentration of halogens (e.g., iodine), rare earth elements and many transition metal elements are remarkably higher in seaweed than in land vegetables, including kale and spinach (Hou and Yan, 1998). The concentrations of rare earth elements in seaweed were 10–20 times higher than those in terrestrial plants. However, these authors found that the major trace elements content (K, Na, Ca, Mg) and some trace elements content (Zn and Mn) were not significantly different from terrestrial plants. Their results didn't confirm that algae could concentrate Sr selectively (Hou and Yan, 1998).

Table 6.1 shows contents of Ca, Na, K, Mg and P in several species of Chlorophyta, Rhodophyta, and Phaeophyta, mentioned in the present review. Two different kinds of studies can be found in the bibliography related to seaweed and trace and ultratrace element content: those that consider seaweed as biomonitor for pollution in seawater, and those concerning seaweed use as foodstuffs. Sometimes, seaweed can have both applications, as shown in Tables 6.1 and 6.2; e.g. *Fucus vesiculosus* (bladderwrack) and *Ulva* sp. For seaweed, it is difficult to create a classification to differentiate between macroelements, trace elements and ultratrace elements. The reason is that different species of marine macroalgae from various locations can have very different levels of an element. For example, levels of iodine can range from µg/g to 5000 µg/g. Therefore, bibliographic data are included in Table 6.2 for 23 elements that are arranged in alphabetical order. Concentrations, when not specified, are expressed in d.w.

## 6.3 Trace and ultratrace elements in seaweeds

### 6.3.1 Legislation concerning seaweed consumption

#### European legislation

Seaweeds have been consumed in coastal areas since ancient times, mainly in the Far East and the Pacific area. Seaweeds are used directly as food, mainly dried or canned, but also as additives (phycocolloids) in a large range of edible products, or as fodder (Zemke-White and Ohno, 1999; Burtin, 2003; Dhargalkar and Pereira, 2005). Trace element content in edible seaweed also provides information about the environment where the seaweed was collected or harvested. Some authors affirm that pollution, specifically heavy metal contamination, must be extreme and chronic to cause significant problems with quality and productivity of marine plant resources (Sharp *et al.*, 1988). The concern about maintaining a healthy diet has caused an increase in

**Table 6.1** Content of Ca, Na, K, Mg and P in seaweeds (mg/g d.w.)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
<b>Chlorophyta</b>									
1	<i>Anadyomene stellata</i>	Malea <i>et al.</i> , 1995	Antikyra Gulf	BM	34.398	5.189	94.650	8.886	
2	<i>Bryopsis pennata</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	10.855	28.535	80.800	6.660	
3	<i>Caulerpa lentillifera</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	7		9.5	16.5	1.6
4	<i>Caulerpa racemosa</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	19.625	155.950	70.300	0.765	
5	<i>Caulerpa racemosa</i>	Santoso <i>et al.</i> , 2006	Seribu Islands, Indonesia	EU	3.2±0.2	27.5±1.2	18.5±5.3	3.8±0.3	
6	<i>Caulerpa sertularioides</i>	Santoso <i>et al.</i> , 2006	Seribu Islands, Indonesia	EU	0.3±0.0	0.7±0.4	12.0±4.4	3.7±1.0	
7	<i>Caulerpa taxifolia</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	15.810	110.400	14.757	6.870	
8	<i>Cladophora albida</i>	Malea <i>et al.</i> , 1995	Antikyra Gulf	BM	14.119	2.459	71.447	8.538	
9	<i>Cladophoropsis vaucheriae-formis</i>	Santoso <i>et al.</i> , 2006	Seribu Islands, Indonesia	EU	9.9±0.4	23.9±1.2	22.3±3.3	7.1±0.6	
10	<i>Codium bursa</i>	Malea <i>et al.</i> , 1995	Antikyra Gulf	BM	86.465–118.536	7.817–25.067	14.374–33.434	5.464–6.623	
11	<i>Codium fragile</i>	Hou and Yan, 1998	Qingdao, China	E	14.2	92.3	25.3	15.2	
12	<i>Codium iyengaraii</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	231.700	9.605	14.730	9.605	
13	<i>Codium reediae</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	7.7		9.4	17.2	1.1
14	<i>Codium reediae</i>	McDermid and Stuercke, 2003	Maui, Hawai, USA	E	8.2		9.2	17	1.2
15	<i>Codium yezoensis</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
16	<i>Dasycladus vermicularis</i>	Malea <i>et al.</i> , 1995	Antikyra Gulf	BM	3.744–11.293	2.502–7.621	25.380–161.960	7.110–9.019	
17	<i>Enteromorpha</i> sp.	Carballeira <i>et al.</i> , 2000	Galicia, NW Spain	BM					
18	<i>Enteromorpha</i> sp.	Astorga <i>et al.</i> , 2008	Strait of Magellan, Chile	BM					
19	<i>Enteromorpha flexuosa</i>	McDermid and Stuercke, 2003	O'ahu, Hawai, USA	E	16		7.4	11.7	1

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
20	<i>Enteromorpha intestinalis</i>	Hou and Yan, 1998	Weihai, China	E	8.5	56.4	7.8	24.1	
21	<i>Enteromorpha intestinalis</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	18.590	13.400	4.745	13.400	
22	<i>Enteromorpha intestinalis</i>	Tuzen <i>et al.</i> , 2009	Rize, Black Sea, Turkey	E					
23	<i>Enteromorpha intestinalis</i>	Tuzen <i>et al.</i> , 2009	Trabzon, Black Sea, Turkey	E					
24	<i>Enteromorpha intestinalis</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	E					
25	<i>Enteromorpha prolifera</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
26	<i>Enteromorpha prolifera</i>	Muse <i>et al.</i> , 1999	Punta Maqueda, Argentina	BM					
27	<i>Enteromorpha prolifera</i>	Muse <i>et al.</i> , 1999	Punta Borja, Argentina	BM					
28	<i>Monostroma fragile</i>	Hou and Yan, 1998	Qingdao, China	E	24	87.2	8.1	16.8	
29	<i>Monostroma hariotti</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
30	<i>Monostroma oxyspermum</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	31.4		5.8	13.6	3.5
31	<i>Ulva</i> sp.	Maher, 1986	St Vincent Gulf, South Australia	BM					
32	<i>Ulva</i> sp.	Carballeira <i>et al.</i> , 2000	Galicia, NW Spain	BM					
33	<i>Ulva fasciata</i>	McDermid and Stuercke, 2003	O'ahu, Hawaii, USA	E	28.7		4.7	21.9	2.2
34	<i>Ulva fasciata</i>	McDermid and Stuercke, 2003	Maui, Hawaii, USA	E	31.5		3.9	29.4	2.2
35	<i>Ulva fenestrata</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
36	<i>Ulva lactuca</i>	Hou and Yan, 1998	Weihai, China	E	50.4	57.3	4.9	32	
37	<i>Ulva lactuca</i>	Muse <i>et al.</i> , 1998	Punta Borja, Argentina	BM					
38	<i>Ulva lactuca</i>	Muse <i>et al.</i> , 1999	Punta Maqueda, Argentina	BM					
39	<i>Ulva lactuca</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	32.550	36.900	8.545	36.900	
40	<i>Ulva lactuca</i>	Tuzen <i>et al.</i> , 2009	Rize, Black Sea, Turkey	E					

(Continued)

Table 6.1 (Continued)

Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
41 <i>Ulva lactuca</i>	Tuzen <i>et al.</i> , 2009	Trabzon, Black Sea, Turkey	E					
42 <i>Ulva lactuca</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	E					
43 <i>Ulva pertusa</i>	Hou and Yan, 1998	Weihai, China	E	56	39.1	12.2	41.1	
44 <i>Ulva pertusa</i>	Hou and Yan, 1998	Qingdao, China	E	21.1	62.6	8.7	12.6	
45 <i>Ulva reticulata</i>	Santoso <i>et al.</i> , 2006	Seribu Islands, Indonesia	EU	12.6±0.3	26.4±0.8	17.9±5.3	21.5±2.8	
46 <i>Ulva rigida</i>	Moreda-Piñeiro <i>et al.</i> , 2007	Galicia, North-West Spain	E	26±1	22.7±0.5	3.7±0.2	18.5±0.54	
47 <i>Ulva rigida</i>	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					
48 <i>Ulva rigida</i>	Taboada <i>et al.</i> , 2010	Galicia, NW Spain	E	15.61	15.95	5.245	20.941	2.1
49 <i>Ulva rigida</i>	Dominguez <i>et al.</i> , 2010	Galicia, NW Spain	E					
50 <i>Ulva rigida</i>	Romarís <i>et al.</i> , 2010	Galicia, NW Spain	E	17±3	29±20	7.2±3.5	29±4.4	
51 <i>Ulvaria splendens</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
<b>Phaeophyta</b>								
52 <i>Adenocystis utricularis</i>	Muse <i>et al.</i> , 1995	Patagonia, Argentina	BM					
53 <i>Adenocystis utricularis</i>	Muse <i>et al.</i> , 1995	Patagonia, Argentina	BM					
54 <i>Adenocystis utricularis</i>	Muse <i>et al.</i> , 1995	Patagonia, Argentina	BM					
55 <i>Adenocystis utricularis</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
56 <i>Adenocystis utricularis</i>	Astorga <i>et al.</i> , 2008	Strait of Magellan, Chile	BM					
57 <i>Agarum cribrosum</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
58 <i>Alaria marginata</i>	Van Netten <i>et al.</i> , 2000	Bamfield, Canada	E					
59 <i>Ascophyllum nodosum</i>	Sharp, Samant, Vaidya, 1988	Point Edwards, Canada	E					
60 <i>Ascophyllum nodosum</i>	Sharp, Samant, Vaidya, 1988	Pumpkin island (control site), Canada	E					
61 <i>Ascophyllum nodosum</i>	Sharp, Samant, Vaidya, 1988	Belledune, Canada	E					
62 <i>Ascophyllum nodosum</i>	Riget <i>et al.</i> , 1998	West Greenland (64°N)	BM					
63 <i>Ascophyllum nodosum</i>	Carballeira <i>et al.</i> , 2000	Galicia, NW Spain	BM					
64 <i>Ascoseira mirabilis</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					

Table 6.1 (Continued)

Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
65 <i>Chorda filum</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
66 <i>Colpomenia sinuosa</i>	Muse <i>et al.</i> , 1995	Patagonia, Argentina	BM					
67 <i>Colpomenia sinuosa</i>	Hou and Yan, 1998	Weihai, China	EU	87.7	35.8	51.3	87.8	
68 <i>Costaria costata</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
69 <i>Cystoseira</i> sp.	Conti <i>et al.</i> , 2010	Linosa Island, Sicily, Italy	BM					
70 <i>Cystoseira barbata</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	EU					
71 <i>Cystoseira indica</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	118.125	80.563	19.050	9.425	
72 <i>Cystoseira zosteroides</i>	Malea <i>et al.</i> , 1995	Antikyra Gulf	BM	44.001	13.194	65.825	7.412	
73 <i>Desmarestia anceps</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
74 <i>Desmarestia antarctica</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
75 <i>Desmarestia viridis</i>	Hou and Yan, 1998	Weihai, China	EU	41.4	47.2	2.2	47.3	
76 <i>Dictyota acutiloba</i>	McDermid and Stuercke, 2003	O'ahu, Hawaii, USA	E	72.6		10.3	13.6	1.6
77 <i>Dictyota dichotoma</i>	Malea <i>et al.</i> , 1995	Antikyra Gulf	BM	1.827–6.961	6.130–6.465	46.400–89.475	8.611–10.215	
78 <i>Dictyota sandvicensis</i>	McDermid and Stuercke, 2003	O'ahu, Hawaii, USA	E	55.7		18.1	9.1	1.3
79 <i>Ecklonia radiata</i>	Maher, 1986	St Vicent Gulf, South Australia	BM					
80 <i>Eisenia bicyclis</i>	Van Netten <i>et al.</i> , 2000	Mitoku, Japan	E					
81 <i>Eisenia bicyclis</i>	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					
82 <i>Fucus</i> sp.	Sharp, Samant, Vaidya, 1988	Sydney St1, Canada	E					
83 <i>Fucus</i> sp.	Sharp, Samant, Vaidya, 1988	Sydney St2 (steel plant wharf), Canada	E					
84 <i>Fucus</i> sp.	Sharp, Samant, Vaidya, 1988	Point Edwards, Canada	E					
85 <i>Fucus</i> sp.	Sharp, Samant, Vaidya, 1988	Madden Pt, Canada	E					
86 <i>Fucus</i> sp.	Sharp, Samant, Vaidya, 1988	Mulgrave, Canada	E					
87 <i>Fucus ceranoides</i>	Carballeira <i>et al.</i> , 2000	Galicia, NW Spain	BM					

(Continued)

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
88	<i>Fucus spiralis</i>	Carballeira <i>et al.</i> , 2000	Galicia, NW Spain	BM					
89	<i>Fucus vesiculosus</i>	Riget <i>et al.</i> , 1997	West Greenland (64°N)	BM					
90	<i>Fucus vesiculosus</i>	Carvalho <i>et al.</i> , 1997	Tagus estuary, northern industrial belt	BM	8.519		11.058		
91	<i>Fucus vesiculosus</i>	Carvalho <i>et al.</i> , 1997	Tagus estuary, estuary center, Portugal	BM	1.114		13.296		
92	<i>Fucus vesiculosus</i>	Carvalho <i>et al.</i> , 1997	Tagus estuary, southern part, Portugal	BM	8.635		13.865		
93	<i>Fucus vesiculosus</i>	Struck, 1998	North Sea	BM	41.1	32.0	12	8.0	3.1
94	<i>Fucus vesiculosus</i>	Struck, 1998	Baltic Sea	BM	33.2	26.7	16.4	10.4	3.72
95	<i>Fucus vesiculosus</i>	Van Netten <i>et al.</i> , 2000	Norway	E					
96	<i>Fucus vesiculosus</i>	Rupérez, 2002	Galicia, NW Spain	E	43.22±0.46	54.69±0.60	9.38±0.07	9.94±0.13	
97	<i>Fucus vesiculosus</i>	Truus <i>et al.</i> , 2004	Kakumäe Bay, Baltic Sea, North Estonia		2.000–0.7000	0.480	1.200–0.400	5.100	
98	<i>Fucus vesiculosus</i>	Romarís <i>et al.</i> , 2010	Galicia, NW Spain	E	31±0.51	36±0.72	17±0.88	8.0±0.21	
99	<i>Heterochordaria abietina</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
100	<i>Himantalia elongata</i>	Moreda-Piñeiro <i>et al.</i> , 2007	Galicia, North-West Spain	E	78±5	18.2±0.5	11.5±0.2	2.2±0.12	
101	<i>Himantalia elongata</i>	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					
102	<i>Himantalia elongata</i>	Domínguez <i>et al.</i> , 2010	Galicia, NW Spain	E					
103	<i>Himantalia elongata</i>	Romarís <i>et al.</i> , 2010	Galicia, NW Spain	E	40±2	40±2.2	10±1.0	8.6±0.47	
104	<i>Himantothallus grandifolius</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
105	<i>Hizikia fusiforme</i>	Dawczynski <i>et al.</i> , 2007	China, Japan and Korea	E, FM	35.4±10.1	14.0±3.4	13.3±0.6	6.67±0.87	1.01±0.04
106	<i>Hizikia fusiforme</i>	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					
107	<i>Hizikia fusiformis</i>	Van Netten <i>et al.</i> , 2000	Mitoku, Japan	E					

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
108	<i>Laminaria</i> sp.	Dawczynski <i>et al.</i> , 2007	China, Japan and Korea	E, FM	102±30.6	25.9±3.2	7.42±1.04	5.70±0.58	2.66±1.39
109	<i>Laminaria</i> sp.	Ródenas <i>et al.</i> , 2009	Brittany, France	E					
110	<i>Laminaria</i> sp.	Ródenas <i>et al.</i> , 2009	Galicia, Spain	E					
111	<i>Laminaria</i> sp.	Ródenas <i>et al.</i> , 2009	Yang-Tse, Korea	E					
112	<i>Laminaria</i> sp.	Ródenas <i>et al.</i> , 2009	Kunga and Mitoku, Japan	E					
113	<i>Laminaria</i> sp.	Ródenas <i>et al.</i> , 2009	Europe, Asia	E					
114	<i>Laminaria</i> sp.	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					
115	<i>Laminaria cichorioides</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
116	<i>Laminaria digitata</i>	Sharp, Samant, Vaidya, 1988	Point Edwards, Canada	E					
117	<i>Laminaria digitata</i>	Sharp, Samant, Vaidya, 1988	Pumpkin island (control site), Canada	E					
118	<i>Laminaria digitata</i>	Rupérez, 2002	Galicia, NW Spain	E	115.79±1.28	38.18±0.43	10.05±0.05	6.59±0.06	
119	<i>Laminaria longicrucis</i>	Sharp, Samant, Vaidya, 1988	Sydney St1, Canada	E					
120	<i>Laminaria japonica</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
121	<i>Laminaria japonica</i>	Hou and Yan, 1998	Qingdao, China	E	96.3	29.2	12.7	6.4	
122	<i>Laminaria japonica</i>	Van Netten <i>et al.</i> , 2000	Japan	E					
123	<i>Laminaria longicrucis</i>	Sharp, Samant, Vaidya, 1988	Belledune, Canada	E					
124	<i>Laminaria longicrucis</i>	Sharp, Samant, Vaidya, 1988	Sydney St2 (steel plant wharf), Canada	E					
125	<i>Laminaria longicrucis</i>	Sharp, Samant, Vaidya, 1988	Point Edwards, Canada	E					
126	<i>Laminaria longicrucis</i>	Sharp, Samant, Vaidya, 1988	Madden Pt, Canada	E					
127	<i>Laminaria longicrucis</i>	Sharp, Samant, Vaidya, 1988	Mulgrave, Canada	E					
128	<i>Laminaria longicrucis</i>	Sharp, Samant, Vaidya, 1988	Pumpkin island (control site), Canada	E					

(Continued)

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
129	<i>Laminaria ochroleuca</i>	Moreda-Piñeiro <i>et al.</i> , 2007	Galicia, North-West Spain	E	52±2	13.3±0.4	9.7±0.2	4.1±0.26	
130	<i>Laminaria ochroleuca</i>	Romarís <i>et al.</i> , 2010	Galicia, NW Spain	E	78±21	30±0.66	9.6±0.87	6.3±1	
131	<i>Laminaria ochroleuca</i> + <i>L. sacharina</i>	Domínguez <i>et al.</i> , 2010	Galicia, NW Spain	E					
132	<i>Laminaria saccharina</i>	Van Netten <i>et al.</i> , 2000	Bamfield, Canada	E					
133	<i>Laminaria setchellii</i>	Van Netten <i>et al.</i> , 2000	Bamfield, Canada	E					
134	<i>Leathesia difformis</i>	Muse <i>et al.</i> , 1995	Patagonia, Argentina	BM					
135	<i>Lessonia fuscescens</i>	Muse <i>et al.</i> , 1995	Patagonia, Argentina	E, BM					
136	<i>Macrocystis integrifolia</i>	Van Netten <i>et al.</i> , 2000	Bamfield, Canada	E					
137	<i>Macrocystis pyrifera</i>	Muse <i>et al.</i> , 1995	Patagonia, Argentina	E, BM					
138	<i>Mazzaella laminarioides</i>	Astorga <i>et al.</i> , 2008	Strait of Magellan, Chile	BM					
139	<i>Nereocystis leutkeana</i>	Van Netten <i>et al.</i> , 2000	Bamfield, Canada	E					
140	<i>Nereocystis leutkeana</i>	Van Netten <i>et al.</i> , 2000	Bamfield, Canada	E					
141	<i>Padina australis</i>	Santoso <i>et al.</i> , 2006	Seribu Islands, Indonesia	EU	0.5±0.2	1.0±0.9	28.3±4.3	4.0±1.6	
142	<i>Padina durvillaei</i>	Rodriguez <i>et al.</i> , 2009	North mining zone, Santa Rosalía, Mexico	BM					
143	<i>Padina durvillaei</i>	Rodriguez <i>et al.</i> , 2009	Mining zone, Santa Rosalía, Mexico	BM					
144	<i>Padina durvillaei</i>	Rodriguez <i>et al.</i> , 2009	South mining zone, Santa Rosalía, Mexico	BM					
145	<i>Padina pavonica</i>	Malea <i>et al.</i> , 1995	Antikyra Gulf	BM	4.263–12.177	3.466–12.588	93.825–233.907	6.837–17.729	
146	<i>Padina pavonica</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	EU					
147	<i>Padina pavonica</i>	Conti <i>et al.</i> , 2010	Linosa Island, Sicily, Italy	BM					

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
148	<i>Padina tetrastromatica</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	26.620	20.530	46.950	24.500	
149	<i>Pelvetia wrightii</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
150	<i>Phaeurus antarcticus</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
151	<i>Puncyaria plantaginea</i>	Hou and Yan, 1998	Weihai, China	EU	80.1	63.3	108	48.2	
152	<i>Sargassum carpophyllum</i>	Hou and Yan, 1998	Beihai, China	EU	129	37.6	10.5	6.6	
153	<i>Sargassum echinocarpum</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	95		13.1	11.6	1.4
154	<i>Sargassum henslowianum</i>	Hou and Yan, 1998	Zhanjiang, China	EU	97.6	38.3	16.3	9.9	
155	<i>Sargassum kjellmanianum</i>	Hou and Yan, 1998	Weihai, China	EU	15.8	41.1	14.8	15	
156	<i>Sargassum kjellmanianum</i>	Hou and Yan, 1998	Qingdao, China	EU	10.7	31	13.2	14.3	
157	<i>Sargassum obtusifolium</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	79		15	9.3	1.4
158	<i>Sargassum pallidum</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
159	<i>Sargassum parvifolium</i>	Hou and Yan, 1998	Beihai, China	EU	100	34.9	13	7.6	
160	<i>Sargassum polycystum</i>	Santoso <i>et al.</i> , 2006	Seribu Islands, Indonesia	EU	17.5±1.4	9.7±1.4	18.7±1.4	5.7±0.7	
161	<i>Sargassum thunbergii</i>	Hou and Yan, 1998	Weihai, China	EU	78.3	34.3	12.7	27.9	
162	<i>Sargassum thunbergii</i>	Hou and Yan, 1998	Qingdao, China	EU	4.6	24.2	29.4	4.9	
163	<i>Sargassum vachellianum</i>	Hou and Yan, 1998	Zhanjiang, China	EU	103.3	32.4	15.8	9.3	
164	<i>Sargassum vulgare</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	60.666	99.022	16.055	14.166	
165	<i>Scytosiphon lomentarius</i>	Hou and Yan, 1998	Weihai, China	EU	57.5	61.5	8.4	31.2	
166	<i>Turbinaria conoides</i>	Santoso <i>et al.</i> , 2006	Seribu Islands, Indonesia	EU	27.9±1.1	11.5±0.5	14.8±2.2	5.7±0.3	
167	<i>Undaria pinnatifida</i>	Hou and Yan, 1998	Weihai, China	E	43.1	48.8	9	16.9	
168	<i>Undaria pinnatifida</i>	Van Netten <i>et al.</i> , 2000	Mitoku, Japan	E					

(Continued)

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
169	<i>Undaria pinnatifida</i>	Van Netten <i>et al.</i> , 2000	Mitoku, Japan	E					
170	<i>Undaria pinnatifida</i>	Rupérez, 2002	Galicia, NW Spain	E	86.99±1.44	70.64±1.66	9.31±0.38	11.81±0.34	
171	<i>Undaria pinnatifida</i>	Dawczynski <i>et al.</i> , 2007	China, Japan and Korea	E, FM	4.80±1.04	98.4±15.7	8.99±1.37	8.68±3.02	3.62±0.72
172	<i>Undaria pinnatifida</i>	Yamada <i>et al.</i> , 2007	Osaka Bay, Japan	M				9.56–27.1	
173	<i>Undaria pinnatifida</i>	Moreda-Piñeiro <i>et al.</i> , 2007	Galicia, North-West Spain	E	63±5	51.7±0.4	13.4±0.2	10.2±0.6	
174	<i>Undaria pinnatifida</i>	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					
175	<i>Undaria pinnatifida</i>	Domínguez <i>et al.</i> , 2010	Galicia, NW Spain	E					
176	<i>Undaria pinnatifida</i>	Romarís <i>et al.</i> , 2010	Galicia, NW Spain	E	64±48	90±10	11±2.1	12±4	
<b>Rhodophyta</b>									
177	<i>Ahnfeltiopsis concinna</i>	McDermid and Stuercke, 2003	Onakahakaha, Hawai'i, USA	E	30.1		4.4	7.5	1
178	<i>Ahnfeltiopsis concinna</i>	McDermid and Stuercke, 2003	Kona, Hawai'i, USA	E	30		4.9	8.8	1.1
179	<i>Antithamnion cruciatum</i>	Tuzen <i>et al.</i> , 2009	Trabzon, Black Sea, Turkey	EU					
180	<i>Antithamnion cruciatum</i>	Tuzen <i>et al.</i> , 2009	Rize, Black Sea, Turkey	EU					
181	<i>Antithamnion cruciatum</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	EU					
182	<i>Botryocladia leptopoda</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	65.925	202.375	9.055	27.040	
183	<i>Bryocladia thysigera</i>	Sergor-Ahmah <i>et al.</i> , 2001	Southern Ghana	BM					
184	<i>Ceramium boydenoo</i>	Hou and Yan, 1998	Weihai, China	E	34.4	35.1	9.5	24.9	
185	<i>Centroceras clavulatum</i>	Sergor-Ahmah <i>et al.</i> , 2001	Southern Ghana	BM					
186	<i>Ceramium kondoi</i>	Hou and Yan, 1998	Weihai, China	E	73.5	73.9	14.2	30.7	
187	<i>Ceramium rubrum</i>	Tuzen <i>et al.</i> , 2009	Rize, Black Sea, Turkey	E					
188	<i>Ceramium rubrum</i>	Tuzen <i>et al.</i> , 2009	Trabzon, Black Sea, Turkey	E					

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
189	<i>Ceramium rubrum</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	E					
190	<i>Champia compressa</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	21.475	10.085	10.630	7.985	
191	<i>Chondrus crispus</i>	Sharp, Samant, Vaidya, 1988	Belledune, Canada	E					
192	<i>Chondrus crispus</i>	Sharp, Samant, Vaidya, 1988	Sydney St1, Canada	E					
193	<i>Chondrus crispus</i>	Sharp, Samant, Vaidya, 1988	Sydney St2 (steel plant wharf), Canada	E					
194	<i>Chondrus crispus</i>	Sharp, Samant, Vaidya, 1988	Point Edwards, Canada	E					
195	<i>Chondrus crispus</i>	Sharp, Samant, Vaidya, 1988	Madden Pt, Canada	E					
196	<i>Chondrus crispus</i>	Sharp, Samant, Vaidya, 1988	Mulgrave, Canada	E					
197	<i>Chondrus crispus</i>	Sharp, Samant, Vaidya, 1988	Pumpkin island (control site), Canada	E					
198	<i>Chondrus crispus</i>	Rupérez, 2002	Galicia, NW Spain	E	31.84±0	42.70±0.62	4.20±0.22	7.32±0.06	
199	<i>Chondrus crispus</i>	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					
200	<i>Chondrus ocellatus</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	22.6		4.4	9.2	2.7
201	<i>Chondrus yendoii</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
202	<i>Corallina elongate</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	EU					
203	<i>Corallina pilulifera</i>	Hou and Yan, 1998	Weihai, China	EU		12.3	184	43.7	
204	<i>Dictyopteris divaricate</i>	Hou and Yan, 1998	Weihai, China	EU	56.7	40.9	48	37.6	
205	<i>Dictyopteris divaricate</i>	Hou and Yan, 1998	Qingdao, China	EU	96.3	29.2	12.7	9.61	
206	<i>Eucheuma denticulatum</i>	McDermid and Stuercke, 2003	O'ahu, Hawaii, USA	E	124		4.5	7.6	0.8
207	<i>Gelidium</i> sp.	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					

(Continued)

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
208	<i>Gelidium amansil</i>	Hou and Yan, 1998	Weihai, China	E		33.7			
209	<i>Gelidium latifolium</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	E					
210	<i>Gelidium sesquipedale</i>	Domínguez <i>et al.</i> , 2010	Galicia, NW Spain	E					
211	<i>Georgiella confluens</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
212	<i>Gigartina acicularis</i>	Sergor-Ahmah <i>et al.</i> , 2001	Southern Ghana	BM					
213	<i>Gigartina skottsbergii</i>	Muse <i>et al.</i> , 1995	Patagonia, Argentina	E, BM					
214	<i>Gloeosiphonia capillaris</i>	Hou and Yan, 1998	Weihai, China	EU	32.6	48.6	6.2	40.5	
215	<i>Gracilaria confervoides</i>	Hou and Yan, 1998	Qingdao, China	E	11.2	27.8	16.2	6.57	
216	<i>Gracilaria coronopifolia</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	221.6		1.8	3.4	3.8
217	<i>Gracilaria corticata</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	114.750	26.290	11.725	4.580	
218	<i>Gracilaria parvispora</i>	McDermid and Stuercke, 2003	Moloka'i, Hawaii, USA	E	160		3.8	4.9	1.5
219	<i>Gracilaria salicornia</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	179.7		7.3	5.1	1.7
220	<i>Halymenia formosa</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	46		5.3	12.5	2.1
221	<i>Hyalosiphonia caespitosa</i>	Hou and Yan, 1998	Weihai, China	EU	46.1	46.3	16.8	26.8	
222	<i>Hypnea musciformis</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	62.125	129.688	7.978	4.930	
223	<i>Hypnea musciformis</i>	Sergor-Ahmah <i>et al.</i> , 2001	Southern Ghana	BM					
224	<i>Hypnea valentiae</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	112.938	154.188	10.350	15.260	
225	<i>Iridaea cordata</i>	Grotti <i>et al.</i> , 2008	Terra Nova Bay, Antarctica	BM					
226	<i>Iridaea cordata</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
227	<i>Jania rubens</i>	Sergor-Ahmah <i>et al.</i> , 2001	Southern Ghana	BM					

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
228	<i>Kappaphycus alvarezii</i>	Santoso <i>et al.</i> , 2006	Seribu Islands, Indonesia	EU	87.1±5.8	11.9±2.5	2.8±0.3	2.9±0.3	
229	<i>Laurencia okamurai</i>	Hou and Yan, 1998	Weihai, China	EU	24.3	63.6	2.1	31.9	
230	<i>Leathesia difformes</i>	Hou and Yan, 1998	Weihai, China	EU	71.4	42.6	48.9	39	
231	<i>Myelophycus siruplex</i>	Hou and Yan, 1998	Weihai, China	EU	24	31.3	14.6	98.6	
232	<i>Myriogramme mangini</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
233	<i>Palmaria</i> sp.	Moreda-Piñeiro <i>et al.</i> , 2007	Galicia, North-West Spain	E	44±3	2.7±0.1	6.5±0.2	1.6±0.08	
234	<i>Palmaria decipiens</i>	Farias <i>et al.</i> , 2002	King George Island, Antarctica	BM					
235	<i>Palmaria palmata</i>	Domínguez <i>et al.</i> , 2010	Galicia, NW Spain	E					
236	<i>Palmaria palmata</i>	Romarís <i>et al.</i> , 2010	Galicia, NW Spain	E	81±25	10±10	3.8±1.2	1.6±0.67	
237	<i>Phorphyra columbina</i>	Astorga <i>et al.</i> , 2008	Strait of Magellan, Chile	BM					
238	<i>Phyllophora antarctica</i>	Grotti <i>et al.</i> , 2008	Cape Evans, Antarctica	BM					
239	<i>Phyllophora antarctica</i>	Grotti <i>et al.</i> , 2008	Terra Nova Bay, Antarctica	BM					
240	<i>Phyllophora nervosa</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	EU					
241	<i>Polycavernosa dentata</i>	Sergor-Ahmah <i>et al.</i> , 2001	Southern Ghana	BM					
242	<i>Polysiphonia japonica</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
243	<i>Polysiphonia urceolate</i>	Hou and Yan, 1998	Weihai, China	EU	32.7	23.7		86.6	
244	<i>Porphyra</i> sp.	Hou and Yan, 1998	Qingdao, China	E	32	19.8	2.4	14.5	
245	<i>Porphyra</i> sp.	Dawczynski <i>et al.</i> , 2007	Japan, Korea	E, FM	27.2±11.4	5.87±3.31	3.39±0.92	3.50±0.50	5.10±1.56
246	<i>Porphyra</i> sp.	Dawczynski <i>et al.</i> , 2007	China	E, FM	29.0±3.1	7.06±3.11	3.11±0.73	3.48±0.32	5.57±0.31
247	<i>Porphyra</i> sp.	Moreda-Piñeiro <i>et al.</i> , 2007	Galicia, North-West Spain	E	13±2	4.0±0.1	2.2±0.1	2.3±0.09	
248	<i>Porphyra</i> sp.	Ródenas <i>et al.</i> , 2009	Brittany, France	E					
249	<i>Porphyra</i> sp.	Ródenas <i>et al.</i> , 2009	Galicia, Spain	E					

(Continued)

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
250	<i>Porphyra</i> sp.	Ródenas <i>et al.</i> , 2009	Yang-Tse, Korea	E					
251	<i>Porphyra</i> sp.	Ródenas <i>et al.</i> , 2009	Kunga and Mitoku, Japan	E					
252	<i>Porphyra</i> sp.	Ródenas <i>et al.</i> , 2009	Europe, Asia	E					
253	<i>Porphyra columbina</i>	Muse <i>et al.</i> , 1999	Punta Borja, Argentina	BM					
254	<i>Porphyra columbina</i>	Muse <i>et al.</i> , 1999	Punta Maqueda, Argentina	BM					
255	<i>Porphyra tenera</i>	Van Netten <i>et al.</i> , 2000	Japan	E					
256	<i>Porphyra tenera</i>	Van Netten <i>et al.</i> , 2000	Japan	E					
257	<i>Porphyra tenera</i>	Rupérez, 2002	Galicia, NW Spain	E	35.00±0.71	36.27±1.15	3.90±0.17	5.65±0.11	
258	<i>Porphyra umbilicales</i>	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					
259	<i>Porphyra umbilicales</i>	Romarís <i>et al.</i> , 2010	Galicia, NW Spain	E	29±8	8.2±1.4	3.7±0.79	2.9±0.26	
260	<i>Porphyra umbilicalis</i>	Tuzen <i>et al.</i> , 2009	Rize, Black Sea, Turkey	E					
261	<i>Porphyra umbilicalis</i>	Tuzen <i>et al.</i> , 2009	Trabzon, Black Sea, Turkey	E					
262	<i>Porphyra umbilicalis</i>	Tuzen <i>et al.</i> , 2009	Sinop, Black Sea, Turkey	E					
263	<i>Porphyra umbilicales</i> + <i>P. linearis</i>	Domínguez <i>et al.</i> , 2010	Galicia, NW Spain	E					
264	<i>Porphyra vietnamensis</i>	McDermid and Stuercke, 2003	Hawai'i, USA	E	39.7		2.9	7.8	2.5
265	<i>Porphyra vietnamensis</i>	Subba rao, Mantri and Ganesan, 2007	Central west coast of India	E	2.49±0.64	52.3±3.2	3.35±0.84	4.95±1.01	
266	<i>Ptilota filicina</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
267	<i>Rhodomela larix</i>	Saenko <i>et al.</i> , 1976	Vostok Bay, Sea of Japan	BM					
268	<i>Rhodomela confervoides</i>	Hou and Yan, 1998	Weihai, China	EU	93.6	66.6	10.8		
269	<i>Sarconema furcellatum</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	136.375	220.188	7.448	19.820	

Table 6.1 (Continued)

	Species	Reference	Geographical origin	Use	K	Na	Ca	Mg	P
270	<i>Scinaia saifullahii</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	14.925	18.690	8.350	12.350	
271	<i>Solieria robusta</i>	Rizvi and Shameel, 2001	Karachi, Pakistan	BM	70.063	184.313	34.088	8.463	
<b>Commercial foods</b>									
272	Cooked <i>H.elongata</i> + <i>Saccorhiza</i> polyschides	Romarís <i>et al.</i> , 2010	Galicia, NW Spain	E	20±0.84	83±0.88	13±0.37	4.8±0.12	
273	Cooked <i>H.elongata</i> + <i>Saccorhiza</i> polyschides	Domínguez <i>et al.</i> , 2010	Galicia, NW Spain	E					
274	Kelp tablets (Species not listed)	Van Netten <i>et al.</i> , 2000	Undisclosed	E					
275	Laver, for rice roll	Choi, Kang and Kim, 2009	Food purchased in Korea	E					
276	Laver, seasoned	Choi, Kang and Kim, 2009	Food purchased in Korea	E					
277	Me-hijiki, leaves of hijiki	Sakao <i>et al.</i> , 1997	Japan	E	46.3±0.9	14.0±0.1	13.9±0.1	6.34±0.22	
278	Sea lettuce	Choi, Kang and Kim, 2009	Food purchased in Korea	E					
279	Sea mustard, dried	Choi, Kang and Kim, 2009	Food purchased in Korea	E					
280	Sea mustard, stem, salted	Choi, Kang and Kim, 2009	Food purchased in Korea	E					
281	Sea tangle, dried	Choi, Kang and Kim, 2009	Food purchased in Korea	E					
282	Sea tangle, raw	Choi, Kang and Kim, 2009	Food purchased in Korea	E					
283	Seaweed	Shiraishi, 2005, 2006	Foods marketed in Japan	E, FM	10.5±0.3	34.2±1.2	2.62±0.08	2.52±0.06	1.5±0.04
284	Seaweed, dry (cochayuyo)	Olivares <i>et al.</i> , 2004	Food marketed in Santiago, Chile	E					
285	Wakame, nori and sea lettuce (Salad)	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					
286	Wak., ogonori, kombu, agar and akamodoki (Salad)	Besada <i>et al.</i> , 2009	Spanish market; Pacific or Atlantic Ocean	E					

E: edible seaweed. EU: edibility unknown; the study was performed to evaluate edibility.

BM: the study was performed using the seaweed as a bioindicator of pollution.

FM: the results are given for fresh matter.

ND: not detected.

**Table 6.2** (Continued) Content of trace and ultra trace element in seaweed ( $\mu\text{g/g d.w.}$ ; samples from Table 6.1)

Species	Al	As	Au	Ba	Br	Cd	Co	Cr	Cu	Fe	Hg	I
<b>Chlorophyta</b>												
1 <i>Anadyomene stellata</i>						31.5			7.1	2146		
2 <i>Bryopsis pinnata</i>						3.15	8.05	9.925	12.9	3795		
3 <i>Caulerpa lentillifera</i>									6	167		
4 <i>Caulerpa racemosa</i>						2.2	6.8	12.525	11.25	2542.5		
5 <i>Caulerpa racemosa</i>									8 $\pm$ 3	813 $\pm$ 237		
6 <i>Caulerpa sertularioides</i>									251 $\pm$ 62	41 $\pm$ 10		
7 <i>Caulerpa taxifolia</i>						0.977	4.2	10.427	8.7	2840		
8 <i>Cladophora albida</i>									61.3	4426		
9 <i>Cladophoropsis vaucheriaeformis</i>												
10 <i>Codium bursa</i>				392	670	1.3–7.3			227 $\pm$ 3	111 $\pm$ 56		154
11 <i>Codium fragile</i>	95.7	15.9					2.73	16.8	2.4–7.3	476–4393		
12 <i>Codium iyengarii</i>						1.925	9.55	2.825	5.65	862.5		
13 <i>Codium recedae</i>									1	91		
14 <i>Codium recedae</i>									1	196		
15 <i>Codium yesoensis</i>							0.3	21.9	1.2	376		
16 <i>Dasycladus vermicularis</i>						0.9–12.5			12.8–253	5334–8567		
17 <i>Enteromorpha</i> sp.							6.0	19.8	44.7		<0.01–0.02	
18 <i>Enteromorpha</i> sp.												
19 <i>Enteromorpha flexuosa</i>				41	662		0.349	1.78	3	104		114.8
20 <i>Enteromorpha intestinalis</i>	1981						0.5	23.325	14	1085		
21 <i>Enteromorpha intestinalis</i>						0.5	0.00663 $\pm$ 0.00043	1.49 $\pm$ 0.12	9.08 $\pm$ 0.51	2695		
22 <i>Enteromorpha intestinalis</i>						0.0273 $\pm$ 0.0021	0.00156 $\pm$ 0.00015	2.48 $\pm$ 0.20	7.14 $\pm$ 0.46	2747 $\pm$ 240		
23 <i>Enteromorpha intestinalis</i>						0.0119 $\pm$ 0.0011	0.00156 $\pm$ 0.00015	2.07 $\pm$ 0.14	1.70 $\pm$ 0.10	343 $\pm$ 21		
24 <i>Enteromorpha intestinalis</i>						0.00185 $\pm$ 0.00010	0.00625 $\pm$ 0.00041	23.4	7.6	585 $\pm$ 43		
25 <i>Enteromorpha prolifera</i>							9.8			152		
26 <i>Enteromorpha prolifera</i>						0.70 $\pm$ 0.07		3.05 $\pm$ 0.08	4.60 $\pm$ 0.70			
27 <i>Enteromorpha prolifera</i>						0.14 $\pm$ 0.02		4.60 $\pm$ 0.08	7.30 $\pm$ 0.12			
28 <i>Monostroma fragile</i>	3530	14.6		24.2	730	<0.1	1	5.11		2380		63.6
29 <i>Monostroma hartiotti</i>		6.59 $\pm$ 0.29					357 $\pm$ 10	11.1 $\pm$ 0.6	15.2 $\pm$ 0.6	3095 $\pm$ 35		
30 <i>Monostroma oxyspermum</i>									28	142		
31 <i>Ulva</i> sp.						0.4 $\pm$ 0.02			1.5 $\pm$ 0.3			
32 <i>Ulva</i> sp.							4.1	4.1	21.0			
33 <i>Ulva fasciata</i>									5	86		
34 <i>Ulva fasciata</i>									1	141		
35 <i>Ulva fenestrata</i>							0.9	3.5	21.0	835		
36 <i>Ulva lactuca</i>	3077			24.9	501		0.564	4.09		2034		53.8
37 <i>Ulva lactuca</i>						0.12 $\pm$ 0.01		1.56 $\pm$ 0.08	5.50 $\pm$ 0.35			
38 <i>Ulva lactuca</i>						0.55 $\pm$ 0.04		0.33 $\pm$ 0.02	4.20 $\pm$ 0.30			
39 <i>Ulva lactuca</i>						2.3	2.3	1.85	7.125	382.5		
40 <i>Ulva lactuca</i>						0.0050 $\pm$ 0.00042	0.00291 $\pm$ 0.00016	1.04 $\pm$ 0.10	9.52 $\pm$ 0.55	425 $\pm$ 24		
41 <i>Ulva lactuca</i>						0.00404 $\pm$ 0.00020	0.00663 $\pm$ 0.00047	0.50 $\pm$ 0.05	4.95 $\pm$ 0.15	277 $\pm$ 20		

42	<i>Ulva lactuca</i>	1294	44.9	534	0.0218±0.0019	0.0322±0.0025	1.02±0.10	6.78±0.42	306±25	
43	<i>Ulva pertusa</i>	23 100	518	480		0.426	2.64	1578	33.2	
44	<i>Ulva pertusa</i>					1.19	7.55	5790	12.9	
45	<i>Ulva reticulata</i>							179±1	280±60	
46	<i>Ulva rigida</i>		0.79±0.01		<0.1	0.4±0.02	1.11±0.2			
47	<i>Ulva rigida</i>		6.41–7.06		0.031–0.033			3.05–3.15		0.018–0.019
48	<i>Ulva rigida</i>							5	2830	8
49	<i>Ulva rigida</i>		3.97±1.47			0.63±0.010	6.4±0.11	14.4±2.97		
50	<i>Ulva rigida</i>			14.6±11.6	504.6±28.56		1.1	14.3	1669	100.1±29.79
51	<i>Ulvaria splendens</i>									
<b>Phaeophyta</b>										
52	<i>Adenocystis utricularis</i>	290–320			0.20–0.32	0.9	11.8	2.2	1077	<0.01
53	<i>Adenocystis utricularis</i>	290–360			0.28–0.35	0.151	<0.5	<0.5	60	<0.05
54	<i>Adenocystis utricularis</i>	330–380			0.36–0.92		1	3.1±0.4	370±190	
55	<i>Adenocystis utricularis</i>		32.6±1.4		10.4±0.67	50.2±2.7	1.80±0.09	1.54±0.07	325±13	
56	<i>Adenocystis utricularis</i>									
57	<i>Agarum cribrosum</i>									
58	<i>Alaria marginata</i>	7.3	39.5		0.45					
59	<i>Ascophyllum nodosum</i>				0.5±0.1					
60	<i>Ascophyllum nodosum</i>				0.6±0.2		1.8±1.1	2.2±1.3	160±200	
61	<i>Ascophyllum nodosum</i>				9.0±2.3		1.9±1.6	5.3±1.7	104±50	
62	<i>Ascophyllum nodosum</i>		22.7–26.1		0.2–0.4	0.5–1.0	0.6	2.3–6.0	16–43	
63	<i>Ascophyllum nodosum</i>					5.6	17.1	62.8		
64	<i>Ascosera mirabilis</i>		42.2±1.9		1.66±0.10	26.4±1.2	3.80±0.21	9.11±0.31	75.1±4.0	
65	<i>Clorda filum</i>					2.7	6.2	1.4	40	
66	<i>Colpomenia sinuosa</i>	3010–3060			0.10–0.22					
67	<i>Colpomenia sinuosa</i>	13 960	226	354		1.53	8.79		5222	77.2
68	<i>Costaria costata</i>					0.6	3.5		95	
69	<i>Cystoseira</i> sp.				1.07±0.88	0.00905±0.00060	0.32±0.11	6.78±1.48	242±15	
70	<i>Cystoseira barbata</i>				0.00055±0.00004		0.99±0.05	2.47±0.18		
71	<i>Cystoseira indica</i>				3.95	5.125	4.7	8.125	249	
72	<i>Cystoseira zosteroideis</i>				25.4			3.3	1400	
73	<i>Desmarestia anceps</i>		28.7±1.3		1.00±0.06	155±6	3.25±0.19	4.05±0.22	108±4	
74	<i>Desmarestia antarctica</i>		52.8±2.2		<0.1	301±11	2.70±0.17	<0.2	35.2±1.1	
75	<i>Desmarestia viridis</i>	6765		643		1.02	5.84		3659	379.4
76	<i>Dictyota acutiloba</i>		135					5	438	
77	<i>Dictyota dichotoma</i>				3.1–3.3			11.0–23.0	1960–2120	
78	<i>Dictyota sandwicensis</i>							5	608	
79	<i>Ecklonia radiata</i>				0.78±0.07		<0.5	3.8±0.2	80	<0.05
80	<i>Eisenia bicyclis</i>	6.5	31		0.57	0.2		3.3		0.023–0.047
81	<i>Eisenia bicyclis</i>		27.9–34.1		0.585–0.827			3.06–4.54		
82	<i>Fucus</i> sp.				0.6±0.4			4.5±2.5	620±495	
83	<i>Fucus</i> sp.				1.2±0.2		1.5±0.8	7.3±1.3	1500±230	
84	<i>Fucus</i> sp.				0.6±0.2		2.7±0.8	1.8±0.8	130±50	
85	<i>Fucus</i> sp.				1.0±0.3		1	3.4±1	84±24	
86	<i>Fucus</i> sp.				1.4±0.2		2.5±0.3	5.8±1.6	100±14	
87	<i>Fucus ceranoides</i>					5.8	4.6	25.6		
88	<i>Fucus spiralis</i>					4.5	3.1	25.6		
89	<i>Fucus vesiculosus</i>		25.1–30.7		0.5–2.1	0.5–0.8	0.6	2.1–5.3	33–77	

(Continued)

Table 6.2 (Continued)

Species	Al	As	Au	Ba	Br	Cd	Co	Cr	Cu	Fe	Hg	I
90 <i>Fucus vesiculosus</i>		30			379		13		34.5	1206		
91 <i>Fucus vesiculosus</i>		26.5			273		14.3		17.3	1074		
92 <i>Fucus vesiculosus</i>		39.4			441		12.4		38.8	1047		
93 <i>Fucus vesiculosus</i>		46.4				0.562	3.12		3.37	603.7	0.0104	0.0104
94 <i>Fucus vesiculosus</i>		21.7				0.787	2.24		2.03	454.6	0.00018	
95 <i>Fucus vesiculosus</i>	228	20				0.34	0.386	11.5	1.4	520	1.08	732
96 <i>Fucus vesiculosus</i>									<5	42.0±1.7		
97 <i>Fucus vesiculosus</i>	130	330	0.011–0.012	372–381	140–160			1.8	20	280–320	0.0063–0.0074	130–160
98 <i>Fucus vesiculosus</i>		37.9±1.22		13.0±0.324	438.2±19.60				4.86±0.245			548.6±19.21
99 <i>Heterochordaria abietina</i>						0.64±0.01	0.41±0.01	<0.9		231		
100 <i>Himantalia elongata</i>		8.2±0.2				0.310–0.326						
101 <i>Himantalia elongata</i>		32.9–36.7					1.2±0.040	3.2±0.40	1.14–1.25		0.008–0.016	
102 <i>Himantalia elongata</i>												
103 <i>Himantalia elongata</i>		13.7±2.22		3.93±1.47	393.1±47.25		389±14	3.60±0.19	3.77±0.772			143.3±78.60
104 <i>Himantothallus grandifolius</i>		112±4				<0.1			1.00±0.05	23.6±1.0		
105 <i>Hizikia fusiforme</i>		87.7±8.2				1.25±0.29			2.61±1.16	679±270	0.0266±0.0053	262±70.0
106 <i>Hizikia fusiforme</i>		103–147				0.988–2.50			1.78–7.70		0.015–0.050	
107 <i>Hizikia fusiformis</i>	5.2	88				0.32	0.061	0.8	0.9	30	0.32	436
108 <i>Laminaria</i> sp.		8.42±2.26				0.59±0.21			1.07±0.51	264±317	0.0255±0.0209	2934±1489
109 <i>Laminaria</i> sp.		53.1±2.9				0.46±0.08	0.0728±0.0087	0.98±0.06				
110 <i>Laminaria</i> sp.		30.1±7.0				0.35±0.10	0.0998±0.0088	0.65±0.04				
111 <i>Laminaria</i> sp.		40.02±3.82				0.31±0.07	0.253±0.014	1.09±0.06				
112 <i>Laminaria</i> sp.		50.7±3.8				2.95±0.19	0.287±0.008	0.73±0.04				
113 <i>Laminaria</i> sp.		43.5±10.3				1.0±1.1	0.178±0.096	0.9±0.2				
114 <i>Laminaria</i> sp.		51.7–68.3				0.085–1.83			0.91–2.50		0.001–0.005	
115 <i>Laminaria cichorioides</i>							2.6	6.7	4	174		
116 <i>Laminaria digitata</i>						0.2±0.1		4.85±5.4	1.5±0.6	10±96		
117 <i>Laminaria digitata</i>						0.4±0.1		1.3±0.4	2.9±1.0	47±37		
118 <i>Laminaria digitata</i>									<5	32.9±5.4		
119 <i>Laminaria longicruris</i>						0.5±0.3		1	1.6±0.2	270±150		
120 <i>Laminaria japonica</i>				25	6770			2.9	0.9	27		
121 <i>Laminaria japonica</i>	320						0.27	1.99		210		3040
122 <i>Laminaria japonica</i>	8.9	29					0.449	1	<0.5	80	0.4	2110
123 <i>Laminaria longicruris</i>						0.02						
124 <i>Laminaria longicruris</i>						2.1±0.4		1.2±0.3	1.7±0.4	86±60		
125 <i>Laminaria longicruris</i>						0.9±0.6		2.0–1.0	2.5±0.3	8700±760		
126 <i>Laminaria longicruris</i>						0.5±0.3		2.3±2.2	1	90±50		
127 <i>Laminaria longicruris</i>						0.5±0.3		2.7±0.4	2.1±0.8	41±5		
128 <i>Laminaria longicruris</i>						1.2±1.2		3.0±0.7	5.8±2.9	370±420		
129 <i>Laminaria longicruris</i>						0.8±0.5		1.4±0.3	2.8±0.6	56±33		
130 <i>Laminaria ochroleuca</i>		48.8±5.0				3.36±0.1	<0.2	<0.9				4870±1102
131 <i>Laminaria ochroleuca</i> + <i>L. sacharina</i>		245±87.6		5.03±2.20	1137±183.3		0.12±0.040	6.8±0.49	1.81±0.565			
132 <i>Laminaria sacharina</i>	7	76.2				2.8	0.084	<0.5	<0.5	40	<0.05	238

133	<i>Laminaria setchellii</i>	2.3	58.5				0.1	0.185	<0.5	<0.5	<10	<0.05	1070
134	<i>Leathesia difformis</i>	720–760					0.20–0.31						
135	<i>Lessonia fuscens</i>	<50					2.03–2.45						
136	<i>Macrocystis integrifolia</i>	51.4	53.1				0.9	0.163	0.12	<0.5	110	<0.05	240
137	<i>Macrocystis pyrifera</i>	<50					1–01–1.15						
138	<i>Mazzaella laminarioides</i>												
139	<i>Nereocystis leutkana</i>	5	79				2.76	0.449	1.3	0.9	20	<0.01	
140	<i>Nereocystis leutkana</i>	9.8	66.3				0.3	0.042	<0.5	<0.5	40	<0.05	734
141	<i>Padina australis</i>												80
142	<i>Padina durvillaei</i>						3.6	4.2			446±16		
143	<i>Padina durvillaei</i>						3.5	5.93			2898		
144	<i>Padina durvillaei</i>						3.5	11.4			984		
145	<i>Padina pavonica</i>						7.3–24.5			82	2508		
146	<i>Padina pavonica</i>						0.00227±0.00016	0.0257±0.0022	3.04±0.19	4.6–103	255–2827		
147	<i>Padina pavonica</i>						0.86±0.11		0.68±0.32	3.35±0.20	591±50		
148	<i>Padina tetraspomatica</i>						2.876	6.376	16.15	12.375	3105		
149	<i>Pelvetia wrightii</i>							2.2	6.5	1.2	112		
150	<i>Phaeurus antarcticus</i>		61.4±2.9				0.70±0.04	182±7	12.1±0.77	4.11±0.22	1400±30		234.8
151	<i>Puncyaria plantaginea</i>	7460		81	1006			1.08	6.53		3901		1985
152	<i>Sargassum carpophyllum</i>	243	127	0.0073	18	664		0.614	<0.09	11	226		
153	<i>Sargassum echinocarpum</i>										92		5197
154	<i>Sargassum henslowianum</i>	725	230	0.0346	26.8	806		1.52	0.436		479		203.6
155	<i>Sargassum kjellmanianum</i>	1413			99.9	700		0.238	1.27		492		273
156	<i>Sargassum kjellmanianum</i>	3680	65.3		81.1	380		0.84	5.66		1820		
157	<i>Sargassum obtusifolium</i>									9	129		
158	<i>Sargassum pallidum</i>							1.6	3.9	4.3	115		2157
159	<i>Sargassum parvifolium</i>	212	145	0.0063	18	666		<0.011	<0.09	2±1	167		
160	<i>Sargassum polycystum</i>										277±214		336.3
161	<i>Sargassum thunbergii</i>	2500		54.9	447			0.807	3.39		1513		110.6
162	<i>Sargassum thunbergii</i>	5350			27 900			2.07	6.05		2970		5939
163	<i>Sargassum vachellianum</i>	799	182.8	0.0212	27.4	712		1.15	0.699		489		
164	<i>Sargassum vulgare</i>						1.2	7	0.18	8.6	1740		116.9
165	<i>Scytosiphon lomentarius</i>	2625		90.2	1006			0.787	3.54		2231		
166	<i>Turbiniaria conoides</i>									3±1	62±17		1571
167	<i>Undaria pinnatifida</i>	1512		108	6491			0.6	4.13		1434		102
168	<i>Undaria pinnatifida</i>	3	55				0.71	0.026	0.7	1.1	40	0.24	60
169	<i>Undaria pinnatifida</i>	1.9	20				0.51	0.098	0.8	<0.5	20	<0.05	
170	<i>Undaria pinnatifida</i>									<5	75.6±11.3		
171	<i>Undaria pinnatifida</i>		6.21±1.17				2.15±1.07			1.51±0.57	184±107	0.0189±0.0051	163±75.3
172	<i>Undaria pinnatifida</i>		7.1–19				0.018–1.300	0.037–0.260	0.48–3.18	3.20–43.8		ND–0.150	
173	<i>Undaria pinnatifida</i>		13.2±0.2				<0.1	<0.2	1.84±0.1				
174	<i>Undaria pinnatifida</i>		42.1–76.9				0.267–4.82			1.07–1.70		0.010–0.057	
175	<i>Undaria pinnatifida</i>												
176	<i>Undaria pinnatifida</i>		31.4±8.32	13.8±13.4	403.7±215.6			0.36±0.010	6.3±0.30	4.30±0.698			139.1±95.66

(Continued)

(Continued)

Table 6.2 (Continued)

Species	Al	As	Au	Ba	Br	Cd	Co	Cr	Cu	Fe	Hg	I
<b>Rhodophyta</b>												
177 <i>Abrifeliopsis concinna</i>									3	86		
178 <i>Abrifeliopsis concinna</i>									3	72		
179 <i>Antithamion cruciatum</i>						0.00463±0.00023	0.00442±0.00032	3.00±0.14	7.74±0.26	2873±150		
180 <i>Antithamion cruciatum</i>						0.0175±0.0014	0.00276±0.00022	4.13±0.32	6.83±0.34	1524±75		
181 <i>Antithamion cruciatum</i>						0.0446±0.0035	0.0819±0.0053	11.6±0.9	17.1±0.9	3949±200		
182 <i>Boryocladia lepiopoda</i>						2.9	8.05	3.4	13.6	499.5		
183 <i>Bryocladia thysigera</i>	509.95±14.30	8.75±1.5			495.10±14.0	ND				<50	ND	
184 <i>Ceramium boydenoo</i>	2403	ND	11.9		873	6.52±0.09	0.469	2.66		1378		71.1
185 <i>Centroceras clavulatum</i>	8103.0±69.7				11645.0±340.0					<50	0.22±0.009	
186 <i>Ceramium kondoi</i>	2210		39.1		2070		0.632	1.99		1431		34.1
187 <i>Ceramium rubrum</i>						0.00902±0.00050	0.00720±0.00050	1.99±0.12	7.17±0.45	1479±45		
188 <i>Ceramium rubrum</i>						0.00064±0.00005	0.00542±0.00045	1.95±0.15	7.28±0.32	1953±65		
189 <i>Ceramium rubrum</i>						0.00050±0.00004	0.00103±0.00008	3.29±0.22	6.55±0.45	996±50		
190 <i>Champia compressa</i>						2	10.6	6.826	8.275	587.5		
191 <i>Chondrus crispus</i>						2.4±2.2		1.5±0.6	5.7±1.6	390±41		
192 <i>Chondrus crispus</i>						0.4±0.1		1.7±0.4	5.8±0.6	430±470		
193 <i>Chondrus crispus</i>						0.5±0.2		2.6±0.2	9.1±4.6	1100±200		
194 <i>Chondrus crispus</i>						0.4		1.4	3.9	350±250		
195 <i>Chondrus crispus</i>						0.3		3.1±0.1	2.3±0.2	60±2		
196 <i>Chondrus crispus</i>						0.4±0.1		3.2±2.1	4.8±1.9	7.6±4.4		
197 <i>Chondrus crispus</i>						0.5±0.1		1.3±0.1	2.6±0.4	120±45		
198 <i>Chondrus crispus</i>									<5	39.7±1.1		
199 <i>Chondrus crispus</i>		23.2–25.5				0.718–0.742			1.55–2.21		0.006–0.007	
200 <i>Chondrus ocellatus</i>									39	142		
201 <i>Chondrus yendoii</i>							1.1	9	6.1	193		
202 <i>Corallina elongate</i>						0.00404±0.00032	0.00752±0.00055	4.48±0.34	3.84±0.17	99±6		161.3
203 <i>Corallina pilulifera</i>	3813		86.8		227		1.21	6.08		3086		
204 <i>Dictyopteris divaricate</i>	4776		126		334		0.933	6.22		3167		93.3
205 <i>Dictyopteris divaricate</i>	8870	11	17.8		370		0.4	2.23		630		28.8
206 <i>Eucheuma denticulatum</i>									2	112		
207 <i>Gelidium sp.</i>		<0.05–0.21				0.025–0.046			0.410–1.55		0.005–0.009	
208 <i>Gelidium amansil</i>			159		59 260		0.784	4.18		2535		203.8
209 <i>Gelidium larifolium</i>						0.0124±0.0011	0.0167±0.0013	1.95±0.15	6.84±0.44	618±26		
210 <i>Gelidium sesquipedale</i>							0.08±0.01	2.4±0.20				
211 <i>Georgiella confluent</i>		4.99±0.32				0.45±0.02	2.40±0.1	7.23±0.47	11.4±0.5	37.0±1.0		
212 <i>Gigartina acicularis</i>	3556.75±65.0	9.25±2.6			705.85±13.5	ND				<50	0.058±0.006	
213 <i>Gigartina skottsbergii</i>	<50					0.95–1.31						
214 <i>Gloesiphonia capillaris</i>	6112		99.7		1298		1.09	4.29		2109		81.1
215 <i>Gracilaria confervoides</i>	1280				1140		0.59	2.55		900		353
216 <i>Gracilaria coronopifolia</i>									2	136		
217 <i>Gracilaria corticata</i>						1.875	5.55	7.1	8.375	1105		
218 <i>Gracilaria parvispora</i>									3	198		

[illegible]

Table 6.2 (Continued)

Species	Al	As	Au	Ba	Br	Cd	Co	Cr	Cu	Fe	Hg	I
256 <i>Porphyra tenera</i>	2.6	29				0.83	0.47	2	8.9	190	0.24	185
257 <i>Porphyra tenera</i>		28.9–49.5				0.253–3.10			<5	103±4.1		
258 <i>Porphyra umbilicalis</i>		15.3±4.97		2.96±1.78	77.36±29.02				5.50–14.1		0.008–0.032	
259 <i>Porphyra umbilicalis</i>							0.0426±0.0034	0.50±0.04	8.61±2.88			58.73±29.39
260 <i>Porphyra umbilicalis</i>						0.0236±0.0015	0.00659±0.00043	1.48±0.10	3.93±0.10	784±24		
261 <i>Porphyra umbilicalis</i>						0.0114±0.0006	0.00798±0.00064	4.92±0.23	4.92±0.10	330±16		
262 <i>Porphyra umbilicalis</i>						0.00343±0.00024		0.84±0.06	4.19±0.15	114±10		
263 <i>Porphyra umbilicalis</i> + <i>P. linearis</i>						0.23±0.030	1.9±0.10					
264 <i>Porphyra vietnamensis</i>									7	154		
265 <i>Porphyra vietnamensis</i>		16.0±1.8				2.9±0.1	1.2±0.1	1.8±0.0	8.3±0.2	1370±9.9	0.2±0	
266 <i>Ptilota filicina</i>							4.5	6	12.9	2260		
267 <i>Rhodomela larix</i>							5.4	15.7	4.8	875		
268 <i>Rhodomela confervoides</i>				87.3	8057		2.28	4.78		2255		431.2
269 <i>Sarcocnema furcellatum</i>	<0.30					2.55	7.45	5.425	12.7	340.75		
270 <i>Scinia saifullahii</i>						1.15	2.85	4.55	4.2	1070		
271 <i>Solieria robusta</i>						2.8	6.9	7.7	11.9	1900		
<b>Commercial foods</b>												
272 Cooked <i>H. elongata</i> + <i>Sacchariza polyschides</i>		15.6±0.565		20.8±0.0512	111.6±8.253				3.27±0.305			36.07±1.193
273 Cooked <i>H. elongata</i> + <i>Sacchariza polyschides</i>							1.02±0.0380	6.8±0.59				
274 Kelp tablets (Species not listed)	280	17				0.13	0.594	11.4	2.2	1040	0.24	815
275 Laver, for rice roll												
276 Laver, seasoned									11.4			
277 Me-hijiki, leaves of hijiki	1300±120	126±10		24.1±2.1		0.62±0.03		0.72–1.35	4.9			
278 Sea lettuce									1.84±0.24	965±74		
279 Sea mustard, dried									1.13			
280 Sea mustard, stem, salted									1.35			
281 Sea tangle, dried									0.19			
282 Sea tangle, raw									0.83			
283 Seaweed									0.34			
284 Seaweed, dry (cochayuyo)				4.61±0.33		0.295±0.002	0.0397±0.0027	0.461±0.022	2.50±0.16	41.1±0.8		
285 Wakame, nori and sea lettuce (Salad)		19.7–23.0				0.683–0.709			1.36±0.73	15.88±3.85		
286 Wak, ogonori, kombu, agar and akamodoki (Salad)		18.6–19.1				1.69–1.80			7.00–7.08		0.006–0.017	
									1.47–1.56		0.024–0.035	

(Continued)

**Table 6.2** (Cont.). Content of trace and ultra trace element in seaweed ( $\mu\text{g/g d.w.}$ ; samples from Table 6.1)

Species	Hg	I	Mn	Mo	Ni	Pb	Rb	Sb	Se	Sr	U	V	Zn
<b>Chlorophyta</b>													
1 <i>Anadyomene stellata</i>						41.5							38.5
2 <i>Bryopsis pennata</i>						43.875							37.425
3 <i>Caulerpa lentillifera</i>			10										17
4 <i>Caulerpa racemosa</i>						23.675							21.725
5 <i>Caulerpa racemosa</i>													10 $\pm$ 2
6 <i>Caulerpa sertularioides</i>													3 $\pm$ 0
7 <i>Caulerpa taxifolia</i>						19.1							25.05
8 <i>Cladophora albida</i>						450							50.3
9 <i>Cladophoropsis vaucheriaeformis</i>													227 $\pm$ 3
10 <i>Codium bursa</i>						11.5–34.5							13.4–24.4
11 <i>Codium fragile</i>	154		277				41.5	<0.027	<0.15	337	<0.10	24.6	48.8
12 <i>Codium tyengarii</i>						26.075							18.25
13 <i>Codium rediae</i>			26										3
14 <i>Codium rediae</i>			26										3
15 <i>Codium yezoensis</i>			153		0.11							1.46	36.2
16 <i>Dasycladus vermicularis</i>						71.7–737							28.6–61.9
17 <i>Enteromorpha</i> sp.			30.1		27.4	10.4							36.8
18 <i>Enteromorpha</i> sp.	<0.01–0.02				<1–12.60	<0.5–11.20		<0.2–1.97				<1.2–11.34	35.80 $\pm$ 29.94
19 <i>Enteromorpha flexuosa</i>			5										6
20 <i>Enteromorpha intestinalis</i>	114.8		23.8				8.45	0.0678	<0.15	206	<0.10	<0.54	9.75
21 <i>Enteromorpha intestinalis</i>						19.6							81.55
22 <i>Enteromorpha intestinalis</i>			48.1 $\pm$ 3.2		3.16 $\pm$ 0.21	1013 $\pm$ 76			0.694 $\pm$ 0.035				12.4 $\pm$ 1.1
23 <i>Enteromorpha intestinalis</i>			60.6 $\pm$ 5.2		4.62 $\pm$ 0.32	596 $\pm$ 47			0.033 $\pm$ 0.003				9.50 $\pm$ 0.65
24 <i>Enteromorpha intestinalis</i>			37.6 $\pm$ 2.5		2.75 $\pm$ 0.20	67.4 $\pm$ 5.2			0.017 $\pm$ 0.002				3.64 $\pm$ 0.23
25 <i>Enteromorpha prolifera</i>			453	2.69	0.24								33.1
26 <i>Enteromorpha prolifera</i>						4.10 $\pm$ 0.40							4.92 $\pm$ 0.50
27 <i>Enteromorpha prolifera</i>						6.10 $\pm$ 0.90							12.50 $\pm$ 0.50
28 <i>Monostroma fragile</i>	63.6		58.4				12.9	<0.027	<0.15	110	<0.10	7.53	21.7
29 <i>Monostroma hariotti</i>			4.09 $\pm$ 0.20	<0.6	4.68 $\pm$ 0.24	1.57 $\pm$ 0.08			6.54 $\pm$ 0.27	47.4 $\pm$ 2.2		38.4 $\pm$ 0.8	15.0 $\pm$ 0.6
30 <i>Monostroma oxyspermum</i>			10										32
31 <i>Ulva</i> sp.						2 $\pm$ 0.5							24 $\pm$ 8
32 <i>Ulva</i> sp.			17.8		7.2								22.0
33 <i>Ulva fasciata</i>			12										9
34 <i>Ulva fasciata</i>			17										6
35 <i>Ulva fenestrata</i>			280	0.55	0.93							0.54	2.2
36 <i>Ulva lactuca</i>	53.8		37.1				20.1	0.31	<0.15	61.4	<0.10	6.91	16
37 <i>Ulva lactuca</i>						3.68 $\pm$ 0.25							5.20 $\pm$ 0.30
38 <i>Ulva lactuca</i>						1.73 $\pm$ 0.33							1.98 $\pm$ 0.40
39 <i>Ulva lactuca</i>						8.55							19.15
40 <i>Ulva lactuca</i>			17.2 $\pm$ 1.2		2.16 $\pm$ 0.13	1354 $\pm$ 50			0.354 $\pm$ 0.022				15.6 $\pm$ 1.3
41 <i>Ulva lactuca</i>			9.98 $\pm$ 0.67		2.06 $\pm$ 0.11	1.54 $\pm$ 0.10			0.162 $\pm$ 0.013				6.50 $\pm$ 0.32
42 <i>Ulva lactuca</i>			11.7 $\pm$ 1.1		2.72 $\pm$ 0.14	22.2 $\pm$ 2.1			0.029 $\pm$ 0.002				19.1 $\pm$ 1.6

(Continued)

**Table 6.2** (Cont.). Content of trace and ultra trace element in seaweed ( $\mu\text{g/g}$  d.w.; samples from Table 6.1)

[illegible]

**Table 6.2** (Cont.). Content of trace and ultra trace element in seaweed ( $\mu\text{g/g d.w.}$ ; samples from Table 6.1)

Species	Hg	I	Mn	Mo	Ni	Pb	Rb	Sb	Se	Sr	U	V	Zn
131 <i>Laminaria ochroleuca</i> + <i>L. sacharina</i>			5.3 $\pm$ 1.4									<0.18	
132 <i>Laminaria saccharina</i>	<0.05	238	3.04	<0.01	0.57	<0.01			5.7		<0.01	0.66	8.5
133 <i>Laminaria setchellii</i>	<0.05	1070	3.67	<0.01	1.31	<0.01			<0.1		<0.01	0.43	22.3
134 <i>Leathesia difformis</i>						(2.3–2.9)							
135 <i>Lessonia fuscens</i>						(5.5–5.8)							
136 <i>Macrocystis integrifolia</i>	<0.05	240	9.96	0.08	1.48	0.19			6.2		<0.01	3.66	6.9
137 <i>Macrocystis pyrifera</i>						(6.9–7.7)							
138 <i>Mazzaella laminarioides</i>	<0.01				<1–7.60	<0.5–7.40		<0.2–1.02				<1.2–4.43	41.7 $\pm$ 15.70
139 <i>Nereocystis leuckana</i>	<0.05	734	5.55	0.2	2.83	<0.01			6		<0.01	1.05	10
140 <i>Nereocystis leuckana</i>	<0.05	80	3.9	<0.01	0.94	0.08			<0.1		0.26	1.43	8.1
141 <i>Padina australis</i>													13 $\pm$ 3
142 <i>Padina durvillaei</i>			108		10.5	7.2							30
143 <i>Padina durvillaei</i>			427		7.8	9							88
144 <i>Padina durvillaei</i>			485		11.5	7.2							96
145 <i>Padina pavonica</i>						13.2–525							17.1–71.1
146 <i>Padina pavonica</i>			156 $\pm$ 12		5.20 $\pm$ 0.42	2859 $\pm$ 145			0.030 $\pm$ 0.003				15.7 $\pm$ 0.8
147 <i>Padina pavonica</i>						2.94 $\pm$ 1.19							37.3 $\pm$ 12.2
148 <i>Padina tetrastrum</i>						15.25							44.475
149 <i>Pelvetia wrightii</i>			77	1.09	0.21							0.62	1.6
150 <i>Phaeurus antarcticus</i>			17.1 $\pm$ 0.8	<0.6	8.76 $\pm$ 0.42	7.59 $\pm$ 0.42			<0.6	400 $\pm$ 11		15.0 $\pm$ 0.6	5.04 $\pm$ 0.30
151 <i>Puncyaria plantaginea</i>		234.8	179				37.3	0.128	0.375	585	<0.10	<0.54	23.2
152 <i>Sargassum carpophyllum</i>		1985	30.7				34.2	<0.027	<0.15	751	0.162	1.22	11.1
153 <i>Sargassum echinocarpum</i>			6										7
154 <i>Sargassum henslowianum</i>		5197	166				29.7	0.149	<0.15	1497	0.262	1.69	32.4
155 <i>Sargassum kjellmanianum</i>		203.6	15.4				10.4	0.0917	<0.15	998	1.5	<0.54	11.8
156 <i>Sargassum kjellmanianum</i>		273	83.8				25.7	<0.027	<0.15	848	<0.10	5.78	25.4
157 <i>Sargassum obusifolium</i>			15										16
158 <i>Sargassum pallidum</i>			46	1.15								0.59	2.7
159 <i>Sargassum parvifolium</i>		2157	117				32.9	0.192	<0.15	1195	0.411	<0.54	7.68
160 <i>Sargassum polycystum</i>													4 $\pm$ 1
161 <i>Sargassum thunbergii</i>		336.3	92.5				26.7	<0.027	<0.15	566	0.57	<0.54	19.4
162 <i>Sargassum thunbergii</i>		110.6	110				16.6	<0.027	<0.15	<13	<0.10	9.39	38.7
163 <i>Sargassum vachellianum</i>		5939	124				26.7	0.135	<0.15	1474	0.214	1.14	23.8
164 <i>Sargassum vulgare</i>						6.8							274.8
165 <i>Scytosiphon lomentarius</i>		116.9	43.6				31.4	0.0567	<0.15	850	1.34	<0.54	22.5
166 <i>Turbinaria conoides</i>													6 $\pm$ 3
167 <i>Undaria pinnatifida</i>		1571	25.8				28.4	0.0828	0.406	875	<0.10	<0.54	17.6
168 <i>Undaria pinnatifida</i>	0.24	102	6.46	0.25	<0.05	0.14			3		0.03	0.39	13
169 <i>Undaria pinnatifida</i>	<0.05	60	3.3	0.07	<0.05	0.21			3		0.72	0.46	14
170 <i>Undaria pinnatifida</i>			8.7 $\pm$ 20										17.4 $\pm$ 0
171 <i>Undaria pinnatifida</i>	0.0189 $\pm$ 0.0051	163 $\pm$ 75.3	7.46 $\pm$ 28.9			0.86 $\pm$ 0.38			61.3 $\pm$ 17.1				33.1 $\pm$ 9.8
172 <i>Undaria pinnatifida</i>	ND-0.150		0.39–6.7		0.77–5.94	0.14–3.53			0.61–3.5				11.3–86.8
173 <i>Undaria pinnatifida</i>			1.08 $\pm$ 0.1			1.01 $\pm$ 0.1				87.3 $\pm$ 0.6			<4.0

174	<i>Undaria pinnatifida</i>	0.010–0.057	6.8±0.11	<0.008–1.28	<0.18	8.25–26.6
175	<i>Undaria pinnatifida</i>		5.68±0.804			70.6±87.8
176	<i>Undaria pinnatifida</i>	139.1±95.66			8.68±2.12	
Rhodophyta						
177	<i>Ahnfeltiopsis concinna</i>		72			22
178	<i>Ahnfeltiopsis concinna</i>		16			10
179	<i>Antithamion cruciatum</i>		78.1±4.5	146±10	0.030±0.003	11.6±0.8
180	<i>Antithamion cruciatum</i>		43.5±3.3	2768±100	0.042±0.002	16.2±1.4
181	<i>Antithamion cruciatum</i>		285±10	3969±250	0.247±0.015	48.9±2.8
182	<i>Boryocladia leptopoda</i>			7.2		26.375
183	<i>Bryocladia thysigera</i>	NID			2.43±0.40	31.90±2.10
184	<i>Ceramium boydenoo</i>	71.1		14.8	<0.10	12.1
185	<i>Centroceras clavulatum</i>		71.55±3.05		20.45±1.90	28.24±1.70
186	<i>Ceramium kondoi</i>	34.1	29.3	15.4	<0.10	1.66
187	<i>Ceramium rubrum</i>		31.2±2.4	9.28±0.52	<0.15	16.9±1.3
188	<i>Ceramium rubrum</i>		74.3±4.6	534±42	0.012±0.001	12.5±0.9
189	<i>Ceramium rubrum</i>		92.5±6.7	83.8±5.5	0.016±0.002	41.6±2.3
190	<i>Champia compressa</i>			22.55		15.05
191	<i>Chondrus crispus</i>			33.1±0.6		76±10
192	<i>Chondrus crispus</i>			6.1±1.6		3.8±2.8
193	<i>Chondrus crispus</i>			8.3±1.5		53±14
194	<i>Chondrus crispus</i>			3.0±1.3		26±1
195	<i>Chondrus crispus</i>			3.1±0.9		28±15
196	<i>Chondrus crispus</i>			7.6±4.4		35±9
197	<i>Chondrus crispus</i>			4.6±0.9		48±3
198	<i>Chondrus crispus</i>		13.2±0			71.4±1.3
199	<i>Chondrus crispus</i>			0.403–0.726		51.8–53.3
200	<i>Chondrus ocellatus</i>		70			284
201	<i>Chondrus yendoii</i>		167			17.1
202	<i>Corallina elongate</i>		27.7±2.2	1065±68	2.11	26.4±2.4
203	<i>Corallina pilulifera</i>	161.3		14.4	0.139	31.4
204	<i>Dictyopteris divaricate</i>	93.3	84.2	31.5	0.113	20.5
205	<i>Dictyopteris divaricate</i>	28.8	23.4	2.85	<0.027	5.92
206	<i>Eucheuma denticulatum</i>		9		<0.10	36.4
207	<i>Gelidium</i> sp.			0.381–0.861		7
208	<i>Gelidium amansil</i>	203.8	37.7	29.7	0.0891	1.30–6.29
209	<i>Gelidium latifolium</i>		77.6±4.7	1446±90	<0.10	16.7
210	<i>Gelidium sesquipedale</i>		10.0±1.3			64.8±5.2
211	<i>Georgiella confuens</i>		0.33±0.02		<0.18	
212	<i>Gigartina acicularis</i>		39.90±2.05	1.23±0.05	4.84±0.24	<0.1
213	<i>Gigartina skottsbergii</i>			(4.9–5–2)	4.90±0.80	21.24±0.66
214	<i>Gloeosiphonia capillaris</i>	81.1	102	23.1	0.143	<0.10
215	<i>Gracilaria confervoides</i>	353	94.4	13.3	<0.027	<0.10
216	<i>Gracilaria coronopifolia</i>		57		4.98	35.2
						48.7
						42

**Table 6.2** (Cont.). Content of trace and ultra trace element in seaweed ( $\mu\text{g/g d.w.}$ ; samples from Table 6.1)

Species	Hg	I	Mn	Mo	Ni	Pb	Rb	Sb	Se	Sr	U	V	Zn
217 <i>Gracilaria corticata</i>						13.325							35.3
218 <i>Gracilaria parvispora</i>			48										8
219 <i>Gracilaria salicornia</i>			10										16
220 <i>Halymenia formosa</i>			11										22
221 <i>Hyalosiphonia caespitosa</i>		54.7	58.9				26.5	0.0997	<0.15	164	<0.10	<0.54	55.4
222 <i>Hypnea musciformis</i>	0.11 $\pm$ 0.007				15.33 $\pm$ 0.98	3.85						ND	15.675
223 <i>Hypnea musciformis</i>			54.50 $\pm$ 12.0										13.60 $\pm$ 1.00
224 <i>Hypnea valentiae</i>			7.53 $\pm$ 0.23		2.83 $\pm$ 0.34	0.33 $\pm$ 0.12							29.925
225 <i>Iridaea cordata</i>			1.51 $\pm$ 0.10	<0.6	3.36 $\pm$ 0.14	1.60 $\pm$ 0.08			<0.6	38.6 $\pm$ 1.6		1.59 $\pm$ 0.11	80 $\pm$ 2
226 <i>Iridaea cordata</i>			24.20 $\pm$ 4.60		19.72 $\pm$ 1.25							2.10 $\pm$ 0.14	<0.1
227 <i>Iania rubens</i>	ND											9.20 $\pm$ 1.00	11.83 $\pm$ 0.77
228 <i>Kappaphycus alvarezii</i>		224.9	26.6				27.8	<0.027	0.865	197	<0.10	<0.54	18 $\pm$ 4
229 <i>Laurencia okamurai</i>		130	45				55.8	0.102	<0.15	951	<0.10	4.95	15.9
230 <i>Leathesia difformis</i>		95.5	133				41.9	0.307	0.582	564	0.876	14.3	30.7
231 <i>Myelophycus siruplex</i>									<0.6	6.05 $\pm$ 0.25		0.50 $\pm$ 0.03	<0.1
232 <i>Myriogramme mangini</i>			6.54 $\pm$ 0.40	<0.6	2.40 $\pm$ 0.13	4.90 $\pm$ 0.23				30.7 $\pm$ 0.6			46.2 $\pm$ 1.3
233 <i>Palmaria</i> sp.			34.8 $\pm$ 0.8			0.52 $\pm$ 0.02			1.54 $\pm$ 0.08	12.5 $\pm$ 0.6		6.29 $\pm$ 0.28	8.02 $\pm$ 0.30
234 <i>Palmaria decipiens</i>			4.01 $\pm$ 0.20	0.80 $\pm$ 0.05	8.16 $\pm$ 0.42	<0.6						130.1 $\pm$ 11.51	
235 <i>Palmaria palmata</i>			233.1 $\pm$ 6.60							4.86 $\pm$ 0.585			64.0 $\pm$ 42.3
236 <i>Palmaria palmata</i>		110.4 $\pm$ 24.04	67.7 $\pm$ 76.2					<0.2-0.80				<1.2-3.08	21.90 $\pm$ 2.12
237 <i>Phorphyra columbina</i>	<0.01				<1-2.60	<0.5-8.20						5.44 $\pm$ 0.11	102 $\pm$ 2
238 <i>Phyllophora antarctica</i>			26.8 $\pm$ 0.2		32.6 $\pm$ 0.3	0.79 $\pm$ 0.12						10.0 $\pm$ 0.1	67 $\pm$ 2
239 <i>Phyllophora antarctica</i>			12.6 $\pm$ 0.2		20.0 $\pm$ 0.3	0.50 $\pm$ 0.12			0.026 $\pm$ 0.002				48.6 $\pm$ 1.8
240 <i>Phyllophora antarctica</i>			261 $\pm$ 17		36.2 $\pm$ 2.2	2219 $\pm$ 78						39.60 $\pm$ 5.60	32.00 $\pm$ 0.21
241 <i>Polysiphonia dentata</i>	0.10 $\pm$ 0.008		126.96 $\pm$ 11.03	2.95	14.00 $\pm$ 0.28							7.41	21
242 <i>Polysiphonia japonica</i>			149				29.8	0.197	<0.15	193	<0.10	7.21	45.4
243 <i>Polysiphonia urceolata</i>		292.8	103				5.73	<0.027	<0.15	94.7	0.26	<0.54	27.1
244 <i>Porphyra</i> sp.		35.8	50.3						39.9 $\pm$ 20.1				37.9 $\pm$ 17.6
245 <i>Porphyra</i> sp.	0.00536 $\pm$ 0.00191	26.7 $\pm$ 12.4	31.2 $\pm$ 19.7			0.24 $\pm$ 0.29			55.8 $\pm$ 6.3				35.6 $\pm$ 8.7
246 <i>Porphyra</i> sp.	0.00695 $\pm$ 0.00332	33.2 $\pm$ 21.4	54.6 $\pm$ 4.9			2.23 $\pm$ 2.56				37.7 $\pm$ 0.5			51.2 $\pm$ 1.5
247 <i>Porphyra</i> sp.			14.7 $\pm$ 0.3			<0.3							
248 <i>Porphyra</i> sp.						0.312 $\pm$ 0.022			0.0230 $\pm$ 0.0057			5.06 $\pm$ 0.13	
249 <i>Porphyra</i> sp.						0.408 $\pm$ 0.013			0.0170 $\pm$ 0.0054			3.05 $\pm$ 0.16	
250 <i>Porphyra</i> sp.						0.174 $\pm$ 0.048			0.0110 $\pm$ 0.0044			0.66 $\pm$ 0.11	
251 <i>Porphyra</i> sp.						0.140 $\pm$ 0.017			0.07100 $\pm$ 0.00652			0.70 $\pm$ 0.08	
252 <i>Porphyra</i> sp.						0.438 $\pm$ 0.368			0.0305 $\pm$ 0.0249			2.4 $\pm$ 1.9	
253 <i>Porphyra columbina</i>						<0.2			0.463 $\pm$ 0.067				36.08 $\pm$ 0.70
254 <i>Porphyra columbina</i>						<0.2							4.11 $\pm$ 0.70
255 <i>Porphyra tenera</i>	0.44	17	32.3	0.59	10.17	0.28			2		0.27	3.96	31
256 <i>Porphyra tenera</i>	0.24	185	48.9	0.61	<0.05	0.14			2		<0.01	1.53	37
257 <i>Porphyra tenera</i>			27.2 $\pm$ 0										22.1 $\pm$ 1.7
258 <i>Porphyra umbilicales</i>	0.008-0.032					<0.008-0.270							39.5-73.8
259 <i>Porphyra umbilicales</i>		58.75 $\pm$ 29.39	32.0 $\pm$ 9.40							4.36 $\pm$ 1.36			68.4 $\pm$ 9.36

260	<i>Porphyra umbilicalis</i>		19.1±1.2	4.04±0.15	648±30	0.134±0.010		22.4±1.1
261	<i>Porphyra umbilicalis</i>		22.3±2.1	0.27±0.01	6.13±0.25	0.011±0.001		22.8±1.2
262	<i>Porphyra umbilicalis</i>		13.3±0.8	2.24±0.20	282±15	0.020±0.002		19.4±1.5
263	<i>Porphyra umbilicalis</i> +		27.0±2.8				1.4±0.20	
264	<i>P. linearis</i> <i>Porphyra</i>	41						11
265	<i>Porphyra vietnamensis</i>	0.2±0	70.9±0.3	1.3±0.1	0.7±0.1			1.95±0.01
266	<i>Porphyra vietnamensis</i>		1138	0.87			5.73	32.3
267	<i>Ptilota filicina</i>		619	2.65			1.27	15.3
268	<i>Rhodomela larix</i>		431.2				<0.10	19.9
269	<i>confervoides</i>						488	
270	<i>Sarconema</i>				16.05			20.775
271	<i>furcellatum</i>							
272	<i>Scinaia saifullahii</i>				13.45			47.875
273	<i>Solieria robusta</i>				18.1			23.6
274	<b>Commercial foods</b>							
275	Cooked	36.07±1.193	29.0±0.395				18.0±0.688	49.0±2.78
276	<i>H. elongata+</i>							
277	<i>Sacchariza polyschides</i>							
278	Cooked	34.7±0.910						7.9±0.70
279	<i>H. elongata+</i>							
280	<i>Sacchariza polyschides</i>							
281	Kelp tablets (Species not listed)	0.24	815	34.9	1.13	9.82	0.57	1.01
282	Laver, for rice roll				0.0629			2.1
283	Laver, seasoned				0.0289			
284	Me-hijiki, leaves of hijiki		29.7±2.4	0.22±0.02	0.90–2.24	1.29±0.12	18.5±10	0.62–3.51
285	Sea lettuce				<0.00002			27.8±1.9
286	Sea mustard, dried				0.0036			
287	Sea mustard, stem, salted				<0.00002			
288	Sea tangle, dried				0.0161			
289	Sea tangle, raw				<0.00002			
290	Seaweed		7.97±0.42					
291	Seaweed, dry (cochayuyo)							
292	Wakame, nori and sea lettuce (Salad)	0.006–0.017						14.6±0.12
293	Wak., ogonori, kombu, agar and akamodoki (Salad)	0.024–0.035						5.85±1.32
294								26.2–27.00
295								42.8–43.4

ND: not detected.

consumption of seaweed in Western Europe in recent years. That is one of the reasons why the European Commission has recently legislated about the production rules for seaweed (collection of wild seaweeds or farming in coastal areas) (Council Regulation (EC) 834/2007, 2007; Commission Regulation (EC) 889/2008, 2008; Commission Regulation (EC) 710/2009, 2009).

France was the first country in Europe that established a regulation concerning seaweed for human consumption (Burtin, 2003). The consumption of the following algae was authorized: six Phaeophyta (*Ascophyllum nodosum*, *Fucus vesiculosus*, *Fucus serratus*, *Himanthalia elongata*, *Undaria pinnatifida*), four Rhodophyta (*Porphyra umbilicalis*, *Palmaria palmate*, *Gracilaria verrucosa*, *Chondrus crispus*), two Chlorophyta (*Ulva* spp., *Enteromorpha* spp.) and two microalgae (*Spirulina* sp., *Odontella aurita*). The concentration limits for toxic minerals in seaweed were: 3.0 mg/kg d.w. (inorganic arsenic), 5.0 mg/kg d.w. (lead), 0.5 mg/kg d.w. (cadmium), 5.0 mg/kg d.w. (tin), 0.1 mg/kg d.w. (mercury) and 5 mg/kg d.w. (iodine).

The most specific laws from the European Commission concerning seaweed regard food additives extracted from seaweed, such as “E400 alginic acid”, “E401 sodium alginate”, “E402 potassium alginate”, “E403 ammonium alginate”, “E404 calcium alginate”, “E405 propane-1,2-diol alginate”, “E406 agar”, “E407 carrageenan” and “E407a processed *Euchema* seaweed”. The concentration limits are 3 mg/kg for arsenic, 5 mg/kg for lead, 1 mg/kg for mercury and 1 mg/kg for cadmium (Commission Directive 2008/84/CE, 2008; Commission Directive 2009/10/EC, 2009). For the additive “E406 agar”, the amount of heavy metals (expressed as lead) should not exceed 20 mg/Kg (Commission Directive 2008/84/CE, 2008). When setting the maximum levels for certain contaminants in foodstuff, the European Commission regulates the maximum level of cadmium in food supplements consisting mainly of dried seaweed as 3 mg/kg w.w., lead in food supplements as 3 mg/Kg w.w., and mercury in food supplements as 0.1 mg/kg w.w. Maximum levels of lead permitted in vegetables are 0.10–0.30 mg/kg w.w. and of cadmium 0.05–0.2 mg/kg w.w. (Commission Regulation (EC) 1181/2006, 2006; Commission Regulation 629/2008, 2008).

Concerning animal feed, the EU includes seaweed meal, in particular brown seaweed that may have been washed to reduce the iodine content (Commission Regulation (EU) 242/2010, 2010). Calcareous marine algae are not permitted to have a concentration bigger than 10 mg/kg of arsenic (Commission Directive 2009/141/EC, 2009); 15 mg/kg of lead and 1000 mg/kg of fluorine (Commission Directive 2005/87/EC, 2005); seaweed meals and feed materials derived from seaweed are not permitted to have a concentration of arsenic higher than 40 mg/kg. Levels of inorganic

arsenic should be lower than 2 mg/kg; this analysis is vital if the food is composed of *Hizikia fusiforme* (Commission Directive 2009/141/EC, 2009). The amount of lead permitted in feed materials is 10 mg/kg, the amount of mercury is 0.1 mg/kg, and the amount of cadmium in feed materials of vegetable origin is 1 mg/kg (Directive 2002/32/EU, 2002). All those concentrations are relative to a feeding stuff with a moisture content of 12%.

### US Legislation

Several seaweed or seaweed-derived products are on the list of specific substances affirmed as GRAS (Generally Recognized As Safe) by FDA, and that can be added directly to human food: alginic acid, agar-agar, brown algae, red algae, ammonium alginate, calcium alginate, potassium alginate and sodium alginate (Code of Federal Regulations, 2009).

Alginic acid can be used as an emulsifier, stabilizer or thickener in soup and soup mixes. Alginic acid must meet the following specifications: <3 mg/kg of arsenic (as As), <0.004% of heavy metals (as Pb), <10 mg/kg of Pb, <4% of ash d.w. Agar-agar can be used in baked goods and baking mixes (maximum percentage: 0.8%), in confections and frostings (maximum 2%), soft candies (maximum 1.25%) and in other food categories (maximum 0.25%). Agar-agar must meet the following specifications: <3 mg/kg of arsenic (as As), <10 mg/kg of heavy metals (as Pb), <10 mg/kg of Pb, <6.5% of ash (total) d.w., <0.5% of ash (acid-insoluble) d.w. Ammonium alginate extracted from brown algae can be used as a stabilizer, thickener or humectant in food with maximum % ranging from 0.1–0.5%. Ammonium alginate must meet the following specifications: <3 mg/kg of arsenic (as As), <0.004% of heavy metals (as Pb), <10 mg/kg of Pb, <4% of ash d.w. Calcium alginate from brown algae can be used as stabilizer or thickener with maximum percentages ranging from 0.002 to 0.5%. It must meet the following specifications: <3 mg/kg of arsenic (as As), <0.004% of heavy metals (as Pb), <10 mg/kg of Pb, 12–18% of ash d.w. Sodium alginate from brown algae can be used with a maximum percentage ranging from 0.3–10% in foods. Sodium alginate must meet the following specifications: <3 mg/kg of arsenic (as As), <0.004% of heavy metals (as Pb), <10 mg/kg of Pb, ash between 18% and 27% after drying. Potassium alginate from brown algae can be used with a maximum percentage ranging from 0.01 to 0.7%. Potassium alginate must meet the following specifications: <3 mg/kg of arsenic (as As), <0.004% of heavy metals (as Pb), <10 mg/kg of Pb, ash between 22% and 33% after drying (Code of Federal Regulations, 2009; Committee on Codex Specifications, 1981).

Edible brown algae include *Analipus japonicus*, *Eisenia bicyclis*, *Hizikia fusiforme*, *Kjellmaniella gyrate*, *Laminaria*

*angustata*, *Laminaria claustronia*, *Laminaria digitata*, *Laminaria japonica*, *Laminaria longicuris*, *Laminaria longissima*, *Laminaria ochotensis*, *Laminaria saccharina*, *Macrocystis pyrifera*, *Petalonia fascia*, *Scytosiphon lomentaria* and *Undaria pinnatifida*. The ingredients have to meet the specifications for kelp in the Food Chemicals Codex, 3d ed. (1981), p. 157. The ingredients can be used as spices, seasoning or flavoring. Red algae are seaweeds of the species *Gloiopeltis furcata*, *Porphyra crispata*, *Porphyra deutata*, *Porphyra perforata*, *Porphyra suborbiculata*, *Porphyra tenera* and *Rhodomenia palmata*. The ingredients have to meet the specifications for kelp in the Food Chemicals Codex, 3rd ed. (1981), p. 157, except that the loss on drying should be not more than 20% and the maximum allowable level for iodine is 0.05%. They can be used also as spices, seasoning or flavoring. The specifications for kelp (dehydrated *Macrocystis* and *Laminaria*) are the following: <3 mg/kg of arsenic (as As, inorganic), <0.004% of heavy metals (as Pb), <10 mg/kg of Pb, iodine content between 0.1 and 0.5%, <45% of ash d.w. (Code of Federal Regulations, 2009; Committee on Codex Specifications, 1981).

### 6.3.2 Trace and ultratrace elements in seaweed: studies concerning seaweed edibility

A large number of studies have determined macrominerals and trace and ultratrace elements in seaweed in order to characterize the different meals or to establish the edibility of a macroalgae. Some of these macroalgae can only be used as spices or food supplements due to their high content of certain elements. For example, using more than 1.3 g d.w. of *Porphyra vietnamensis* a day is not recommended due to the high arsenic content (Subba-Rao *et al.*, 2007). Many of these publications are written in Japanese and Chinese, but the literature in English has increased in recent years all round the world and include works from Hawaii (McDermid and Stuercke, 2003), Chile (dry cochayuyo) (Olivares *et al.*, 2004), Japan (Shiraishi, 2005, 2006), Indonesia (Santoso *et al.*, 2006), India (Subba Rao *et al.*, 2007) and the Black Sea (Turkey) (Tuzen *et al.*, 2009). Mineral content in various edible seaweeds and foodstuffs are shown in Tables 6.1 and 6.2.

Several authors have performed studies about edible seaweed collected in Galicia (northwest Spain). Rupérez (2002) determined macrominerals (Na, K, Ca, Mg) and trace elements (Fe, Zn, Mn, Cu) in several brown (*Fucus vesiculosus*, *Laminaria digitata*, *Undaria pinnatifida*) and red (*Chondrus crispus*, *Porphyra tenera*) seaweed. As these macroalgae showed a mineral content much bigger than those reported for edible land plants, the author concluded that they could

be used as food supplements. Taboada *et al.* (2010) studied the composition of *Ulva rigida*, one of the most common edible green seaweed. Romarís-Hortas *et al.* (2010) characterized edible seaweed harvested on the Galician coast using pattern recognition techniques and major and trace element data. High differences could be observed in elemental composition among the samples, especially for some elements such as arsenic and iodine, which were present in high concentrations in brown seaweed, mainly in Kombu samples. Thus, arsenic concentration varied from 5.8 µg/g in red seaweed Dulse (*Palmaria palmata*) to 245 µg/g in Kombu (*Laminaria* sp.), and iodine varied from 59 µg/g in Nori to 4870 µg/g in Kombu. Chemometrics allowed classifying the edible seaweed according to their type (Rhodophyta, Chlorophyta, or Phaeophyta).

Part of the bibliography is devoted to the comparison of commercially available seaweed from various origins. Van Netten *et al.* (2000) analyzed trace elements and radionuclides in 15 seaweed samples (six local samples from British Columbia, seven from Japan, one from Norway and one undisclosed). The authors found that six of eight imported seaweeds had levels of mercury much higher than the local products. *Laminaria japonica* and *Laminaria setchellii* had the highest concentrations of iodine, and arsenic levels were also elevated. Dawczynski *et al.* (2007) studied the mineral content in 34 edible dried seaweed products marketed in Germany and imported from Korea, China, and Japan. Seaweed products included Phaeophyta (*Laminaria* sp., *Undaria pinnatifida* and *Hizikia fusiforme*) and Rhodophyta (*Porphyra* sp.). Assuming a daily intake of 5 g of fresh algae, the content of Pb, Cd and Hg was assessed as harmless, and the contribution of the essential elements to the diet was considered low with the exception of iodine. More than 4000 mg iodine/kg fresh matter were found in several *Laminaria* sp. (Kombu). Moreover, some brown algae, such as *Hizikia fusiforme* (Hijiki) had high contents of total arsenic, which could pose a health risk. Ródenas de la Rocha *et al.* (2009) determined As, Cd, Co, Cr, Mo, Ni, Pb, Sb, Se and V in samples of *Porphyra* and *Laminaria* from Europe and Asia. *Porphyra* contained high concentrations of all the studied elements, except for arsenic. In general, seaweed from Korea and Japan had the highest concentrations of Pb and Cd, possibly due to differences in environmental pollution between sampling stations, whereas Spanish and French samples showed the highest levels of some essential trace elements. Besada *et al.* (2009) examined the quantities of heavy metals (Cd, Pb, Hg, Cu, Zn, As and inorganic As) present in 52 samples from 11 algae-based products from the Atlantic and the Pacific Oceans that were commercialized in Spain. The authors performed univariate and multivariate studies (principal component analysis (PCA), cluster analysis and linear discriminant analysis (LDA)) for

their characterization. PCA yielded three principal components (PCs) that explained 73.3% of the variance. PC1 was associated to total As and inorganic As, which characterized *Hizikia* samples; PC2 was associated with Cu and Zn (*Porphyra umbilicales*), and PC3 was associated with Pb and Cu. Two-way ANOVA (analysis of variance) showed statistical differences for Cu, Hg, Zn and total As in samples from the two production areas, at 95% confidence level. LDA also had an 80% of success when classifying the products as a function of their geographical origin. The authors observed that most of the samples exceeded the limits set for cadmium by the French legislation, and *Hizikia fusiforme* exceeded the limits for total and inorganic arsenic. However, the concentrations for Hg and Pb were below the limits. Four *Porphyra* samples and the salads had high concentrations of Zn and Cu, but there is not yet any legislation concerning the maximum levels for those elements.

### 6.3.3 Radionuclides in edible seaweed

Radionuclides are one of the possible food contaminants in seaweeds. Van Netten *et al.* (2000) analyzed  $^{131}\text{I}$ ,  $^{137}\text{Cs}$ ,  $^{125}\text{Sn}$ ,  $^{226}\text{Ra}$  and  $^{40}\text{K}$  in 15 edible seaweed by ICP-MS. Traces of  $^{137}\text{Cs}$  ( $6.1 \pm 3.2$  Bq/kg in *Porphyra tenera*) were found in a product from Norway, and of  $^{226}\text{Ra}$  in products from Japan ( $58 \pm 37$  Bq/kg in *Eisenia bicyclis*,  $206 \pm 203$  Bq/kg in *Undaria pinnatifida*) and from Norway ( $5 \pm 4$  Bq/kg in *Fucus vesiculosus*).  $^{137}\text{Cs}$  is probably related to the Chernobyl accident, whereas  $^{225}\text{Ra}$  could be a consequence of naturally occurring uranium decay. Levels of  $^{40}\text{K}$  varied from “not detected” to  $4370 \pm 189$  Bq/kg. These radionuclide levels were not of health significance due to the low consumption of the products. Intake of  $^{40}\text{K}$  by Japanese subjects was also studied by Shiraishi (2005, 2006), because this radionuclide provides the highest percentage of internal doses to the Japanese.  $^{40}\text{K}$  and stable potassium were observed in all the foods in their natural ratio (K:  $10.5 \pm 0.3$  mg/g;  $^{40}\text{K}$ :  $317 \pm 2$  mBq/g). The anthropogenic radionuclides,  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$ , were measured in Wakame (*Undaria pinnatifida*) and edible kelp (*Laminaria longissima*), collected in four coastal areas of Japan during 1998–2008 (Morita *et al.*, 2010a). These isotopes were at low levels ( $^{90}\text{Sr} \leq 0.0031 \pm 0.0027$  Bq/kg w.w.;  $^{137}\text{Cs} \leq 0.062 \pm 0.0050$  Bq/kg w.w.) or below detection limits, and showed no much variation among different sampling sites. As a conclusion, it seems that the radiation levels found in the typical Japanese (Sugiyama *et al.*, 2007) or Korean (Choi *et al.*, 2008) foodstuffs which include seaweed, are safe and in the same level as that in other countries.

## 6.4 Trace and ultratrace elements in seaweed: pollution biomonitoring

### 6.4.1 Seaweeds as bioindicators

Seaweeds (or marine macroalgae) have the ability of concentrating trace and ultratrace elements dissolved or bound to particulate matter; thus, the levels in seaweed can be up to thousands of times higher than the levels in seawater. Moreover, macroalgae have several of the characteristics needed to be good bioindicators: they are easy to collect due to their benthic nature, are available during all year round, have an adequate longevity, species can be identified easily, and are present at sites prone to pollution (e.g., estuaries) (Astorga-España *et al.*, 2008; Conti *et al.*, 2010).

Several studies in the bibliography have compared the characteristics of seaweeds with other well established bioindicators such as mussels (*Mytilus edulis*). Thus, Ostapezuk *et al.* (1997) collected results during more than ten years of levels of Hg, As, Se, Cd, Pb, Cu, Na, K, S, P, Zn, Mn, Fe, Sr, Ca, Mg, Tl, Ni, and Co in *Fucus vesiculosus* and *Mytilus edulis* from the North Sea and the Baltic Sea. Macroalgae showed the greatest variation of element concentration with sampling time; for example, differences between maximum and minimum concentrations of nickel were more than 300%. The authors observed several groups of elements with different long-term tendencies of their concentrations. For As, Mn, Co, Ni and Ba the biomagnifications in *Fucus* were significantly higher than in mussels, whereas Hg and Se were more accumulated in mussels than in algae. No significant concentration differences were observed for P, S, Na, K, Mg, Cu, Cd, Pb, and Zn. Both bioindicators represent different pathways of element uptake (Ostapezuk *et al.*, 1997). Algae are especially responsive to the soluble trace element content, whereas bivalves are filter-feeders that also ingest inorganic particulate material. In a later study, Struck *et al.* (1997) performed factor analysis to evaluate the concentration patterns in the bioindicators and salinity as a function of location. Factors describing most of the variance were strongly correlated to salinity for seaweed and less for mussels. The salinity correlated factors controlled the uptake by seaweed of As, Co, Cu, Hg, Mn, Pb, and Zn, and of the macroelements Ca, K, Mg and Na. Results of the cluster analysis on the geographical element concentration showed that arsenic and mercury concentrations varied in parallel within seaweed and mussel. All other elements showed different patterns in both bioindicators. Cluster analysis and LDA indicated a clear separation between North and Baltic Sea locations and the different salinities when taking into account macroelement concentration patterns, for both seaweed and mussels. However,

location groups based on the trace-element concentration patterns were less and not similarly arranged to the ones based on macroelement concentration patterns.

Even when some authors have been able to distinguish among trace metal concentrations in seaweed from different locations, other parameters seem to be more influential than the geographical origin. Some of these influential parameters are the sampling time, the part of the specimen that is collected, and the type and species of seaweed.

#### ***Influence of sampling time and part of the specimen collected***

When performing seaweed sampling, it is important to decide when the seaweed is going to be collected (sampling season, age or size of the specimen). Malea *et al.* (1995) used *Dasycladus vermicularis* from the Greek coast to evaluate seasonal variation of metal concentration. Fe, Zn, Cd and Ca in this Chlorophyta increased from March to July, with a maximum concentration in summer or autumn (for cadmium). It seems that concentrations of Ca, Fe and Zn increase with tissue age, because metals are irreversibly absorbed and more binding sites are formed; however, contents of other elements such as copper seems to decrease with age (Malea, 1994). Copper, lead and magnesium showed the opposite seasonal variation with maximum concentration in winter when the seaweed biomass was minimum (Malea *et al.*, 1995). Hou and Yan (1998) studied the seasonal variation of several inorganic elements in *Sargassum kjellmanianum*. The authors did not observe significant variations for the levels of major elements (Na, Ca, and Mg) during a full growth period of algae. However, concentrations of Cl and K were slightly higher in the later growth stage. Trace elements (Sc, Cs, V, Cr, Th, Al, and Fe) and rare earth elements had a similar seasonal variation, with the lowest concentrations in the initial and later growth stages, while the highest concentrations in February, which is the middle growth stage. Iodine showed a similar behavior, with the highest concentration in March. Manganese concentration was lowest in March and highest in the later growth stage. Other elements, such as Br, Rb, and Zn, did not show significant seasonal variations (Hou and Yan, 1998).

The longevity of the species sampled could also be linked to different accumulation patterns for the various elements. *Ascophyllum nodosum* can live for more than 12 years, *Chondrus crispus* fronds for 2 or 3 years, and *Laminaria* sp. for 1.5–2 years. In areas where there are not chronic problems, it is recommended that a large number of samples be collected to register the differences between element concentrations in tissues of various ages (Sharp *et al.*, 1988).

Different results can also be obtained if the whole specimen is homogenized and afterwards analyzed, or if only a part of it is going to be selected to undergo the treatment. It is essential to decide which parts of the seaweed are going to be used for monitoring. Carvalho *et al.* (1997) used X-ray fluorescence to analyze the concentration of K, Ca, Ti, Mn, Fe, Co, Ni, Cu, Zn, As, Br, Sr, and Pb in the different parts of *Fucus vesiculosus* from the Tagus estuary (Lisbon). The pattern of accumulation in the various parts (base, stipe, reproductive organs, and growing tips) was similar for Cu, Pb, and Zn, whereas iron accumulated mainly in the base of the algae, and arsenic accumulated in the reproductive organs and the growing tips. Yamada *et al.* (2007) determined metal concentrations in the sporophytes of samples of *Undaria pinnatifida* collected at 15 localities in Osaka Bay (Japan). Most metals, except for Mg, Cd, and Hg, showed higher concentrations in the blade than in the sporophyll. The concentrations of most of the elements examined were in the mg/kg or µg/kg range (including Sn: 100–1200 µg/kg).

#### ***Influence of type of algae (algal division)***

The cell walls of the three marine macroalgae divisions (Chlorophyta, Phaeophyta, and Rhodophyta) possess electrostatic and chelating properties which are responsible for biosorption. Typical algal cell walls include a fibrillar skeleton and an amorphous embedding matrix. The most common fibrillar skeleton material is cellulose, but it can be constituted by xylan as in the case of Rhodophyta, and xylan and mannan as in the case of Chlorophyta. The Phaeophyta algal embedding matrix is predominantly alginic acid or alginate with a smaller amount of fucoidan. The Rhodophyta matrix contains galactans (agar, carrageenan, porphyran, etc.). Thus, these algae are effective biosorbents due to the presence of these large amounts of anionic polysaccharides that possess a strong ion exchange capacity. In fact, brown algae such as *Laminaria* sp. and *Fucus* sp. are considered as one of the most promising substrates to act as a low-cost sorbent for heavy metals (Davis *et al.*, 2003).

Several studies in the literature report that the three divisions of seaweed differ in their elemental composition. In an early study, Saenko *et al.* (1976) determined Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Mo, and Zn in 16 species of Rhodophyta, Chlorophyta and Phaeophyta, and two species of higher water plants from Voskok Bay, Sea of Japan. The authors observed that Rhodophyta possessed the highest ability to concentrate elements in general, while Chlorophyta and higher water plants also had this ability with respect to Ti, Fe, Mn, and Cu. Dawczynski *et al.* (2007) observed that the mean contents of Na, K, Mg and Ca were higher in

Phaeophyta than in Rhodophyta, whereas P content was higher in Rhodophyta. Hou and Yan (1998) observed that the concentrations of K and Ca in Chlorophyta were lower than Phaeophyta or Rhodophyta, whereas concentrations of Na and Mg were slightly higher in Chlorophyta than in Rhodophyta and Phaeophyta. Regarding trace elements, values were significantly different among the seaweed studied, and vary with sampling site and collecting season. The concentrations of rare earth elements in Chlorophyta and Rhodophyta were higher, and strontium and iodine were lower than those in Phaeophyta. The average concentration of bromine in Rhodophyta was double that in Phaeophyta, and 12 times higher than in Chlorophyta. The concentration factors were higher for tri- and tetravalent elements than those of mono- and divalent elements. Thus, concentration factors for Al, Th, Fe, Sc, and rare earth elements were usually higher than  $10^5$ , and higher for light rare earth elements than for heavy rare elements. For the same family of elements, the concentration factors increased gradually with the atomic radius of the element – from Mg to Ba, Na to Rb, and Cl to I. Fluorine was an exception; its preconcentration factor was bigger than for chlorine and lower than for iodine. The elements with large atomic radii could be combined with molecules of the algae cell and be absorbed afterwards. However, the elements with lower atomic radii easily exist as ions, and their concentration in algae could only be affected by osmotic pressure (Hou and Yan, 1998).

Romarís-Hortas *et al.* (2010) used chemometrics to classify edible seaweed from the Galician coast according to their type and variety. Three groups of samples (Phaeophyta, Rhodophyta, and Chlorophyta) were observed using PCA and cluster analysis. Arsenic, bromine and iodine concentrations had the highest loadings in PC1 (38% total variance), sodium and calcium contents in PC2 (19% total variance), and potassium and copper contents in PC3 (14%). One hundred percent of correct assignments in the groups were achieved when using LDA. However, a satisfactory classification when using SIMCA (soft independent modelling of class analogy) was obtained only for Rhodophyta (100% of the cases correctly classified), whereas percentages of 89 and 90% were obtained for brown seaweed for recognition and prediction, respectively.

### *Influence of the species*

As it can be observed, different species accumulate metals in different ways. *Fucus vesiculosus* (Phaeophyta) is one of the most established seaweed used as a bioindicator (see Tables 6.1 and 6.2, and the section entitled “Seaweeds as bioindicators of radioactive pollution”). Moreover, the character-

istics of many other species have been studied. For example, among the 16 species examined by Saenko *et al.* (1976) in Japan, *Ptilota filicina* concentrated Ti, V, Mn, Fe, Ni, Cu, Zn, and Mo; *Polysiphonia japonica* Ti, V, Mn, Fe, Zn, Mo and Cr; *Rhodomela larix* Ti, V, Mn, Fe, Ni, Zn and Cr; *Agarum cribrosum* Ti, V, Mn, Fe, Ni, Zn, and Cr; *Ulvaria splendens* Ti, V, Mn, Fe, Ni, and Cu; *Ulva fenestrata* Mn, Fe, Ni, and Cu; *Enteromorpha prolifera* Mn, Co, Zn, Mo, and Cr; *Codium yessoensis* V, Mn, Fe, Zn, and Cr. The accumulation coefficients values were also variable. Maximum coefficients were found for Mn (*Ptilota filicina*:  $1.8 \times 10^5$ ), Ti (*Ptilota filicina*:  $4.4 \times 10^4$ ), Fe (*Polysiphonia japonica*:  $2.4 \times 10^4$ ) and Cr (*Polysiphonia japonica*:  $1.2 \times 10^6$ ). Serfor-Armah *et al.* (2001) evaluated six *Rhodophyta* seaweed species as bioindicators for monitoring toxic element pollutants in the coast of Ghana. Data showed high variability in and between specie and among sites of collection. Al and Br showed the highest concentrations, and Al, Br, Ni and Zn were found in all the seaweed species analyzed. Cd was only determined in *Centroceras clavulatum*, and high levels of Fe were found in *Polycavernosa dentata* and *Hypnea musciformis*. Recently, Conti *et al.* (2010) determined Cd, Cr, Cu, Pb, and Zn in two brown algae *Padina pavonica* (L.) Thivy and *Cystoseira* sp. They concluded that the pollutant distribution in seaweed correlated better with species than with sites. Thus, *Padina* species had higher Cr concentrations whereas *Cystoseira* had higher Pb levels. *Padina* had potential as a bioindicator of trace metals in marine ecosystems but more studies are needed to confirm *Cystoseira* as a useful bioindicator. Other authors such as Fariás *et al.* (2002) and Rodenas de la Rocha *et al.* (2009), have also reported a high variation in mineral content among seaweed species even when the difference in the environmental factors was minimum.

More data about mineral concentration in the various seaweed species is included in Tables 6.1 and 6.2, and in the following two sections.

### **6.4.2 Trace and ultratrace elements in seaweed: studies concerning environmental monitoring**

A large number of studies have been performed in the recent decades to establish baseline levels of trace elements in seaweeds from a region or to study the influence of anthropogenic sources of pollution.

*Fucus vesiculosus* (Phaeophyta) is one of the species most often used to perform studies about trace element pollution, specifically to monitor heavy metal pollution (Truus *et al.*, 2004) and radionuclides (see Section 6.4.3). However, when this species is not available, other seaweed species have

been used. Therefore, one of the objectives of these environmental studies is to identify natural differences in element concentration between seaweed species and establish baseline concentration levels (Riget *et al.*, 1997). Jayasekera and Rossbach (1996) analyzed the content of As, Cd, Co, Cr, Hf, Ni, Th, U, Zn, and the rare earth elements Ce, Eu, Sm, Tb, and Yb in the brown algae *Fucus vesiculosus* from the North Sea and the Baltic Sea, using as a reference an unpolluted control station in Sri Lanka. As *Fucus vesiculosus* was not available in this tropical environment, the authors compared concentrations with *Sargassum filipendula* (Phaeophyta) and with *Ulva* sp. (Chlorophyta). Cd, Co, Ni, and Zn concentrations were lowest in *Sargassum filipendula* from Sri Lanka, whereas levels of Hf, Th, U, and rare earth elements were highest in this seaweed. Elevated concentrations of Hf, Th, U, and rare earth elements in *Sargassum* and *Ulva* samples from Sri Lanka, could be due to an environment rich in those elements. Compared with *Ulva*, brown alga *Sargassum filipendula* accumulates Ag, As, Br, and Sr. Trace element concentrations in *Ascophyllum nodosum* (egg wrack) have also been compared with *Fucus* sp. in Canada (Sharp *et al.*, 1998), and with *Fucus vesiculosus* and *Fucus distichus* in a West Greenland fiord (Riget *et al.*, 1997). Concentrations of most elements in that region, especially of the heavy metals, were relatively low compared to other areas in Europe and North America, and can be considered natural background levels.

Data are also available from unpolluted areas of Australia and Asia. Maher (1986) determined concentrations of Cd, Cu, Pb, and Zn in *Ecklonia radiata* and *Ulva* sp. from St. Vincent Gulf (South Australia). Hou and Yan (1998) determined the concentration of five major and twenty eight trace elements in thirty five seaweeds collected along the coast of China, in sampling sites where contamination from human activity is negligible. *Colpomenia sinuosa* was the Phaeophyta that showed the highest concentrations of most of the trace elements. The concentrations of Br, Al, Cr, Fe, U, Th, and rare earth elements in the brown algae *Sargassum vachellianum*, *Sargassum henslowianum*, *Sargassum parvifolium* and *Sargassum carpophyllum*, were lower than in other Phaeophyta; however, the concentrations of iodine were much higher. In Rhodophyta, the highest concentrations of trace elements were found in *Myelophycus siruplex* and *Polysiphonia urceolata*, while the lowest were in *Porphyra* sp. For *Corallina pilulifera*, a typical calcareous alga, the concentration of calcium was 18.4% d.w., but levels of other alkaline earth metal, such as Mg, Sr, and Ba were not high.

Other studies concern the Antarctic coast. Fariás *et al.* (2002) examined the accumulation ability in the uptake of metals and metalloids of 11 Antarctic macroalgae. The highest levels of trace elements were found in *Monostroma*

*hariotti* and *Phaeurus antarcticus*. *Monostroma hariotii* offer the advantage as bioindicator due to its wide distribution. However, the main drawback was its inability to accumulate As, Cd, and Pb. The characteristics as bioindicators of *Phyllophora antarctica* and *Iridaea cordata* were reported by Grotti *et al.* (2008). The metal accumulation for *Phyllophora antarctica* was higher than that found for *Iridaea*, probably due to their different characteristics and the different sampling period. *Phyllophora* accumulated mainly manganese and nickel. Astorga-España, Calisto-Ulloa and Guerrero (2008) collected *Adenocystis utricularis*, *Enteromorpha* sp., *Mazzaella laminarioides* and *Porphyra columbina* from the coast of the Strait of Magellan (Chile). The authors analyzed Ag, Hg, Ni, Pb, Sb, V, and Zn to establish baseline levels of these elements with the data obtained in that location. *Enteromorpha* sp. showed the highest capacity for accumulating metals and could be selected as the best environmental bioindicator among these four species. Zn showed the highest concentration and the widest range for all the species studied. Mercury was only detected in *Enteromorpha* sp. There were also a large number of samples where Sb, Ag, Pb, and Ni were below detection limits offered by the analytical techniques used.

Studies have been performed to evaluate the influence of anthropogenic sources of pollution. Muse *et al.* (1995) determined Cd and Pb in eight common seaweed from Golfo Nuevo (an area influenced by an industrial city and close to an aluminum works) and Bahía Camarones (a traditional area for seaweed harvesting) in Patagonia (Argentina). Accumulation of Cd and Pb was found even when their levels in the water corresponded to an unpolluted environment. *Lessonia*, *Macrocystis* and *Gigantina* from Bahía Camarones showed lower levels of metals than seaweed from Golfo Nuevo. However, high values of Al (300–3000 mg Al/kg d.w.) were determined in the brown algae from the industrialized area, especially for *Colpomenia sinuosa*. Muse *et al.* (1999) also used *Ulva lactuca* and *Enteromorpha prolifera* that showed different metal concentrations between two sites studied. *Porphyra columbina* was only useful as an accumulator for Zn. Malea *et al.* (1995) evaluated pollution near to an aluminum factory in Antikyra Gulf (Greece). Fe and Pb were the elements with the highest concentration (Fe > Pb) after Ca and the macroelements K, Mg, and Na. Other elements, such as Cu, Zn, Cd, and K in *D. vermicularis* were correlated with levels in the sediment and not with their dissolved levels in seawater. However, element concentrations in the Phaeophyta *Padina durvillaei* from Santa Rosalía (Baja California, Mexico) do not follow the drastic increases and gradients of Cu, Co, Mn, and Zn detected in surface sediments (mainly smelter wastes which present low geochemical mobility) (Rodríguez-Figueroa *et al.*, 2009).

### 6.4.3 Seaweeds as bioindicators of radioactive pollution

Seaweeds have been widely used as bioindicators of radioactive pollution, due to their ability to concentrate isotopes. In fact, several products with acid-alginates (mostly from brown seaweed), have also been used to decorporate strontium or cesium after body contamination (Nesterenko and Nesterenko, 2009). Many studies deal with pollution of pollution from coastal discharges into the North Atlantic Ocean and use Phaeophyta as bioindicator. *Fucus vesiculosus* is the most common seaweed used in these studies. *Fucus vesiculosus* has been used in the United Kingdom for more than 40 years to monitor radioactivity in surface and coastal waters (Mitchell, 1969); likewise, "The Norwegian Fucus Project" which started in 1980, consists of monitoring radioactive pollution along the Norwegian coast using *Fucus vesiculosus* and *Ascophyllum nodosum* (IFE, 2011). Wallberg and Moberg (2002) evaluated 20 years worth of data concerning environmental quality near Swedish nuclear power plants and the Studsvik Research Facility. In the marine environment, *Fucus vesiculosus* usually had higher activity concentrations and a wider range of radionuclides ( $^{54}\text{Mn}$ ,  $^{58}\text{Co}$ ,  $^{60}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{95}\text{Nb}$ ,  $^{110\text{m}}\text{Ag}$ , and  $^{137}\text{Cs}$ ; occasionally  $^{152}\text{Eu}$ ) than other bioindicators, such as *Mytilus edulis*. Moreover, close to the discharge point, the  $^{60}\text{Co}$  activity concentration in *Fucus vesiculosus* and in discharge water were correlated. Manjón and García-León (Manjón *et al.*, 1995) collected a great variety of seaweed (Chlorophyta, Rhodophyta, and Phaeophyta) on the Andalusian Coast (Spain) during 1988–1989, and analyzed  $^{137}\text{Cs}$ ,  $^{99}\text{Tc}$ ,  $^{238}\text{Pu}$ ,  $^{239+240}\text{Pu}$ , and  $^{241}\text{Am}$ . The typical range for  $^{137}\text{Cs}$  in the samples was 1–2 Bq/kg, whereas values of  $^{99}\text{Tc}$  were less than 0.5 Bq/kg d.w. However, the authors found in *Fucus* (*spiralis* and *vesiculosus*), collected near the site of the aircraft accident in Palomares, radionuclides concentrations 20 times higher than in the surrounding areas ( $^{238}\text{Pu}$ : up to  $49 \pm 5$  mBq/kg d.w.,  $^{239+240}\text{Pu}$ :  $2020 \pm 20$  mBq/kg d.w.,  $^{241}\text{Am}$ :  $420 \pm 50$  mBq/kg d.w.).

The main sources of contamination in the environment due to man-made radionuclides have been the global fallout after atmospheric weapon tests in the 1950s and the 1960s, coastal discharges, and Chernobyl accident in 1986, that released large amounts of  $^{137}\text{Cs}$ . Radionuclides have been used as tracers of pollution, for example to study the transfer of European coastal pollution from the nuclear reprocessing plants in Sellafield (United Kingdom) and La Hague (France) to the Arctic (Dahlgård *et al.*, 1995). Some of these man-made radionuclides are  $^{99}\text{Tc}$ ,  $^{137}\text{Cs}$ ,  $^{134}\text{Cs}$ ,  $^{90}\text{Sr}$ ,  $^{129}\text{I}$ ,  $^{239,240}\text{Pu}$ .

The number of nuclear facilities, either in operation or in process of decommission in the OSPAR catchment in

2007 was 92, a number that remained stable for 10 years. The 2010 quality status report from the OSPAR convention for the Protection of the Marine Environment of the North-East Atlantic (Ospar Commission, 2010) shows that Region III (Celtic Seas) has benefitted from the reduction of the discharges in the nuclear sector, in particular technetium from Sellafield. Also the amount of  $^{137}\text{Cs}$  has diminished in the Celtic Seas. These decreases can be observed in seaweed, but some monitoring areas in sectors I and II (Greater North Sea and Arctic Waters) still have contaminated waters with radionuclides from the Chernobyl accident. Up to now, many studies have been performed to evaluate pollution in those areas. Kershaw *et al.* (1999) analyzed  $^{99}\text{Tc}$  in seaweed from six sampling sites near British Nuclear Group Sellafield nuclear reprocessing plant and on the coasts of Scotland and England. The concentration of  $^{99}\text{Tc}$  increased after the discharges of the EARP (Enhanced Actinide Removal Plant) that could not remove that isotope. Technetium has a concentration factor in *Fucus* approximately of  $10^5$ , which permits its detection even when water concentration is low. The maximum concentrations were observed at the pipeline (85 kBq/kg w.w.), and the levels decreased with distance. The  $^{99}\text{Tc}$  is bioabsorbed by *Fucus* and most of it is not released, in contrast with  $^{129}\text{I}$  and  $^{137}\text{Cs}$ . A periodicity of approximately 12 months was found in some of the time series for each sampling point. This was explained because lower concentrations in *Fucus* occur in the spring and summer as a result of increased growth that dilutes the isotope.  $^{99}\text{Tc}$  is not absorbed easily onto particles and can thus be used as an oceanographic tracer to examine how pollution evolves. Brown *et al.* (1999) observed a clear increase in  $^{99}\text{Tc}$  concentration in seaweed samples (*Fucus serratus* and *Fucus vesiculosus*) collected in the outer Oslofjord between 1996 and 1997. Maximum concentration observed was 170 Bq/kg d.w., being the concentration factor ( $1.21 \times 10^5$ ), higher than for other bioindicators such as shrimp, lobster and mussels. In a recent study (Bryan *et al.* 2008), high  $^{99}\text{Tc}$  concentrations in seaweed (*Fucus vesiculosus* and *Ascophyllum nodosum*) were still found in samples from Menai Straits (North Wales) near the Sellafield site.  $^{99}\text{Tc}$  activity concentrations in seaweed ranged from 457 to 1397 Bq/kg. High concentrations were also analyzed in the sediment. Therefore, seaweed seems to have an important role in concentrating elements from seawater and transferring them to the sediments.

Ryan *et al.* (1999) determined plutonium activity concentrations in *Fucus vesiculosus* collected in nine locations around the Irish coast. The activity concentrations of  $^{238}\text{Pu}$  and  $^{239,240}\text{Pu}$  showed a decreasing trend down the east coast from Ardglass (502 and 2755 mBq/kg d.w., respectively) to Dunmore East (8.4 and 66 mBq/kg d.w.). Studying  $^{238}\text{Pu}/^{239,240}\text{Pu}$  ratios, the authors also concluded that

the plutonium in the west coast of Ireland came mainly from Sellafield. A mathematical model was also proposed to simulate the annually average transport of radionuclides from Sellafield across the Irish Sea. The historical reconstruction since the early 1970s was provided at various locations on the east coast of Ireland for  $^{137}\text{Cs}$  in seaweed using *Fucus vesiculosus* (concentration factor approximately 50) and herring (*Clupea harengus*) (concentration factor approximately 120). Plutonium is predominantly associated with the sediments leading to extended transport times, whereas cesium is predominantly found in the dissolved phase (Smith *et al.*, 2000). The time series of concentration of  $^{137}\text{Cs}$  reached a maximum in the late seventies (approximately 70 Bq/kg) and afterwards values diminished to less than 5 Bq/kg w.w. Greenland is another region affected by the discharges of Sellafield. Dahlgaard *et al.* (2004) determined the levels of  $^{137}\text{Cs}$  and  $^{99}\text{Tc}$  in *Fucus vesiculosus*, *Fucus distichus* and *Ascophyllum nodosum* from the coast of Greenland during 1999–2001. Values ranged from 0.23 to 3.06 Bq/kg d.w. for  $^{137}\text{Cs}$  and from 1.6 to 29.62 Bq/kg d.w. for  $^{99}\text{Tc}$ . The concentration of these two isotopes in seawater and biota followed the typical oceanic patterns of transport of pollutants: concentrations in North-East Greenland and the coastal East Greenland current > South-West Greenland > Central West Greenland and North-West Greenland > Irmiger Sea ~ Faroe Islands.

Another source of radionuclides in the United Kingdom was the steam-generating heavy water reactor at AEA Winfrith (Dorset, UK). Cundy *et al.* (1999) examined the decline of the radionuclides  $^{60}\text{Co}$  and  $^{65}\text{Zn}$  along the southern UK coast over the period of 1988–1998, after the closure of that facility. The authors used *Fucus vesiculosus* and *Fucus serratus* as bioindicators. The rate of decline of  $^{65}\text{Zn}$  in *Fucus serratus* was similar to that of  $^{60}\text{Co}$ . Effective half-lives ranged from 1.9 to 3.9 years, which were significantly longer than the physical half-life of  $^{65}\text{Zn}$  (245 days). Thus, even when activities of  $^{60}\text{Co}$  and  $^{65}\text{Zn}$  decreased exponentially in seaweed after reactor closure, contamination persisted probably due to the absorption of radionuclides from sediments and recycling of contaminated organic matter.

Less data are available from Russian sources concerning North Atlantic and Antarctic Ocean pollution. Matishev *et al.* (2000) studied concentrations of several radioisotopes in seaweed (*Fucus vesiculosus*, *Laminaria* sp., *Fucus distichus*) collected in the region of the civilian nuclear icebreaker RTP "Atomflot" (Kola Bay, Northwest Russia). Levels of  $^{137}\text{Cs}$  in seaweed (0.3–2.3 Bq/kg w.w.) were similar to levels in Northern Norway (<2 Bq/kg w.w.) after 1990. Before the Chernobyl accident,  $^{137}\text{Cs}$  concentrations were the result of the discharges from European nuclear facilities. After 1986, the levels were influenced by the Chernobyl accident. These levels decreased from 8 Bq/kg to

less than 2 Bq/kg in the 1990s. Only *Laminaria* sp. samples collected next to the Atomflot area had high concentrations of  $^{137}\text{Cs}$  ( $46 \pm 5$  Bq/kg w.w.). These samples also contained  $^{134}\text{Cs}$  ( $1.2 \pm 4$  Bq/kg w.w.),  $^{60}\text{Co}$  ( $1.6 \pm 5.0$  Bq/kg w.w.),  $^{152}\text{Eu}$  ( $4.6 \pm 2.5$  Bq/kg w.w.), and detectable levels of  $^{58}\text{Co}$ ,  $^{103}\text{Ru}$ , and  $^{154}\text{Eu}$ .

Brown algae are vital in the iodine biogeochemical cycle, also from the point of view of radionuclide  $^{129}\text{I}$ , which is one of the most persistent radionuclides in the environment (half-life of  $1.6 \times 10^7$  years) (Leblanc *et al.*, 2006). Brown algal kelp species are the most efficient iodine accumulators among all living systems; for example, *Laminaria digitata* contains an average content of 1.0% d.w. of iodine (preconcentration factor 30 000). Cooper *et al.* (1998) determined  $^{129}\text{I}/^{127}\text{I}$  ratios in 34 Arctic marine algae (mainly *Laminaria* and *Fucus*) collected between 1930 and 1993. Five samples of marine algae were also analyzed to determine Pu isotope ratios. The  $^{129}\text{I}/^{127}\text{I}$  ratio in seaweed from Novaya Zemlya increased as much as three orders of magnitude from the pre-nuclear era (before 1945) to 1993. Sellafield (United Kingdom) and La Hague (France) were also the main source of  $^{129}\text{I}$ , and also responsible for the increase of the  $^{241}\text{Pu}/^{239}\text{Pu}$  ratio. Marine macroalgae collected between 1969 and 1993 in the Bering, Beaufort and East Siberian Seas had a much lower  $^{129}\text{I}/^{127}\text{I}$  ratio than those observed in the European Arctic, and there were no major contributions of non-fallout  $^{129}\text{I}$  to North American Arctic surface waters. The maximum measured specific activity of  $^{131}\text{I}$  measured around Japan by Morita *et al.* (2010b) was  $0.37 \pm 0.010$  Bq/kg w.w. The  $^{137}\text{Cs}$  was also detected in all brown algal samples, with low specific activities, ranging from  $0.0034 \pm 0.00075$  to  $0.090 \pm 0.014$  Bq/kg w.w. There was no correlation between the specific activities of both isotopes.  $^{131}\text{I}$  was detected near big cities with large populations, far from nuclear plants. The authors suggested that the likely pollution source of  $^{131}\text{I}$  was its excretion by nuclear medicine patients.

Others studies in the bibliography concern natural radionuclides. Suriyanarayanan *et al.* (2008) studied the concentrations of  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  in water, sediments and biota from Point Calimere Coast (India) to establish a baseline data on the radiation profile. The activity concentration of  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  in *Sargassum wightii* were  $2.8 \pm 0.5$  Bq/kg and  $13.7 \pm 0.79$  Bq/kg, respectively, while *Padina parvula* showed activity concentrations of  $2.9 \pm 0.5$  Bq/kg and  $95.0 \pm 2.0$  Bq/kg, respectively. These macroalgae concentrated less  $^{210}\text{Po}$  than the soft tissues of bivalves and less  $^{210}\text{Pb}$  than the shells and bones. Goddard and Jupp (2001) analyzed seaweed and seagrass from different sampling stations along the coast of Oman. They determined anthropogenic radionuclides and naturally occurring radionuclides in a wide variety of seaweed. Natural

radionuclides from the 3-decay series,  $^{238}\text{U}$ ,  $^{235}\text{U}$ , and  $^{232}\text{Th}$  were detected, and  $^{137}\text{Cs}$  was the only man-made radionuclide observed. The highest concentrations of  $^{137}\text{Cs}$  (up to 3.9 Bq/kg) were in the green intertidal seaweeds, *Enteromorpha* and *Chaetomorpha*, in Musandam sampling station. The mean  $^{137}\text{Cs}$  concentration in Chlorophyta was 3.3 Bq/kg and in Phaeophyta was 1.8 Bq/kg d.w.

## 6.5 Chemical speciation

### 6.5.1 Importance of the chemical species of an element

Significance of the presence of trace and ultratrace elements in biological and environmental systems depends on the total levels of the element, but also on the chemical forms of the element. This is mainly due to the different toxicity exhibited by specific element species, and also, because of mobility and availability of an element in the different compartments of an ecosystem is dependent on the specific chemical form of that element. It is well-known that mercury species are generally toxic, but inorganic mercury ( $\text{Hg(II)}$ ) is less dangerous than methylated mercury forms (methyl-mercury, Me-Hg). The same occurs for inorganic chromium, for which the trivalent species ( $\text{Cr(III)}$ ) is essential for living organisms, while the hexavalent form ( $\text{Cr(VI)}$ ) is highly toxic and carcinogenic (Florence, 1989). On other occasions, the methylated forms of an element exhibits low toxicity. For arsenic, inorganic forms ( $\text{As(III)}$  and  $\text{As(V)}$ ) are highly toxic, while toxicity decreases for organoarsenic compounds (methylated arsenic forms) and even, some arsenic species such as arsenobetaine ( $\text{AsB}$ ) are non-toxic compounds (Florence, 1989). In addition, bioavailability of essential and toxic elements from foodstuff varies in function of the chemical form. As an example, monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA) species are less bioavailable from seaweed (*Fucus* sp.) and softshell clams (*Mya arenaria*) (within the 1.2–1.9% range) than the arsenosugars bioavailable fraction (between 43 and 46%) (Koch *et al.*, 2007).

### 6.5.2 Sources of organometallic species in the environment and foodstuffs

The presence of specific chemical forms of an element in the different environmental compartments and in foodstuff can be attributed to anthropogenic sources such as the presence of hexavalent chromium ( $\text{Cr(VI)}$ ) (Metze *et al.*, 2005) or organotin compounds (OTCs), especially alkylbutyltin compounds (Rosenberg, 2005). There are chemical species used in different industrial process and/or added to

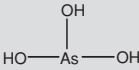
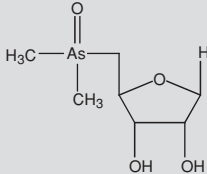
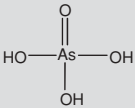
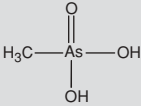
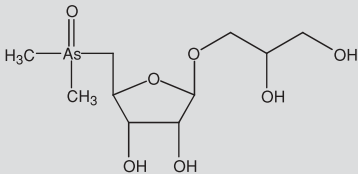
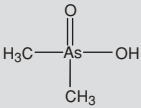
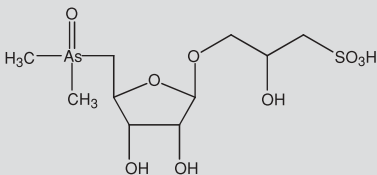
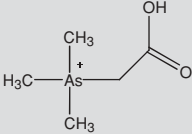
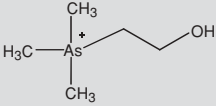
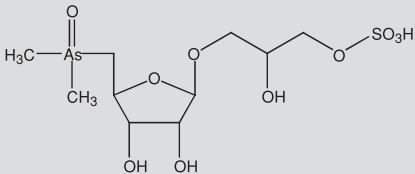
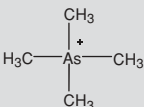
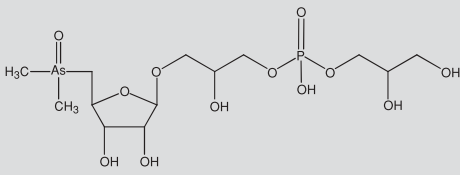
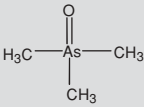
manufactured products. However on other occasions, the presence of certain organometallic species is attributed to biosynthesis from inorganic element forms by biota, mainly as a defence (detoxification) mechanism. This is the case of the presence of certain organometallic substances such as Me-Hg. This species is biosynthesized from inorganic mercury by microorganisms in sediments and by algae in sea water (Hovart and Gibičar, 2005). It then enters the aquatic food chain, and finally, it attains the highest concentrations in mollusks and fish (and even humans) by biomagnification processes. Similarly, biotransformation from inorganic arsenic by microorganisms, mainly by reduction and methylation pathways, and subsequent biomagnification in the food chain also occurs for organoarsenic compounds (Prohaska and Stinger, 2005). As previously mentioned, the biosynthesized products can be more toxic than the inorganic forms initially absorbed by living organisms, such as for Me-Hg. At times, such as for organoarsenic compounds, toxicity of the biosynthesized products is lower than that exhibited by the inorganic forms, or the biosynthesized products can even be non-toxic substances (as for AsB). On other occasions and also as a consequence of a toxic event, plants can biosynthesize certain oligopeptides, which chelate certain metals, mainly copper, cadmium and lead. The mechanism is similar to that reported for animals when biosynthesizing metallothioneins (MTs) and metallothioneins-like proteins (MLPs). The presence and/or increase of these molecular features, referred as phytochelatins (PCs), in terrestrial and marine plants, and even in macro- and micro-algae (Ferrat *et al.*, 2003), are considered as stress biomarkers of metal impregnation.

Finally, there are other several organometallic species which play important physiological functions. These chemical species are biosynthesized by certain living organisms from inorganic element forms present in food. This is the case of iodinated (3-iodotyrosine – MIT – and 3,5-diiodotyrosine – DIT) and selenium (selenocysteine –  $\text{SeCys}_2$  – and selenomethionine – SeMet) amino acids; vitamins, such as vitamin  $\text{B}_{12}$  (cyanocobalamin) containing cobalt; iodinated thyroid hormones (3:3':5:5'-tetraiodothyronine –  $\text{T}_4$  –, and 3:3':5-triiodothyronine –  $\text{T}_3$ ); or more complex structures such as proteins containing certain essential elements such as iron, copper, calcium and zinc.

### 6.5.3 Organometallic compounds (elemental chemical species) in algae

The presence of several organometallic species, mainly arsenic and iodine species, has been reported in algae. Tables 6.3 to 6.6 summarize the structure of the different chemical

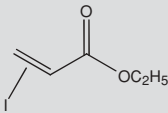
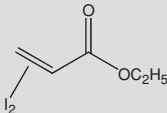
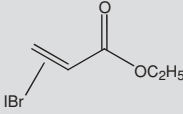
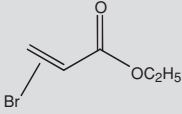
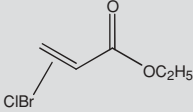
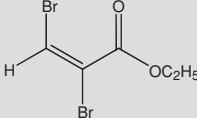
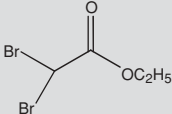
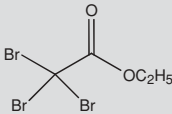
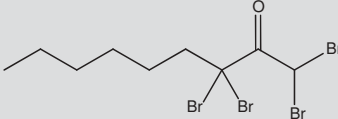
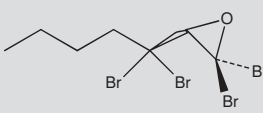
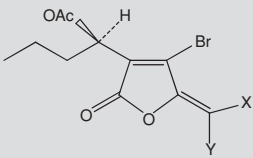
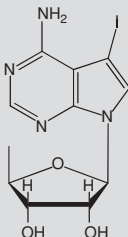
**Table 6.3** Arsenic species found in algae

Name	Structure	Name	Structure
Arsenous acid		5-dimethylarsinoyl- $\beta$ -ribofuranose	
Arsenic acid			
Monomethylarsenic acid (MMA)		3-[5'-deoxy-5'-(dimethylarsinoyl)- $\beta$ -ribofuranosyloxy]-2-hydroxypropylene glycol	
Dimethylarsenic acid (DMA)		3-[5'-deoxy-5'-(dimethylarsinoyl)- $\beta$ -ribofuranosyloxy]-2-hydroxypropane sulfonic acid	
Arsenobetaine (AsB)			
Arsenocholine (AsCo)		3-[5'-deoxy-5'-(dimethylarsinoyl)- $\beta$ -ribofuranosyloxy]-2-hydroxypropyl hydrogen sulfate	
Tetramethylarsonium ion (TMA)		3-[5'-deoxy-5'-(dimethylarsinoyl)- $\beta$ -ribofuranosyloxy]-2-hydroxypropyl-2,3-hydroxypropyl phosphate	
Trimethylarsine oxide (TMAO)			

**Table 6.4** Iodine, bromine and chlorine species found in algae

Name	Structure	Name	Structure
Iodide	$I^-$	Iodate	$IO_3^-$
Methyl iodide	$CH_3I$	Chloriodomethane	$CH_2ICl$
Bromiodomethane	$CH_2IBr$	Bromodiodomethane	$CHI_2Br$
Dibromiodomethane	$CHIBr_2$	Bromochloriodomethane	$CHIClBr$
Diiodomethane	$CH_2I_2$	Triiodomethane	$CHI_3$
Ethyl iodide	$CH_3CH_2I$	<i>n</i> -propyl-iodide	$CH_3CH_2CH_2I$
Isopropyl-iodide	$(CH_3)_2CHI$	1-iodobutane	$CH_3CH_2CH_2CH_2I$
2-iodobutane	$CH_3CH_2CHI CH_3$	1-iodopentane	$CH_3CH_2CH_2CH_2CH_2I$
1-methyl-1-iodopropane	$CH_3CH_2CH(CH_3)I$	1-bromo-2-iodoethane	$CHI_2CH_2Br$
3-iodo-1-propanol		Diiodoformaldehyde	
Bromiodoacetic acid		Diiodoacetamide	
Monoiodotyrosine		Diiodotyrosine	
1,1,3,3-tetrabromo-2-heptanol		1,1,1,3-tetrabromo-2-heptanol	
1,1,3,3-tetrabromo-2-nonanol		1,1,1,3-tetrabromo-2-nonanol	
Z-3-bromo-2-heptenoic acid		Z-3-bromo-2-nonenoic acid	
3,3-dibromo-2-butylacrylic acid		3,3-dibromo-2-hexylacrylic acid	

Table 6.4 (Continued)

Ethyl iodoacrylate		Ethyl diiodoacrylate	
Ethyl bromoiodoacrylate		Ethyl-bromoacrylate	
Ethyl bromochloroacrylate		Ethyl-E-2,3-dibromoacrylate	
Ethyl-2,2-dibromoacetate		Ethyl-2,2,2-tribromoacetate	
1,1,3,3-tetrabromo-2-nonanone		Trans-1,3,3-tribromo-1-heptene oxide	
3-[1-(acetyloxy)butyl]-4-bromo-5-(iodomethylene)-2(5H)-furanone		4-amino-7-(5'-deoxyribos-1'β-gl)-5-iodopyrino[2,3-d]pyridine	

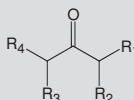
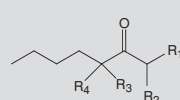
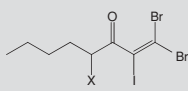
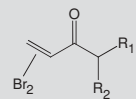
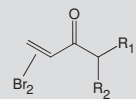
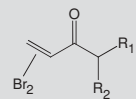
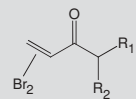
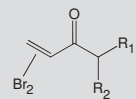
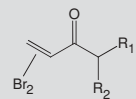
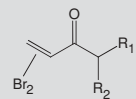
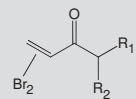
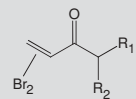
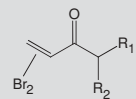
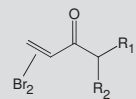
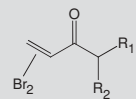
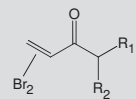
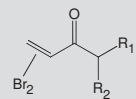
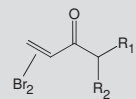
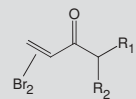
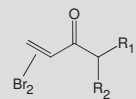
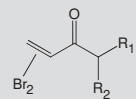
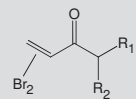
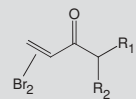
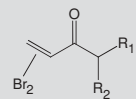
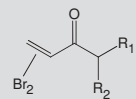
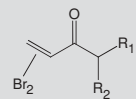
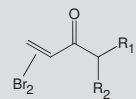
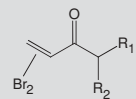
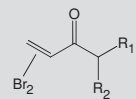
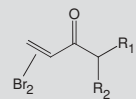
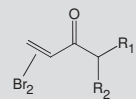
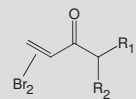
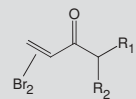
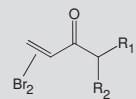
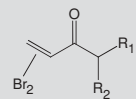
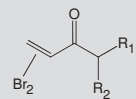
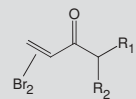
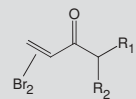
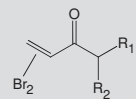
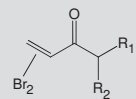
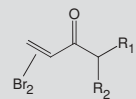
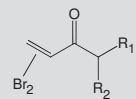
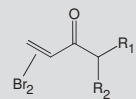
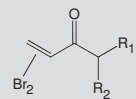
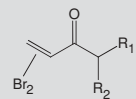
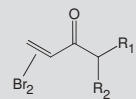
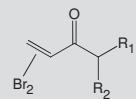
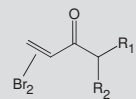
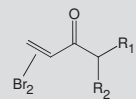
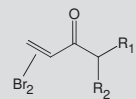
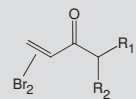
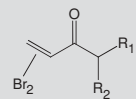
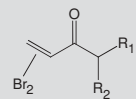
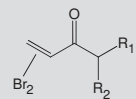
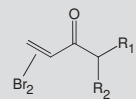
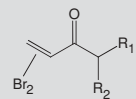
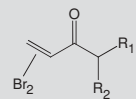
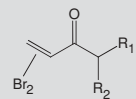
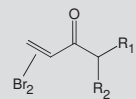
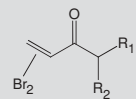
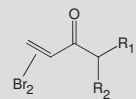
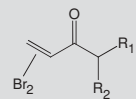
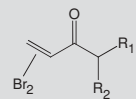
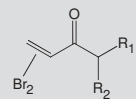
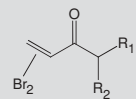
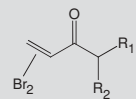
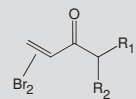
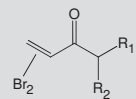
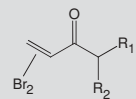
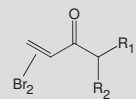
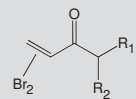
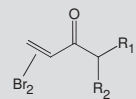
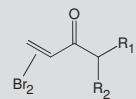
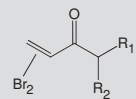
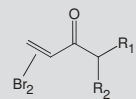
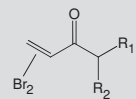
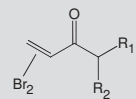
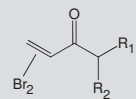
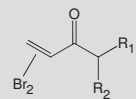
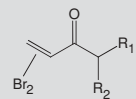
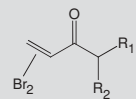
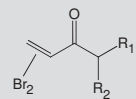
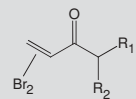
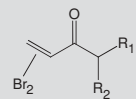
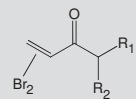
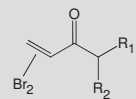
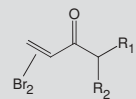
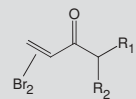
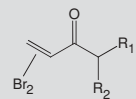
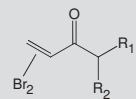
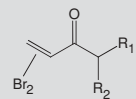
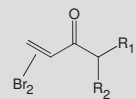
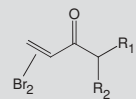
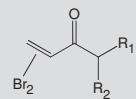
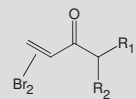
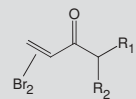
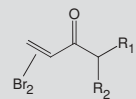
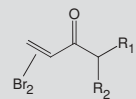
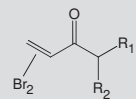
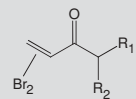
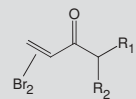
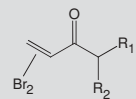
forms of arsenic and selenium, as well as chemical species of non-metallic elements such as iodine, bromine and chlorine.

#### Arsenic species

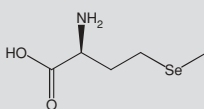
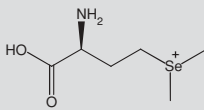
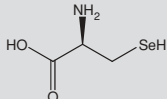
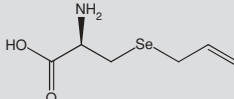
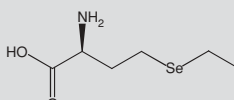
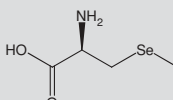
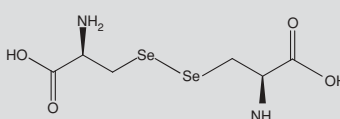
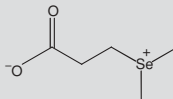
Arsenic occurs as inorganic arsenic (trivalent arsenic or arsenite – As(III), and pentavalent arsenic or arsenate – As(V)), but as mentioned above, methylated arsenic forms such as monomethylarsenic acid (MMA), dimethylarsenic

acid (DMA), arsenobetaine (AsB), arsenocholine (AsC), tetramethylarsonium ion (TETRA) and trimethylarsine oxide (TMAO) are also present (Table 6.3). In addition to organoarsenic species biosynthesized by methylation pathways, marine algae offer another group of organoarsenic compounds referred to as arsenosugars, which are believed to be formed from arsenate in seawater by successive alkylation and adenosylation processes (Miguens-Rodriguez *et al.*, 2002). Four commonly occurring arsenosugars (arsinoylribofuranosides) have been found in marine

**Table 6.5** Iodine, bromine and chlorine (ketones) species found in algae

									
Name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	Name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
1-bromo-3-iodoacetone	Br	H	I	H	1-iodo-3,3-dibromo-2-heptanone	I	H	Br	Br
1-chloro-3-iodoacetone	Cl	H	I	H	1,3-dibromo-2-heptanone	Br	H	Br	H
1,1-dibromo-3-iodoacetone	Br	Br	I	H	1,1,3-tribromo-2-heptanone	Br	Br	Br	H
1,3-dibromoacetone	Br	H	Br	H	1,3,3-tribromo-2-heptanone	Br	Br	H	Br
1,1-dibromo-3-bromoacetone	Br	Br	Br	H	1,1,3,3-tribromo-2-heptanone	Br	Br	H	Br
1,1,3,3-tetrabromoacetone	Br	Br	Br	Br					
1,1-bromochloro-3,3-dibromoacetone	Br	Cl	Br	Br					
1,1-dibromo-3-chloroacetone	Br	Br	Cl	H					
1,1-bromochloro-3-bromoacetone	Br	Cl	Br	H					
1,1-dibromo-3,3-dichloroacetone	Br	Br	Cl	Cl					
1,3-dibromo-1,3-dichloroacetone	Br	Cl	Br	Cl					
1-bromo-3-chloroacetone	Br	H	Cl	H					
1-bromo-3,3-dichloroacetone	Br	H	Cl	Cl	Name	X			
1-bromo-1,3-dichloroacetone	Br	Cl	Cl	H	1,1-dibromo-2-iodo-1-octen-3-one	H			
1-bromo-1,3,3-trichloroacetone	Br	Cl	Cl	Cl	1,1-dibromo-4-chloro-2-iodo-1-octen-3-one	Cl			
1,3-dichloroacetone	Cl	H	Cl	H					
1,1-dichloro-3-chloroacetone	Cl	Cl	Cl	H					
1,1,3,3-tetrachloroacetone	Cl	Cl	Cl	Cl					
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									
									

**Table 6.6** Selenium species found in algae

Name	Structure	Name	Structure
Selenite	$\text{SeO}_3^{2-}$	Selenomethionine	
Selenate	$\text{SeO}_4^{2-}$	Se-methylselenomethionine	
Selenocysteine		Se-allylselenocysteine	
Se-methylselenocysteine		Selenoethionine	
Selenocysteine		Dimethylselenium propionate ion	

### ***Iodine, bromine, and chlorine species***

Algae contain different halogenated compounds, but iodinated forms are the most important from the nutritional and environmental point of view. Iodine is an essential element for many living organisms, and algae offer a great capacity for retaining this element from the simplest iodinated species (inorganic iodine) such as iodide ( $\text{I}^-$ ) and iodate ( $\text{IO}_3^-$ ), to more complex iodinated forms such as iodine amino acids (MIT and DIT) (Table 6.4). In addition, macroalgae play an important role in the iodine cycle in marine environments through the biosynthesis of volatile iodinated species such as methyl-iodide, ethyl-iodide and chloriodomethane, and also methyl-bromide and different organochlorines (Baker *et al.*, 2001), hence its presence in seawater and in the marine atmosphere (Edmonds and Morita, 1998; Gschwend *et al.*, 1985). These iodinated compounds, mainly methyl-iodide, interact with  $\text{O}_3$ ,  $\text{H}_2\text{O}_2$  and  $\text{NO}_x$  and generate molecular iodine ( $\text{I}_2$ ) and other gaseous activated iodine compounds (AIC), such as iodine monochloride (ICl) and hypiodous acid (HOI),

compounds typically found in marine atmospheres (Huang and Hoffmann, 2009). Field and laboratory experiments have recently proved these findings by using brown (*Laminaria digitata* and *Fucus serratus*), red (*Kallymenia antarctica*) and green (*Ulva* sp.) seaweed (Chance *et al.*, 2009). Moreover, iodine has been recognized as an antioxidant element for algae (Chance *et al.*, 2009). This theory is supported by the increase of released iodine, mainly oxidized iodine forms, when algae are subjected to oxidative stress by attacks from alginate-degrading organisms (bacteria and mollusks).

Tables 6.4 and 6.5 list the different iodine (also bromine and chlorine) species found in algae. Iodide ( $\text{I}^-$ ) and iodate ( $\text{IO}_3^-$ ) are present as inorganic forms, and iodide is by far the major species in all algae types. However, algae contains different volatile iodoalkanes, such as methyl-iodide, diiodomethane, triiodomethane, ethyl-iodide, isopropyl-iodide, *n*-propyl-iodide, 1-methyl-1-iodopropane, 1-iodobutane, 1-iodopentane, chloriodomethane, bromiodomethane,

bromodiodomethane, dibromiodomethane, bromochloriodomethane and 1-bromo-2-iodoethane, and other organoiodine compounds such as diiodoformaldehyde, 3-iodo-1-propanol, 1-bromo-3-iodo-2-propanone, 1-bromo-2,3-dichloro-1-iodoethene, bromoiodoacetic acid, and diiodoacetamide (Tables 6.4 and 6.5) (McConnell and Fenical, 1977; Gribble, 2003). Iodinated species of more complex structure such as iodinated ketones (1-iodo-3,3-dibromo-2-heptanone, 1-chloro-3-iodoacetone, and 1,1-dibromo-3-iodoacetone, ethyl-bromiodoacrylate, ethyl-iodoacrylate and ethyl-diiodoacrylate), and iodine amino acids (MIT and DIT) have also been found in algae (McConnell and Fenical, 1977; Edmonds and Morita, 1998). Since iodinated amino acids are present in certain proteins, there are also reported iodine containing proteins in microalgae such as *Chlorella vulgaris* (Gómez-Jacinto *et al.*, 2010) and seaweed such as *Laminaria japonica* (Kombu) and *Undaria pinnatifida* (Wakame) (Shah *et al.*, 2005). Similarly, iodine bound to high molecular weight polyphenolic compounds have also been described (Shah *et al.*, 2005).

Other compounds such as 4-amino-7-(5'-deoxyribose-1' $\beta$ -yl)-5-iodopyrrolo[2,3-d]pyrimidine isolated from *Hypnea valendiae*, gamma-methylene lactones (general structures are given in Table 6.4) extracted from *Delisea fibrata*, and iodobromo aromatic sesquiterpenes from *Laurencia nana*, have also been reported (Edmonds and Morita, 1998).

Regarding bromine and chlorine species, inorganic bromine as bromide ( $\text{Br}^-$ ) and bromate ( $\text{BrO}_3^-$ ), and inorganic chlorine (chloride,  $\text{Cl}^-$ ) are found in algae. In addition, halogenated ketones (Table 6.5) such as 1,2-dibromoacetone, 1,1,2-tribromoacetone, 1,1,2,2-tetrabromoacetone, 1-bromo-2-chloroacetone, 1,2-dibromo-1-chloroacetone, 1,1,2-tribromo-2-chloroacetone, 1,2-dichloroacetone, 1,1,2-trichloroacetone, 1,1,2,2-tetrachloroacetone, 1-bromo-2,2-dichloroacetone, 1-bromo, 1,2-dichloroacetone, 1-bromo-1,2,2-trichloroacetone, 1,1-dibromo-2,2-dichloroacetone, 1,2-dibromo-1,2-dichloroacetone, and ethyl-E-2,3-dibromoacrylate are also reported (McConnell and Fenical, 1977). Different bromine and chlorine haloesters (McConnell and Fenical, 1977) have also been found in algae, such as ethyl 2,2-dibromoacetate and ethyl 2,2,2-tribromoacetate (Table 6.4). Other compounds such as certain halobutenones (general structures are listed in Table 6.5) have also been found in *Asparagopsis armata* (McConnell and Fenical, 1977). Red algae *Bonnemaisonia nootkana*, *B. asparagoides*, *B. hamifera* and *Trailliella intricata* also offer different halogenated species (Table 6.5). Most of them are brominated compounds of  $\text{C}_7$ – $\text{C}_9$  halogen-containing ketones, alcohols and carboxylic acids (McConnell and Fenical, 1980).

### Mercury species

As previously mentioned, inorganic mercury ( $\text{Hg(II)}$ ) in water ecosystems is biomethylated, which leads to the formation of methyl-mercury ( $\text{CH}_3\text{-Hg}^+$ , Me-Hg). Sulfate reducing bacteria are mainly responsible for the biomethylation process either under biotic or abiotic conditions (Ullrich *et al.*, 2001). Formed Me-Hg is then transported in food chains by unicellular and filamentous algae and cyanobacteria, and then in macroalgae and macroinvertebrates, and Me-Hg is pre-concentrated in their tissues. However, as demonstrated by Gorski *et al.* (2006) by using freshwater green algae *Selenastrum capricornutum*, it must be pointed out that the bioavailability of Me-Hg to algae in aquatic systems is influenced by the binding of Me-Hg to various aqueous ligands, colloids and particles.

Mercury and Me-Hg species are biomagnified in aquatic biota. As demonstrated by Coehlo *et al.* (2005), the percentage of organic mercury (mainly Me-Hg) in algae responds to the environmental mercury gradient in the water column, and showed very high concentration factors, hence, a high bioaccumulation ability for mercury. However, the levels of this element (inorganic and methylated forms) in algae from unpolluted areas are low. This occurs even in marine macroalgae (seaweed) (Serfor-Armah *et al.*, 2001) and, in general, recent literature shows mercury contents not higher than 15.8 ng/g in red seaweed such as *Plocamium corallorhiza* and *Gelidium abbottiorum* (Misheer *et al.*, 2006a, b), and ranging from 60 to 190 ng/g in Chlorophyta such as *Caulerpa racemosa* (sea grapes) and *Ulva lactuca* (sea lettuce) (Misheer *et al.*, 2006c, d). Reported total mercury levels in edible seaweed (dried seaweed) are also low, approximately 41 ng/g in the red seaweed *Porphyra umbilicalis* (Nori); and a bit higher in brown seaweed, within the 23.4–86.8 ng/g range in *Undaria pinnatifida* (Wakame), 126.9 ng/g in *Laminaria ochroleuca* (Kombu), and 251.9 ng/g for *Himanthalia elongate* (sea spaghetti) (Fernández-Fernández *et al.*, 2007). Because of the low content of mercury in algae, levels of Me-Hg must be very low, and few reports can be found in the literature. As examples, Campbell *et al.* (2005) have found trace of total mercury in some ice algae, but levels of Me-Hg were not detected; while Žižek *et al.* (2007) have confirmed the presence of Me-Hg in river filamentous algae, and have established a low Me-Hg percentage, approximately 0.5% of the total mercury.

### Selenium species

In addition to inorganic forms of selenium (selenite,  $\text{Se(IV)}$ , and selenate,  $\text{Se(VI)}$ ), selenium occurs in biota as a series of organic species with important physiological roles,

mainly selenium containing amino acids, in which sulphur is replaced by selenium. Although several selenium organic species have been described in biota, approximately nine selenium containing amino acids among them (Larsen *et al.*, 2001), few data are reported in algae. Early works by Bottino *et al.* (1984) suggested the presence of free selenium containing amino acids (Table 6.6) such as selenocysteine (SeCys), Se-methylselenocysteine (MeSeCys), SeCys<sub>2</sub>, SeMet, and Se-methylselenomethionine (MeSeMet) in unicellular marine algae *Dunaliella primolecta*, *Chlorella* sp. and *Porphyridium cruentum*. Other selenium amino acids such as Se-allylselenocysteine (AllSeCys) and selenoethionine (SeEt) (Table 6.6) have been detected in *Chlorella* sp. (Larsen *et al.*, 2001). Some investigations suggest that MeSeCys in marine algae tends to spontaneously decompose with the formation of pyruvate and ammonia via aminoacrylic acid and methaneselenic acid. The latter reacts with sulphhydryls or selenols to generate selenodisulfides or diselenides, respectively (Řezanka and Sigler, 2008). In addition, recent studies (Larsen *et al.*, 2001) show the presence of the selenonium species dimethylselenonium propionate ion (DMSeP) in some algae such as *Chlorella* sp. This chemical species (Table 6.6) is converted to volatile dimethylselenide (DMSe) by algae, which is then transferred to seawater and air (Fan *et al.*, 1997). Therefore, algae play an important role in the cycling of selenium in marine environments.

### Antimony and tin species

Antimony speciation in marine biota is not well documented, and although the organic species trimethylantimony(V) (TMSb(V)) has been identified in molluscs, most of the studies for algae deal with total antimony (Filella *et al.*, 2007). However, there are some speciation studies for antimony, mainly for assessing inorganic antimony species (antimoniate, Sb(V), and antimonite, Sb(III)). Foster *et al.* detected Sb(V) and Sb(III) in algae samples collected from two locations at Kerosene Creek, Waitapu, New Zealand (algae type is not given) (Foster *et al.*, 2005). Both inorganic antimony species have also been detected in *Macrosystis integrifolia* (de Gregori *et al.*, 2007), while speciation analysis of *Schimmelmanna plumose*, *Fotogenia fastigiata* and *Halopectis hordacea* has only shown the presence of the pentavalent species (Sb(V)) (de Gregori *et al.*, 2007). These latter results are consistent with the photo-oxidation of Sb(III) to Sb(V) by marine microalgae (studies performed with *Tetraselmis levis*, *Chlorella autotrophica*, *Nannochloropsis* sp., *Tetraselmis subcordiformis*, *Phaeodactylum tricornutum*, and *Porphyridium purpureum*), which tends to reduce the concentration of the trivalent antimony species (Li *et al.*, 2006). Finally, some studies suggest that anti-

mony can be tightly bound to phytochelatins or may be present as antimony nanocrystallites in algae (Foster *et al.*, 2005).

Concerning tin speciation, there is only one paper describing the presence of inorganic tin (Sn(IV)), methylated tin species such as monomethyltin (MMT), dimethyltin (DMT), trimethyltin (TMT), and tetramethyltin (TeMT), and also organobutyltin compounds (*n*-butyltin, MBT) in seaweed (*Ulva* sp., *Enteromorpha* sp., and *Sargassum* sp.) (Ishii, 1982).

### Metal binding phytochelatins

As previously mentioned, phytochelatins (PCs) are biosynthesized by algae as a detoxification mechanism against heavy metal pollution (Ferrat *et al.*, 2003). PCs consist of small sulfur-rich oligopeptides (molecular weight ranges from 2 to 10 kDa) of the general structure ( $\gamma$ -Glu-Cys) $_n$ -Gly ( $n = 2-11$ ) (Torres *et al.*, 2008). Biosynthesis of PCs involves non-ribosomal mechanisms by the action of the constitutive enzyme phytochelatin synthase (Perales-Vela *et al.*, 2006) from reduced glutathione (GSH) as a substrate. This peptide (GSH) has been reported as the primary peptide involved in binding heavy metals in PCs in higher plants (Rausser, 1995; Zenk, 1996; Mendoza-Cózatl *et al.*, 2005). The chelation of heavy metals by the sulphhydryl-rich peptide (from cysteine) allows the plant to control the intracellular concentration level of metal ions and imparts a certain degree of tolerance to the heavy metal stress (Rosenberg, 2003).

Different studies have been conducted to test the biosynthesis of PCs by algae under a polluted environment. Therefore, PC biosynthesis was proven in *Salvinia minima* after lead exposure (Estrella-Gómez *et al.*, 2008), and in *Stichococcus minor* and *Geminella terricola* under a copper environment (Kalinowska and Pawlik-Skowrońska, 2010). In addition, chromatographic studies have revealed the existence of different PC oligomers in brown and red seaweed (Pawlik-Skowrońska *et al.*, 2007). Therefore, the presence of a dimer oligomer (PC2), with an *m/z* ratio of 540 for the major ion after electrospray ionization-mass spectrometry (ESI-MS), and a trimer oligomer (PC3), *m/z* ratio of 773, were detected in *F. serratus* and *Gracilaria gracilis*. Moreover, a tetramer oligomer (PC4) with an *m/z* ratio of 1004 (ESI-MS) was also measured in *F. serratus*. The study concludes that the concentrations of different PC oligomers are related to the levels of heavy metals such as arsenic, cadmium, copper, lead and zinc, and also to the levels of GSH. High concentrations of heavy metal and GSH in algae correspond to high PCs concentrations. Similarly to characterization of metal binding PCs in terrestrial plants (Vacchina *et al.*, 1999, 2000), further studies are required

for elucidating the levels of heavy metals chelated by these biomolecules in algae.

#### 6.5.4 Analytical chemistry of elemental speciation in algae

According to the International Union for Pure and Applied Chemistry (IUPAC), the chemical species of an element is referred to a specific form (isotopic composition, electronic or oxidation state, and/or complex or molecular structure) of that element (Templeton *et al.*, 2000). Therefore, speciation analysis is understood as all those analytical activities for isolating, identifying and measuring the concentrations of one or more individual chemical species in a sample (Templeton *et al.*, 2000; Milchalke, 2003). Special care must be taken into consideration to avoid chemical species inter-conversion during isolation and determination stages. As examples, artifactual formation of Me-Hg from inorganic mercury can occur when using distillation techniques as sample pre-treatments while analysing environmental samples (Hintelmann *et al.*, 1997); and also, the presence of selenomethionine–Se-oxide (SeOMet) is attributed to conversion of SeMet during sample preparation (Larsen *et al.*, 2001).

##### Sample pretreatment procedures

Different strategies for isolating organometallic species have been proposed. These methodologies vary in function of the sample nature and mainly the specific organometallic form. In some cases, non-exhaustive extraction procedures are adequate for extracting labile element species, such as for isolating methyl-mercury (Me-Hg) (commonly released by using diluted hydrochloric acid and organic solvents), or for extracting organoarsenic compounds (methanol–water mixtures as a solvent and under mild operating conditions). However, more exhaustive methods can be required by other organometallic species such as selenium (SeCys<sub>2</sub> and SeMet) and iodine (MIT and DIT) amino acids, species integrated in large biomolecules which have to be broken down for species releasing. Some reviews can be found on this topic, such as that by Rubio *et al.* (2010) dealing with arsenic speciation in algae and aquatic plants.

Most Me-Hg isolation procedures are based on solvent extraction by mechanical shaking (Cai *et al.*, 1997), mainly using dichloromethane or toluene after acidification with hydrochloric acid (Žižek *et al.*, 2007; Campbell *et al.*, 2005; Coehlo *et al.*, 2005). The acid treatment is needed to breakdown sulphhydryl–mercury bonds. Inorganic antimony is also extracted from algae by solvent extraction (mechanical shaking) with ethylenediaminetetra-acetic acid (EDTA) and citric acid (Foster *et al.*, 2005; de Gregori *et al.*, 2007).

Interconversion of Sb(III) to Sb(V) is avoided when using these extracting solutions because both species form stable complexes with citric acid, and also Sb(III), which tends to be oxidized to Sb(V), is effectively chelated with EDTA (de Gregori *et al.*, 2007). Boiling water and ethanol mixtures have been used to extract organotin compounds (Ishii, 1982). Trichloroacetic acid (TCA) in an ice bath is also used as an extractant for water-soluble selenium metabolites (including DMSeP) from *Chlorella* sp. The extract is then lyophilized to remove TCA before selenium speciation studies (Larsen *et al.*, 2001). Similarly, distillate water (Nischwitz and Pergantis, 2006), methanol/water mixtures (Pergantis *et al.*, 2000; Pickford *et al.*, 2002), and diluted phosphoric acid (Geng *et al.*, 2009) have also been used to extract arsenicals from algae.

Solvent extraction procedures by mechanical shaking are also the chosen treatments for isolating volatile halogenated compounds from algae, mainly using pentane (methyl-iodide, chloriodomethane and dibromiodomethane extraction), dichloromethane (iodinated acetones extraction), ethanol (iodinated acrylate esters), and chloroform–ethanol mixtures (volatile bromine and chlorine species) (McConnell and Fenical, 1977, 1980). Solvent extraction with diluted sodium hydroxide or with diluted hydrochloric acid solutions can also be used to extract iodine bound to high and low molecular weight compounds, or iodine bound to low molecular weight compounds, respectively (Shah *et al.*, 2005). Moreover, Tris-hydrochloric acid (pH 8.0) buffer solutions have also been reported for isolating iodine-containing proteins, while ethanol is mainly used to extract iodine bound to polyphenols (Shah *et al.*, 2005).

Solvent extraction procedures can also be assisted by microwave energy, such as for inorganic iodine and bromine (Chen *et al.*, 2007) or some arsenic compounds (Karadjova *et al.*, 2007) with tetramethylammonium hydroxide (TMAH); or with methanol/water (Kirby *et al.*, 2004; Karadjova *et al.*, 2007; Han *et al.*, 2009). The use of ultrasound energy for assisting organoarsenic compounds from algae has also been proposed. Methanol/water mixtures (Lai *et al.*, 1997; Wangkarn and Pergantis, 2000; McSheehy and Szpunar, 2000; McSheehy *et al.*, 2002; Miguens-Rodriguez *et al.*, 2002; Van Hulle *et al.*, 2002; Wuilloud *et al.*, 2006) and also distillate water (Hirata and Toshimitsu, 2005; Shimoda *et al.*, 2010) were used as extractants; the latter gave successful results even for extracting arsenosugars. On other occasions, pressurized liquid extraction (PLE) is also used, such as for organoarsenic compound extraction (including arsenosugars) with methanol/water mixtures as extractants (Gallagher *et al.*, 1999; Gallagher *et al.*, 2001).

In addition to these single solvent extraction processes, multistage or sequential extractive schemes have also been

proposed to isolate different iodinated forms from algae (Hou *et al.*, 2000; Shah *et al.*, 2005; Gómez-Jacinto *et al.*, 2010), or arsenicals bound to the lipids or water soluble fractions (Thomson *et al.*, 2007a, b; Foster *et al.*, 2008; Cao *et al.*, 2009). For iodinated species extraction, these procedures consist of pigments removal (use of acetone), followed by removal of carbohydrates and polyphenols (use of solutions containing calcium chloride and caffeine), protein isolation (sonication with a Tris-hydrochloric acid buffer at pH 8.0) and precipitation (use of acetone), and redissolution (Tris-hydrochloric acid at a pH 8.0) (Hou *et al.*, 2000; Shah *et al.*, 2005). Other procedures imply a first extraction with water (isolation of iodine water-soluble targets), followed by an extraction with Tris-hydrochloric acid buffer solution (pH 9.0) containing sodium dodecylsulphonate (SDS) for extracting iodine bound to macromolecules, and by a treatment of the filtrate with diammonium sulfate for iodine containing protein precipitation. The final step of this scheme consists of an iodine-linking protein redissolution in water followed by a purification stage by dialysis (Gómez-Jacinto *et al.*, 2010). Concerning arsenicals, algae are treated with chloroform/methanol mixtures (mechanical shaking) (Thomson *et al.*, 2007a, b; Foster *et al.*, 2008) to extract arsenic species bound to the lipid fraction. On other occasions, the lipid fraction is extracted by sonicating with acetone instead of chloroform/methanol (Cao *et al.*, 2009). The residue is then treated with hot water for isolating water-soluble arsenic compounds (Thomson *et al.*, 2007a, b; Foster *et al.*, 2008) or with methanol/water and hydrochloric acid (Cao *et al.*, 2009).

Enzymatic hydrolysis methods have also been proposed for element speciation in algae. As examples, the proteolytic enzyme Proteinase K was used to assess iodine containing high molecular weight compounds in seaweed (Shah *et al.*, 2005).

Finally, PCs can be isolated from algae by solvent extraction with 5-sulfosalicylic acid (SSA) containing 6.3 mM diethylenetriammonopenta-acetic acid (DTPA) in ice-bath. Disrupted cells are then centrifuged (10 000 g), and the supernatants (extracts) are used for PC oligomers separation (Pawlik-Skowrońska *et al.*, 2007).

### Separation and determination procedures

For element species measurement, although some non-chromatographic methods can be addressed in speciation studies (Ishii, 1982; Hou *et al.*, 2000; Coehlo *et al.*, 2005; Misheer *et al.*, 2006c), most of the methods require a separation of the individual chemical species by high performance liquid chromatography (HPLC), gas chromatography (GC) or capillary electrophoresis (CE) coupled to species selective detectors, mainly electrospray ionization-mass spec-

trometry (ESI-MS) or element selective detectors such as inductively coupled plasma-mass spectrometry (ICP-MS).

GC coupled with MS and also with electron capture detector (ECD) is commonly used when assessing volatile halogenated species in algae, mainly methyl iodide, chloroiodomethane, dibromoiodomethane (McConnell and Fenical, 1977), halogenated ketones and acrylate esters (McConnell and Fenical, 1977, 1980). GC-ECD is also used for methyl-mercury determination (Campbell *et al.*, 2005; Žižek *et al.*, 2007), a method commonly based on that developed by Horvat and Byrne (Horvat and Byrne, 1990), although GC can be coupled with cold vapour atomic fluorescence spectrometry (CVAFS) after ethylation (Žižek *et al.*, 2007), a method, in this case, previously developed by Liang *et al.* (Liang *et al.*, 1994). The hyphenation of CE and ICP-MS has also been used by Chen *et al.* (2007) for inorganic iodine and bromine speciation in algae. However, most of the applications for speciation studies in algae use HPLC coupled with ICP-MS. Elemental species separations can be performed with anion exchange chromatography (AEC), cation exchange chromatography (CEC), reverse phase chromatography (RPC), and size exclusion chromatography (SEC). AEC has been used for antimony (Foster *et al.*, 2005) and selenium (Larsen *et al.*, 2001) speciation with ICP-MS, and also for antimony with hydride generation – atomic fluorescence spectrometry (HG-AFS) after UV photolysis (de Gregori *et al.*, 2007); while CEC has been mainly used for selenium speciation with ICP-MS detection (Larsen *et al.*, 2001). Arsenic speciation in algae has been performed mainly by AEC with ICP-MS detection (Gallagher *et al.*, 2001; Van Hulle *et al.*, 2002; Laparra *et al.*, 2004; Hirata and Toshimitsu, 2005; Ichikawa *et al.*, 2006; Wuilloud *et al.*, 2006; Koch *et al.*, 2007; Han *et al.*, 2009; Shimoda *et al.*, 2010), or after HG-ICP-MS (Gallagher *et al.*, 1999). Finally, AEC has also been used for iodine (Shah *et al.*, 2005; Wang and Jiang, 2008; Gómez-Jacinto *et al.*, 2010) and bromine speciation (Wang and Jiang, 2008) with ICP-MS detection. Arsenosugars are commonly resolved by AEC, and their structures elucidation was first established by ESI-MS methods. Molecular mass spectrometry techniques are nowadays powerful detection systems for arsenosugars detection/determination, such as ESI-MS-MS (Gallagher *et al.*, 1999; Van Hulle *et al.*, 2002; Nischwitz and Pergantis, 2006; Geng *et al.*, 2009; Shimoda *et al.*, 2010), time-of-flight mass spectrometry (TOF-MS) (Pergantis *et al.*, 2000; Pickford *et al.*, 2002; Miguens-Rodriguez *et al.*, 2002), and ESI-ion trap MS (Wuilloud *et al.*, 2006).

RPC has also been used for arsenic speciation (Lai *et al.*, 1997; Wangkarn and Pergantis, 2000), although some applications can be found in the literature for iodine speciation studies (Shah *et al.*, 2005). Element binding high molecular compounds have been resolved by SEC combined with AEC

(bidimensional SEC and AEC), such as for arsenic containing biomolecules (McSheehy and Szpunar, 2000; McSheehy *et al.*, 2002) and for iodine-binding proteins (Shah *et al.* 2005; Gómez-Jacinto *et al.*, 2010). Finally, ESI-MS techniques are commonly used for PCs detection/determination after RPC separation of the different oligomers (Pawlik-Skowrońska *et al.*, 2007).

## References

- Afzal-Rizvi, M. and Shameel, M. (2001) Distribution of elements in marine algae of Karachi coast. *Pak. J. Bot.*, **33**, 357–363.
- Astorga-España, M.S., Calisto-Ulloa, N.C. and Guerrero, S. (2008) Baseline concentrations of trace metals in macroalgae from the Strait of Magellan, Chile. *Bull. Env. Contam. Toxicol.*, **80**, 97–101.
- Baker, J.M., Sturges, W.T., Sugier, J. *et al.* (2001) Emissions of CH<sub>3</sub>Br, organochlorines, and organoIodines from temperate macroalgae. *Chemosphere Global Change Science*, **3**, 93–106.
- Barreiro, R., Picado, L. and Real, C. (2002) Biomonitoring heavy metals in estuaries: A field comparison of two brown algae species inhabiting upper estuarine reaches. *Env. Monitor. Assess.*, **75**, 121–134.
- Besada, V., Andrade, J.M., Schultze, F. and González, J.J. (2009) Heavy metals in edible seaweeds commercialised for human consumption. *J. Mar. Syst.*, **75**, 305–313.
- Bottino, N.R., Banks, C.H., Irgolic, K.J. *et al.* (1984) Selenium containing amino acids and proteins in marine algae. *Phytochemistry*, **23**, 2445–2452.
- Bryan, S.E., McDonald, P., Hill, R. and Wilson, R.C. (2008) Sea to land transfer of anthropogenic radionuclides to the North Wales coast, Part I: External gamma radiation and radionuclide concentrations in intertidal sediments, soil and air. *J. Env. Radioactivity*, **99**, 7–19.
- Burtin, P. (2003) Nutritional value of seaweeds. *Electr. J. Env., Agric. Food Chem.*, **2**, 498–503.
- Brown, J.E., Kolstad, A.K., Brungot, A.L. *et al.* (1999) Levels of <sup>99</sup>Tc in seawater and biota samples from Norwegian coastal waters and adjacent seas. *Mar. Poll. Bull.*, **38**, 560–571.
- Cai, Y., Tang, G., Jaffé, R. and Jones, R. (1997) Evaluation of some isolation methods for organomercury determination in soil and fish samples by capillary gas chromatography-atomic fluorescence spectrometry. *Int. J. Env. Anal. Chem.*, **68**, 331–345.
- Campbell, L.M., Norstrom, R.J., Hobson, K.A. *et al.* (2005) Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). *Science of Total Environment*, **351–352**, 247–263.
- Cao, X., Hao, C., Wang, G. *et al.* (2009) Sequential extraction combined with HPLC–ICP–MS for As speciation in dry seafood products. *Food Chem.*, **113**, 720–726.
- Carballeira, A., Carral, E., Puente, X. and Villares, R. (2000) Regional-scale monitoring of coastal contamination. Nutrients and heavy metals in estuarine sediments and organisms on the coast of Galicia (northwest Spain). *Int. J. Env. Poll.*, **13**, 534–572.
- Carvalho, M.L., Ferreira, J., Amorim, P. *et al.* (1997) Study of heavy metals and other elements in macrophyte algae using energy-dispersive x-ray fluorescence. *Env. Toxicol. Chem.*, **16**, 807–812.
- Chance, R., Baker, A.R., Küpper, F.C. *et al.* (2009) Release and transformations of inorganic Iodine by marine macroalgae. *Estuarine Coastal and Shelf Science*, **82**, 406–414.
- Chen, J.H., Wang, K.E. and Jiang, S.J. (2007) Determination of Iodine and bromine compounds in foodstuffs by CE-inductively coupled plasma MS. *Electrophoresis*, **28**, 4227–4232.
- Choi, M., Kang, M. and Kim, M. (2009) The analysis of copper, selenium, and molybdenum contents in frequently consumed foods and an estimation of their daily intake in Korean adults. *Biol. Trace Element Res.*, **128**, 104–117.
- Choi, M., Lin, X., Lee, S.A., *et al.* (2008) Daily intakes of naturally occurring radioisotopes in typical Korean foods. *J. Env. Radioactivity*, **99**, 1319–1323.
- Code of Federal Regulations (2009) *21 CFR184\_Part 184\_Direct food substances affirmed as generally recognized as safe*. Food and Drug Administration HHS, Washington DC.
- Coelho, J.P., Pereira, M.E., Duarte, A. and Pardal, M.A. (2005) Macroalgae response to a mercury contamination gradient in a temperate coastal lagoon (Ria de Aveiro, Portugal). *Estuarine Coastal and Shelf Science*, **65**, 492–500.
- Commission Directive 2005/87/EC of 5 December 2005 amending Annex I to Directive 2002/32/EC on undesirable substances in animal feed as regards lead, fluorine and cadmium. (2005) *Official Journal of the European Communities*, 6/12/2009, L318/19–318/24.
- Commission Directive 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners. (2008) *Official Journal of the European Communities*, 20/9/2008, L253/1–253/75.
- Commission Directive 2009/10/EC of 13 February 2009 amending Directive 2008/84/EC laying down specific purity criteria on food additives other than colours and sweeteners. (2009) *Official Journal of the European Communities*, 14/2/2009, L44/62–L44/78.
- Commission Directive 2009/141/EC of 23 November 2009 amending Annex I to Directive 2002/32/EC as regards

- maximum levels for arsenic, theobromine, *Datura* sp., *Ricinus communis* L., *Croton tiglium* L. and *Abrus precatorius* L. (2009) *Official Journal of the European Communities*, 24/11/2009, L308/20- L308/23.
- Commission Regulation (EC) 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. (2006) *Official Journal of the European Communities*, 20/12/2006, L364/5-L364/24.
- Commission Regulation (EC) 629/2008 of 2 July 2008 amending Regulation (EC) 1881/2006 setting maximum levels for certain contaminants in foodstuffs. (2008) *Official Journal of the European Communities*, 3/7/2008, L173/6-L173/9.
- Commission Regulation (EC) 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. (2008) *Official Journal of the European Communities*, 18/9/2008, L250/1-L250/84.
- Commission Regulation (EC) 710/2009 of 5 August 2009 amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production. (2009) *Official Journal of the European Communities*, 6/8/2008, L204/15-L204/34.
- Commission Regulation (EU) 242/2010 of 19 March 2010 creating the Catalogue of feed materials. (2010) *Official Journal of the European Communities*, 24/3/2010, L77/17-L77/32.
- Committee on Codex Specifications (1981) *Food Chemical Codex*, National Academy Press, Washington DC.
- Conti, M.E., Bocca, B., Iacobucci, M. *et al.* (2010) Baseline trace metals in seagrass, algae, and mollusks in a southern Tyrrhenian ecosystem (Linosa Island, Sicily). *Arch. Env. Contam. Toxicol.*, **58**, 79–95.
- Cooper, L.W., Beasley, T.M., Zhao, X.L. *et al.* (1998) Iodine-129 and plutonium isotopes in Arctic kelp as historical indicators of transport of nuclear fuel-reprocessing wastes from mid-to-high latitudes in the Atlantic Ocean. *Mar. Biol.*, **131**, 391–399.
- Council Regulation (EC) 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. (2007) *Official Journal of the European Communities*, 20/7/2007, L189/1-L189/23.
- Cundy, A.B., Croudace, I.W., Warwick, P.E. and Bains, M.E.D. (1999) Decline of radionuclides in the nearshore environment following nuclear reactor closure: a UK case study. *Env. Sci. Technol.*, **33**, 2841–2849.
- Dahlgaard, H., Chen, Q., Herrmann, J. *et al.* (1995) On the background levels of  $^{99}\text{Tc}$ ,  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$  in the North Atlantic. *J. Mar. Syst.*, **6**, 571–578.
- Dahlgaard, H., Eriksson, M., Nielsen, S.P. and Joensen, H.P. (2004) Levels and trends of radioactive contaminants in the Greenland environment. *Science of the Total Environment*, **331**, 53–67.
- Davis, T.A., Volesky, B. and Mucci, A. (2003) A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res.*, **37**, 4311–4330.
- Dawczynski, C., Schaefer, U., Leiterer, M. and Jahreis, G. (2007) Nutritional and toxicological importance of macro, trace, and ultra-trace elements in algae food products. *J. Agric. Food Chem.*, **55**, 10470–10475.
- Dhargalkar, V.K. and Pereira, N. (2005) Seaweed: promising plant of the millennium. *Sci. Culture*, March–April, 60–66.
- De Gregori, I., Quiroz, W., Pinochet, H., Pannier, F. and Potin-Gautier, M. (2007) Speciation analysis of antimony in marine biota by HPLC-(UV)-HG-AFS: Extraction procedures and stability of antimony species. *Talanta*, **73**, 458–465.
- Dembitsky, V.M. and Levitsky, D.O. (2004) Arsenolipids. *Progr. Lipid Res.*, **43**, 403–448.
- Dembitsky, V.M. and Rezanka, T. (2003) Natural occurrence of arseno compounds in plants, lichens, fungi, algal species, and microorganisms. *Plant Sci.*, **165**, 1177–1192.
- Directive 2002/32/EC of 7 May 2002 on undesirable substances in animal feed (2002). *Official Journal of the European Communities*, 30/5/2002, L140/10-L140/21.
- Domínguez-González, R., Moreda-Piñeiro, A., Bermejo-Barrera, A. and Bermejo-Barrera, P. (2005) Application of ultrasound-assisted acid leaching procedures for major and trace elements determination in edible seaweed by inductively coupled plasma-optical emission spectrometry. *Talanta*, **66**, 937–942.
- Domínguez-González, R., Romarís-Hortas, V., García-Sartal, C. *et al.* (2010) Evaluation of an *in vitro* method to estimate trace elements bioavailability in edible seaweeds. *Talanta*, **82**, 1668–1673.
- Edmonds, J.S. (2000) Diastereoisomers of an “arsenomethionine”-based structure from *Sargassum lacerifolium*: the formation of the arsenic-carbon bond in arsenic-containing natural products. *Med. Chem. Lett.*, **10**, 1105–1108.
- Edmonds, J.S., Morita, M. and Shibata, Y. (1987) Isolation and identification of arsenic-containing ribofuranosides and inorganic arsenic from Japanese edible seaweed. *Hizikia fusiforme*. *J. Chem. Soc., Perkin Trans.*, **1**, 577–580.

- Edmonds, J.S. and Morita, M. (1998) The determination of iodine species in environmental and biological samples. *Pure Appl. Chem.*, **70**, 1567–1584.
- Estrella-Gómez, N., Mendoza-Cóatl, D., Moreno-Sánchez, R. *et al.* (2009) The Pb-hyperaccumulator aquatic fern *Salvinia minima* Baker, responds to  $Pb^{2+}$  by increasing phytochelatin synthesis via changes in SmPCS expression and in phytochelatin synthase activity. *Aquat. Toxicol.*, **91**, 320–328.
- Fan, T.W.M., Lane, A.N. and Higashi, R.M. (1997) Selenium biotransformations by a euryhaline microalga isolated from a saline evaporation pond. *Env. Sci. Technol.*, **31**, 569–576.
- Fariás, S., Pérez-Arisnabarreta, S., Vodopivec, C. and Smichowski, P. (2002) Levels of essential and potentially toxic trace metals in Antarctic macroalgae. *Spectrochim. Acta Part B*, **57**, 2133–2140.
- Fernández-Fernández, A.M., Moreda-Piñeiro, A. and Bermejo-Barrera, P. (2007) On-line preconcentration cold vapour atomic absorption spectrometry for the determination of trace mercury in edible seaweeds. *J. Anal. Atomic Spectrom.*, **22**, 573–577.
- Ferrat, L., Pergent-Martini, C. and Roméo, M. (2003) Assessment of the use of biomarkers in aquatic plants for the evaluation of environmental quality: application to seagrasses. *Aquat. Toxicol.*, **65**, 187–204.
- Filella, M., Belzile, N. and Lett, M.C. (2007) Antimony in the environment: A review focused on natural waters. III. Microbiota relevant interactions. *Earth Sci. Rev.*, **80**, 195–217.
- Florence, T.M. (1989) Trace element speciation in biological systems. In: *Trace Element Speciation: Analytical Methods and Problems* (ed. G.E. Batley), CRC Press, Boca Raton, pp. 319–343.
- Foster, S., Maher, W., Krikowa, F. *et al.* (2005) Observations on the measurement of total antimony and antimony species in algae, plant and animal tissues. *J. Env. Monitor.*, **7**, 1214–1219.
- Foster, S., Thomson, D. and Maher, W. (2008) Uptake and metabolism of arsenate by anoxic cultures of the microalgae *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. *Mar. Chem.*, **108**, 172–183.
- Gallagher, P.A., Wei, X., Shoemaker, J.A. *et al.* (1999) Detection of arsenosugars from kelp extracts via IC-electrospray ionization-MS-MS and IC membrane hydride generation ICP-MS. *J. Anal. Atomic Spectrom.*, **14**, 1829–1834.
- Gallagher, P.A., Shoemaker, J.A., Wei, X. *et al.* (2001) Extraction and detection of arsenicals in seaweed via accelerated solvent extraction with ion chromatographic separation and ICP-MS detection. *Fresenius J. Anal. Chem.*, **369**, 71–80.
- Geng, W., Komine, R., Ohta, T. *et al.* (2009) Arsenic speciation in marine product samples: Comparison of extraction-HPLC method and digestion-cryogenic trap method. *Talanta*, **79**, 369–375.
- Goddard, C.C. and Jupp, B.P. (2001) The radionuclide content of seaweeds and seagrasses around the coast of Oman and the United Arab Emirates. *Mar. Poll. Bull.*, **42**, 1411–1416.
- Gómez-Jacinto, V., Arias-Borrego, A., García-Barrera, T. *et al.* (2010) Iodine speciation in Iodine-enriched microalgae *Chlorella vulgaris*. *Pure Appl. Chem.*, **82**, 473–481.
- Gong, Z., Lu, X., Ma, M. *et al.* (2002) Arsenic speciation analysis. *Talanta*, **58**, 77–96.
- Gorski, P.R., Armstrong, D.E., Hurley, J. P. and Shafer, M.M. (2006) Speciation of aqueous methylmercury influences uptake by a freshwater alga (*Selenastrum capricornutum*). *Env. Toxicol. Chem.*, **25**, 534–540.
- Gribble, G.W. (2003) The diversity of naturally produced organohalogenes. *Chemosphere*, **52**, 289–297.
- Grotti, M., Soggia, F., Lagomarsino, C. *et al.* (2008) Natural variability and distribution of trace elements in marine organisms from Antarctic coastal environments. *Antarctic Science*, **20**, 39–51.
- Gschwend, P.M., MacFarlane, J.K. and Newman, K.A. (1985) Volatile halogenated organic compounds released to seawater from temperate marine macroalgae. *Science*, **227**, 1033–1035.
- Han, C., Cao, X., Yu, J.J. *et al.* (2009) Arsenic speciation in *Sargassum fusiforme* by microwave-assisted extraction and LC-ICP-MS. *Chromatographia*, **69**, 587–591.
- Hintelmann, H., Falter, R., Ilgen, G. and Evans, R.D. (1997) Determination of artifactual formation of monomethylmercury ( $CH_3Hg^+$ ) in environmental samples using stable  $Hg^{2+}$  isotopes with ICP-MS detection: Calculation of contents applying species specific isotope addition. *Fresenius J. Anal. Chem.*, **358**, 363–370.
- Hirata, S. and Toshimitsu, H. (2005) Determination of arsenic species and arsenosugars in marine samples by HPLC-ICP-MS. *Anal. Bioanal. Chem.*, **383**, 454–460.
- Hou, X. and Yan, X. (1998) Study on the concentration and seasonal variation of inorganic elements in 35 species of marine algae. *Science of the Total Environment*, **222**, 141–156.
- Hou, X., Yan, X. and Chai, C. (2000) Chemical species of Iodine in some seaweeds. II. Iodine bound macromolecules. *J. Radioanal. Nucl. Chem.*, **245**, 461–467.
- Horvat, M. and Byrne, A.R. (1990) A modified method for the determination of methylmercury by gas chromatography. *Talanta*, **37**, 207–212.
- Hovart, M. and Gibičar, D. (2005) Speciation of mercury: Environment, food, clinical and occupational health.

- In: *Handbook of Elemental Speciation II. Species in the Environment, Food, Medicine and Occupational Health* (ed. R. Cornelis), John Wiley & Sons, Ltd, Chichester, pp. 281–304.
- Huang, R. and Hoffmann, T. (2009) Development of a coupled diffusion denuder system combined with gas chromatography/mass spectrometry for the separation and quantification of molecular Iodine and the activated Iodine compounds Iodine monochloride and hypiodous acid in the marine atmosphere. *Anal. Chem.*, **81**, 1777–1783.
- Ichikawa, S., Kamoshida, M., Hanaoka, K. *et al.* (2006) Decrease of arsenic in edible brown algae *Hizikia fusiforme* by the cooking process. *Appl. Organometall. Chem.*, **20**, 585–590.
- IFE, Institute for Energy Technology. *The Norwegian Fucus Project*. Available from: [http://www.ife.no/departments/health\\_and\\_safety/projects/tangprosjektet/view?set.language=en&cl=en](http://www.ife.no/departments/health_and_safety/projects/tangprosjektet/view?set.language=en&cl=en) (accessed 18 January 2011).
- Ishii, T. (1982) Tin in marine algae. *Bull. Jap. Soc. Sci. Fish.*, **48**, 1609–1615.
- Jayasekera, R. and Rossbach, M. (1996) Use of seaweeds for monitoring trace elements in coastal waters. *Env. Geochem. Health*, **18**, 63–68.
- Kalinowska, R. and Pawlik-Skowrońska, B. (2010) Response of two terrestrial green microalgae (Chlorophyta, Trebouxiophyceae) isolated from Cu-rich and unpolluted soils to copper stress. *Env. Poll.*, **158**, 2778–2785.
- Karadjova, I.B., Petrov, P.K., Serafimovski, I. *et al.* (2007) Arsenic in marine tissues - The challenging problems to electrothermal and hydride generation atomic absorption spectrometry. *Spectrochim. Acta, Part B*, **62**, 258–268.
- Kershaw, P.J., McCubbin, D. and Leonard, K.S. (1999) Continuing contamination of north Atlantic and Arctic waters by Sellafield radionuclides. *Science of the Total Environment*, **237/238**, 119–132.
- Kirby, J., Maher, W., Ellwood, M. and Krikowa, F. (2004) Arsenic species determination in biological tissues by HPLC–ICP–MS and HPLC–HG–ICP–MS. *Aust. J. Chem.*, **57**, 957–966.
- Koch, I., McPherson, K., Smith, P. *et al.* (2007) Arsenic bioaccessibility and speciation in clams and seaweed from a contaminated marine environment. *Mar. Poll. Bull.*, **54**, 586–594.
- Ladra-Ramos, N., Domínguez-González, R., Moreda-Piñero, A. *et al.* (2005) Determination of major and trace elements in edible seaweeds by AAS after ultrasound-assisted acid leaching. *Atom. Spectrosc.*, **26**, 59–67.
- Lai, V.W.M., Cullen, W.R., Harrington, C.F. and Reimer, K.J. (1997) The characterization of arsenosugars in commercially available algal products including a Nostoc species of terrestrial origin. *Appl. Organometall. Chem.*, **11**, 797–803.
- Laparra, J.M., Vélez, D., Montoro, R. *et al.* (2004) Bioaccessibility of inorganic arsenic species in raw and cooked *Hizikia fusiforme* seaweed. *Appl. Organometall. Chem.*, **18**, 662–669.
- Larsen, E.H., Hansen, M., Fan, T. and Vahl, M. (2001) Speciation of selenoamino acids, selenonium ions and inorganic selenium by ion exchange HPLC with mass spectrometric detection and its application to yeast and algae. *J. Anal. Atom. Spectrom.*, **16**, 1403–1408.
- Leblanc, C., Colin, C., Cosse *et al.* (2006) Iodine transfers in the coastal marine environment: The key role of brown algae and of their vanadium-dependent haloperoxidases. *Biochimie*, **88**, 1773–1785.
- Li, S.X., Zheng, F.Y., Hong, H.S. *et al.* (2006) Photo-oxidation of Sb(III) in the seawater by marine phytoplankton-transition metals-light system. *Chemosphere*, **65**, 1432–1439.
- Liang, L., Horvat, M. and Bloom, N.S. (1994) An improved speciation method for mercury by GC/CVAFS after aqueous phase ethylation and room temperature precollection. *Talanta*, **41**, 371–379.
- Maher, W.A. (1986) Trace metal concentrations in marine organisms from St. Vincent Gulf, South Australia. *Water, Air, Soil Poll.*, **29**, 77–84.
- Malea, P. (1994) Seasonal and local distribution of metals in the seagrass *Halophila stipulacea* (Forsk.) Aschers. in the Antikyra Gulf, Greece. *Env. Poll.*, **85**, 77–85.
- Malea, P., Haritonidis, S. and Kevrekidis, T. (1995) Metal content of some green and brown seaweeds from Antikyra Gulf (Greece). *Hydrobiologia*, **310**, 19–31.
- Manjón, G., Garcia-Leon, M., Ballestra, S. and López, J.J. (1995) The presence of man-made radionuclides in the marine environment in the south of Spain. *J. Env. Radioactivity*, **28**, 171–89.
- Matishov, G.G., Matishov, D.G., Namjatov, A.A. *et al.* (1999) Discharges of nuclear waste into the Kola Bay and its impact on human radiological doses. *J. Env. Radioactivity*, **48**, 5–21.
- McConnell, O. and Fenical, W. (1977) Halogen chemistry of the red alga *Asparagopsis*. *Phytochemistry*, **16**, 367–374.
- McConnell, O.J. and Fenical, W. (1980) Halogen chemistry of the red alga *Bonnemaisonia*. *Phytochemistry*, **19**, 233–249.
- McDermid, K.J. and Stuercke, B. (2003) Nutritional composition of edible Hawaiian seaweeds. *J. Appl. Phycol.*, **15**, 513–524.
- McSheehy, S. and Szpunar, J. (2000) Speciation of arsenic in edible algae by bi-dimensional size-exclusion anion exchange HPLC with dual ICP-MS and electrospray MS/MS detection. *J. Anal. Atom. Spectrom.*, **15**, 79–87.

- McSheehy, S., Pohl, P., Vélez, D. and Szpunar, J. (2002) Multidimensional liquid chromatography with parallel ICP MS and electrospray MS/MS detection as a tool for the characterization of arsenic species in algae. *Anal. Bioanal. Chem.*, **372**, 457–466.
- Mendoza-Cózatl, D., Losa-Tavera, H., Hernández-Navarro, A. and Moreno-Sánchez, R. (2005) Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. *FEMS Microbiol. Rev.*, **29**, 653–671.
- Metze, D., Jakubowski, N. and Klockow, D. (2005) Speciation of chromium in environment and food. In: *Handbook of Elemental Speciation II. Species in the Environment, Food, Medicine and Occupational Health* (ed. R. Cornelis). John Wiley & Sons Ltd, Chichester, pp. 120–135.
- Michalke, B. (2003) Element speciation definitions, analytical methodology, and some examples. *Ecotoxicol. Env. Safety*, **56**, 122–139.
- Miguens-Rodríguez, M., Pickford, R., Thomas-Oates, J.E. and Pergantis, S.A. (2002) Arsenosugars identification in seaweed extracts using high performance liquid chromatography/electrospray ion trap mass spectrometry. *Rapid Commun. Mass Spectrom.*, **16**, 323–331.
- Misheer, N., Kindness, A. and Jonnalagadda, S.B. (2006a) Elemental uptake by seaweed, *Plocamium corallorhiza* along the KwaZulu-Natal coast of Indian Ocean, South Africa. *J. Env. Sci. Health, Part B*, **41**, 1037–1048.
- Misheer, N., Kindness, A. and Jonnalagadda, S.B. (2006b) Elemental distribution in seaweed, *Gelidium abbottiorum* along the KwaZulu-Natal coastline, South Africa. *J. Env. Sci. Health, Part A*, **41**, 1639–1653.
- Misheer, N., Kindness, A. and Jonnalagadda, S.B. (2006c) Seaweeds along KwaZulu-Natal coast of South Africa - 4: elemental uptake by edible seaweed *Caulerpa racemosa* (sea grapes) and the arsenic speciation. *J. Env. Sci. Health, Part A*, **41**, 1217–1233.
- Misheer, N., Kindness, A. and Jonnalagadda, S.B. (2006d) Seaweeds along KwaZulu-Natal coast of South Africa-3: elemental uptake by *Ulva lactuca* (sea lettuce). *J. Env. Sci. Health, Part A*, **41**, 1249–1259.
- Moreda-Piñeiro, J., Alonso-Rodríguez, E., Lopez-Mahía, P. et al. (2007) Development of a new sample pre-treatment procedure based on pressurized liquid extraction for the determination of metals in edible seaweed. *Anal. Chim. Acta*, **598**, 95–102.
- Mitchell, N.T. (1969) *Radioactivity in surface and coastal waters of the British Isles*. Ministry of Agriculture, Fisheries and Food Fisheries Radiobiological Laboratory, Suffolk (UK).
- Morita, T., Fujimoto, K., Kasai, H. et al. (2010a) Temporal variations of  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$  concentrations and the  $^{137}\text{Cs}/^{90}\text{Sr}$  activity ratio in marine brown algae, *Undaria pinnatifida* and *Laminaria longissima*, collected in coastal areas of Japan. *J. Env. Monitor.*, **12**, 1179–1186.
- Morita, T., Niwa, K., Fujimoto, K. et al. (2010b) Detection and activity of Iodine-131 in brown algae collected in the Japanese coastal areas. *Science of the Total Environment*, **408**, 3443–3447.
- Muse, J.O., Tudino, M.B., d'Huicque, L. et al. (1995) A survey of some trace elements in seaweeds from Patagonia, Argentina. *Env. Poll.*, **87**, 249–53.
- Muse, J.O., Stripeikis, J.D., Fernández, F.M. et al. (1999) Seaweeds in the assessment of heavy metal pollution in the Gulf San Jorge, Argentina. *Env. Poll.*, **104**, 315–322.
- Nesterenko, V.B. and Nesterenko, A.V. (2009) Decorporation of Chernobyl radionuclides. *Ann. NY Acad. Sci.*, **1181** (Chernobyl), 303–310.
- Niegel, C. and Matysik, F.M. (2010) Analytical methods for the determination of arsenosugars – A review of recent trends and developments. *Anal. Chim. Acta*, **657**, 83–99.
- Nischwitz, V. and Pergantis, S.A. (2006) Improved arsenic speciation analysis for extracts of commercially available edible marine algae using HPLC-ES-MS/MS. *J. Agric. Food Chem.*, **54**, 6507–6519.
- Olivares, M., Pizarro, F., de Pablo, S. et al. (2004) Iron, zinc and copper: contents in common Chilean foods and daily intakes in Santiago, Chile. *Nutrition*, **20**: 205–212.
- Ospar Commission (2010) *Quality Status Report 2010*. Oskar Commission, London.
- Ostapczuk, P., Burow, M., May, K. et al. (1997) Mussels and algae as bioindicators for long-term tendencies of element pollution in marine ecosystems. *Chemosphere*, **34**, 2049–2058.
- Pawlik-Skowrońska, B., Pirszel, J. and Brown, M.T. (2007) Concentrations of phytochelators and glutathione found in natural assemblages of seaweeds depend on species and metal concentrations of the habitat. *Aquatic Toxicol.*, **83**, 190–199.
- Peña-Farfal, C., Moreda-Piñeiro, A., Bermejo-Barrera, A. et al. (2005) Speeding up enzymatic hydrolysis procedures for the multi-element determination in edible seaweed. *Anal. Chim. Acta*, **548**, 183–191.
- Perales-Vela, H.V., Peña-Castro, J.N. and Cañizares-Villanueva, R.O. (2006) Heavy metal detoxification in eukaryotic microalgae. *Chemosphere*, **64**, 1–10.
- Pergantis, S.A., Wangkarn, S., Francesconi, K.A. and Thomas-Oates, J.E. (2000) Identification of arsenosugars at the picogram level using nanoelectrospray quadrupole time-of-flight mass spectrometry. *Anal. Chem.*, **72**, 357–366.
- Pickford, R., Miguens-Rodríguez, M., Afzaal, S. et al. (2002) Application of the high mass accuracy capabilities of FT-ICP-MS and Q-ToF-MS to the characterisation of

- arsenic compounds in complex biological matrices. *J. Anal. Atom. Spectrom.*, **17**, 173–176.
- Philpott, J. and Bradford, M. (2006) Seaweed: Nature's secret for a long and healthy life?. *The Nutrition Practitioner*, Winter, 1–21.
- Prohaska, T. and Stingeder, G. (2005) Arsenic and arsenic species in environments and human nutrition. In: *Handbook of Elemental Speciation II. Species in the Environment, Food, Medicine and Occupational Health* (ed. R. Cornelis). John Wiley & Sons Ltd, Chichester, pp. 69–85.
- Rao, P.V.S., Mantri, V.A. and Ganesan, K. (2007) Mineral composition of edible seaweed *Porphyra vietnamensis*. *Food Chem.*, **102**, 215–218.
- Rausser, W.E. (1995) Phytochelatin and related peptides. *Plant Physiol.*, **109**, 1141–1149.
- Řezanka, T. and Sigler, K. (2008) Biologically active compounds of semi-metals. *Phytochemistry*, **69**, 585–606.
- Riget, F., Johansen, P. and Asmud, G. (1997) Baseline levels and natural variability of elements in three seaweed species from West Greenland. *Mar. Poll. Bull.*, **34**, 171–176.
- Ródenas-de-la-Rocha, S., Sanchez-Muñiz, F.J., Gomez-Juaristi, M. and Marín, M.T.L. (2009) Trace elements determination in edible seaweeds by an optimized and validated ICP-MS method. *J. Food Comp. Anal.*, **22**, 330–336.
- Rodríguez-Figueroa, G.M., Shumilin, E. and Sánchez-Rodríguez, I. (2009) Heavy metal pollution monitoring using the brown seaweed *Padina durvillaei* in the coastal zone of the Santa Rosalía mining region, Baja California Peninsula, Mexico. *J. Appl. Phycol.*, **21**, 19–26.
- Romaris-Hortas, V., García-Sartal, C., Barciela-Alonso, M.C., Moreda-Piñeiro, A. and Bermejo-Barrera, P. (2010) Characterization of edible seaweed harvested on the Galician Coast (Northwestern Spain) using pattern recognition techniques and major and trace element data. *J. Agric. Food Chem.*, **58**, 1986–1992.
- Rosenberg, E. (2003) The potential of organic (electrospray and atmospheric pressure chemical ionisation) mass spectrometric techniques coupled to liquid-phase separation for speciation analysis. *J. Chromatogr. A*, **1000**, 841–889.
- Rosenberg, E. (2005) Speciation of tin. In: *Handbook of Elemental Speciation II. Species in the Environment, Food, Medicine and Occupational Health* (ed. R. Cornelis). John Wiley & Sons Ltd, Chichester, pp. 422–463.
- Rubio, R., Ruiz-Chancho, M.J. and López-Sánchez, J.F. (2010) Sample pre-treatment and extraction methods that are crucial to arsenic speciation in algae and aquatic plants. *Trends Anal. Chem.*, **29**, 53–69.
- Rupérez, P. (2002) Mineral content of edible marine seaweeds. *Food Chem.*, **79**, 23–26.
- Ryan, T.P., Dowdall, A.M., Long, S. *et al.* (1999) Plutonium and americium in fish, shellfish and seaweed in the Irish environment and their contribution to dose. *J. Env. Radioactivity*, **44**, 349–369.
- Saenko, G.N., Koryakova, M.D., Makienko, V.F. and Dobrosmylova, I.G. (1976) Concentration of polyvalent metals by seaweeds in Vostok Bay, Sea of Japan. *Mar. Biol.*, **34**, 169–76.
- Sakao, S., Ogawa, Y. and Uchida, H. (1997) Determination of trace elements in sea weed samples by inductively coupled plasma mass spectrometry. *Anal. Chim. Acta*, **355**, 121–127.
- Santoso, J., Gunji, S., Yoshie-Stark, Y. and Suzuki, T. (2006) Mineral contents of Indonesian seaweeds and mineral solubility affected by basic cooking. *Food Sci. Technol. Res.*, **12**, 59–66.
- Serfor-Armah, Y., Nyarko, B.J.B., Osa, E.K. *et al.* (2001) Rhodophyta seaweed species as bioindicators for monitoring toxic element pollutants in the marine ecosystem of Ghana. *Water, Air, Soil Poll.* **127**: 243–253.
- Shah, M., Wuilloud, R.G., Kannamkumarath, S.S. and Caruso, J.A. (2005) Iodine speciation studies in commercially available seaweed by coupling different chromatographic techniques with UV and ICP-MS detection. *J. Anal. Atom. Spectrom.*, **20**, 176–182.
- Sharp, G.J., Samant, H.S. and Vaidya, O.C. (1988) Selected metal levels of commercially valuable seaweeds adjacent to and distant from point sources of contamination in Nova Scotia and New Brunswick. *Bull. Env. Contam. Toxicol.*, **40**, 724–30.
- Shimoda, Y., Suzuki, Y., Endo, Y. *et al.* (2010) Speciation analysis of arsenics in commercial Hijiki by high performance liquid chromatography-tandem-mass spectrometry and high performance liquid chromatography-inductively coupled plasma mass spectrometry. *J. Health Sci.*, **56**, 47–56.
- Shiraishi, K. (2005) Dietary intakes of eighteen elements and  $^{40}\text{K}$  in eighteen food categories by Japanese subjects. *J. Radioanal. Nucl. Chem.*, **266**, 61–69.
- Shiraishi, K. (2006) Dietary intakes of eighteen elements and  $^{40}\text{K}$  in eighteen food categories by Japanese subjects. [Erratum to document cited in CA143:477119]. *J. Radioanal. Nucl. Chem.*, **267**, 261–264.
- Smith, C.N., Clarke, S., McDonald, P. *et al.* (2000) Reconstructing historical radionuclide concentrations along the east coast of Ireland using a compartmental model. *Science of the Total Environment*, **254**, 17–30.
- Struck, B.D., Pelzer, R., Ostapczuk, P., Emons, H. and Mohl, C. (1997) Statistical evaluation of ecosystem properties influencing the uptake of As, Cd, Co, Cu, Mercury, Mn, Ni, Pb and Zn in seaweed (*Fucus vesiculosus*) and

- common mussel (*Mytilus edulis*). *Science of the Total Environment*, **207**, 29–42.
- Subba-Rao, P.V., Mantri, V.A. and Ganesan, K. (2007) Mineral composition of *Porphyra vietnamensis*. *Food Chem.*, **102**, 215–218.
- Sugiyama, H., Terada, H., Takahashi, M. *et al.* (2007) Contents and daily intakes of gamma-ray emitting nuclides,  $^{90}\text{Sr}$ , and  $^{238}\text{U}$  using market-basket studies in Japan. *J. Health. Sci.*, **53**, 107–118.
- Suriyanarayanan, S., Brahmanandhan, G.M., Malathi, J. *et al.* (2008). Studies on the distribution of  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  in the ecosystem of Point Calimere Coast (Palk Strait), India. *J. Env. Radioactivity*, **99**, 766–771.
- Taboada, C., Millán, R. and Míguez, I. (2010) Composition, nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. *J. Sci. Food Agric.*, **90**, 445–449.
- Templeton, D.M., Ariese, F., Cornelis, R., *et al.* (2000) Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches (IUPAC Recommendations 2000). *Pure Appl. Chem.*, **72**, 1453–1470.
- Thomson, D., Maher, W. and Foster, S. (2007a) Arsenic and selected elements in marine angiosperms, south-east coast, NSW, Australia. *Appl. Organometall. Chem.*, **21**, 381–395.
- Thomson, D., Maher, W. and Foster, S. (2007b) Arsenic and selected elements in inter-tidal and estuarine marine algae, south-east coast, NSW, Australia. *Appl. Organometall. Chem.*, **21**, 396–411.
- Torres, M.A., Barros, M.P., Campos, S.C.G. *et al.* (2008) Biochemical biomarkers in algae and marine pollution: A review. *Ecotoxicol. Env. Safety*, **71**, 1–15.
- Truus, K., Vaher, M., Koel, M. *et al.* (2004) Analysis of bioactive ingredients in the brown alga *Fucus vesiculosus* by capillary electrophoresis and neutron activation analysis. *Anal. Bioanal. Chem.*, **379**, 849–852.
- Tuzen, M., Verep, B., Ogretmen, A.O. and Soylak, M. (2009) Trace element content in marine algae species from the Black Sea, Turkey. *Env. Monitor. Assess.*, **151**, 363–368.
- Ullrich, S.M., Tanton, T.W. and Abrashitova, S.A. (2001) Mercury in the aquatic environment: a review of factors affecting methylation. *Crit. Rev. Env. Sci. Technol.*, **31**, 241–293.
- Vacchina, V., Połec, K. and Szpunar, J. (1999) Speciation of cadmium in plant tissues by size-exclusion chromatography with ICP-MS detection. *J. Anal. Atom. Spectrom.*, **14**, 1557–1566.
- Vacchina, V., Łobinski, R., Oven, M. and Zenk, M.H. (2000) Signal identification in size-exclusion HPLC-ICP-MS chromatograms of plant extracts by electrospray tandem mass spectrometry (ES MS/MS). *J. Anal. Atom. Spectrom.*, **15**, 529–534.
- Van Hulle, M., Zhang, C., Zhang, X. and Cornelis, R. (2002) Arsenic speciation in Chinese seaweeds using HPLC-ICP-MS and HPLC-ES-MS. *Analyst*, **127**, 634–640.
- Van Netten, C., Hoption, C.S.A., Morley, D.R. and Van, N.J.P. (2000) Elemental and radioactive analysis of commercially available seaweed. *The Science of the Total Environment*, **255**, 169–75.
- Wallberg, P. and Moberg, L. (2002) Evaluation of 20 years of environmental monitoring data around Swedish nuclear installations. *J. Env. Radioactivity*, **63**, 117–133.
- Wang, K.E. and Jiang, S.J. (2008) Determination of iodine and bromine compounds by ion chromatography/dynamic reaction cell inductively coupled plasma mass spectrometry. *Anal. Sci.*, **24**, 509–514.
- Wangkarn, S. and Pergantis, S.A. (2000) High-speed separation of arsenic compounds using narrow-bore high-performance liquid chromatography on-line with inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.*, **15**, 627–633.
- WHO Report (1996) *Trace elements in human nutrition and health*, World Health Organization, Geneva.
- WHO Report (2004) *Vitamin and mineral requirements in human nutrition*, World Health Organization and Food and Agriculture Organization of the United Nations, Geneva.
- Wuilloud, R.G., Altamirano, J.C., Smichowskic, P.N. and Heitkemper, T.D. (2006) Investigation of arsenic speciation in algae of the Antarctic region by HPLC-ICP-MS and HPLC-ESI-Ion Trap MS. *Journal of Analytical Atomic Spectrometry*, **21**, 1214–1223.
- Yamada, M., Yamamoto, K., Ushihara, Y. and Kawai, H. (2007) Variation in metal concentrations in the brown alga *Undaria pinnatifida* in Osaka Bay, Japan. *Phycol. Res.*, **55**, 222–230.
- Zemke-White, W.L. and Ono, M. (1999) World seaweed utilization: An end-of-century summary. *J. Appl. Phycol.*, **11**, 369–376.
- Zenk, M.H. (1996). Heavy metal detoxification in higher plants - a review. *Gene*, **179**, 21–30.
- Žižek, S., Horvat, M., Gibičar, D. *et al.* (2007) Bioaccumulation of mercury in benthic communities of a river ecosystem affected by mercury mining. *Science of Total Environment*, **377**, 407–415.

# **PART II**

**Isolation and Chemical Properties of  
Molecules Derived from Seaweeds**

# 7

## Chemical Composition of Seaweeds

**Ladislava Mišurcová**

*Tomas Bata University in Zlín, Faculty of Technology, Department of Food Technology and Microbiology, Czech Republic*

### 7.1 Introduction

Algae form a diverse group of organisms because of their morphotypes, cellular structure, and their proportion ranging from several centimeters to tens of meters. Seaweed is named as macro algae because of their greater abundance in seas and oceans. These are represented as an extensive group of macroscopic organisms which are classified generally into red, brown, and green seaweed depending on the nature of pigments present in these algae. General classification according to seaweed color is currently used in scientific quarters. According to the systematic classification, seaweed belongs to three phyla: Rhodophyta, Heterocontophyta, and Chlorophyta.

Due to their high nutritional value, seaweeds are traditionally used for various purposes such as food worldwide, and especially in Asian countries (Mabeau and Fleurence, 1993; McHugh, 2003; Norziah and Ching, 2000). World seaweed production was reported to be 15.1 million tonnes worth of 7.2 milliard US\$ in 2006 (FAO, 2006). Nowadays, the utilization of seaweed as food, particularly as spice and delicacies is extended in western countries due to the change of the life style and dietary conventions. The objective of scientific interest is edible seaweed as suitable ingredients in food products (e.g., brown seaweed *Undaria pinnatifida* is used as an ingredient in pasta; Prabhasankar *et al.*, 2009). Because of high content of bioactive compounds, they have been used for the production of pharmaceutical and medicinal products (MacArtain *et al.*, 2007).

Nevertheless, economical significance of seaweed lies in their utilization as raw material for the production of hydrocolloids – agar, carrageenan from red seaweed and alginates from brown seaweed, which are used in food and cosmetics industry and also in medicine and pharmacy. Genera of agar-containing red seaweed *Gelidium* sp. and *Gracilaria*; genera of carrageenan-containing red seaweed *Chondrus crispus*, *Gigartina*, *Iridaea*, *Kappaphycus alvarezii* and *Eucheuma denticulatum* are used most widely (McHugh, 2003). Except from the best-known species, which are mentioned above, lesser-known species are in the scientific objective for the purpose of hydrocolloids production such as green algae *Cladophora* spp. (Mihrianyan *et al.*, 2007).

Earlier, seaweed utilization as animal feed was localized also in coastal European countries such as Ireland, Iceland, Norway, France, Spain, and Portugal (McHugh, 2003; Kumar and Kaladharan, 2007).

Seaweed extracts have been used in agriculture as plant growth supplements because of their cytokinin content (Stirk and Staden, 1997). Cytokinin is known as an important factor of the plant growth and development and promotes cell division and stimulates shoot proliferation (Werner *et al.*, 2001). *Ascophyllum nodosum*, *Laminaria* spp. and *Fucus serratus* are used as fertilizers in Europe (Reitz and Trumble, 1996a, b; McHugh, 2003).

The chemical composition of seaweed provides their high nutritional value contributing to human nutrients – such as proteins with all essential amino acids, minerals and

vitamins. In addition, they consist of bioactive secondary metabolites and many different compounds with health benefits (Cardozo *et al.*, 2007; Alves de Sousa *et al.*, 2007; Wong *et al.*, 2000; Cho *et al.*, 2009; Artan *et al.*, 2008; Choi *et al.*, 2009; Zvyagintseva *et al.*, 2005). Seaweeds possess small amounts of lipids and these are mostly polyunsaturated  $\omega$ -3 and  $\omega$ -6 fatty acids. Seaweed has a high dietary fiber content, which does not belong among the nutritional factors but has protective effects on human health.

However, some species of seaweed may contain native toxic compounds which can limit the utilization of this seaweed as food or animal feed, for example, *Caulerpa saxifolia* contains sesquiterpene caulerpyne that blocks the mitotic cycle of sea urchin embryos and also inhibits stimulation of mitogen-activated protein kinase. Further, caulerpyne shows growth-inhibitory effects in some human cancer cells and its influence on the activity of nerve cells was also observed (Mozzachiodi *et al.*, 2001; Valls *et al.*, 1994).

The chemical composition of seaweed varies, which is dependent on the type of species, the time of collection, geographic habitat, and on many external conditions such as water temperature, light intensity and nutrient concentration in water (Mabeau and Fleurence, 1993; Marinho-Soriano *et al.*, 2006; Marsham *et al.*, 2007).

There are a number of research articles evaluating the changeable composition of seaweed. They show great differences of concentration of nutritional factors such as proteins, minerals, lipids, or dietary fiber determined within diverse genera of seaweed (Mabeau and Fleurence, 1993; Marinho-Soriano *et al.*, 2006; Marsham *et al.*, 2007; Vasconcelos and Leal, 2001; Villares *et al.*, 2002; Diéz *et al.*, 2003; Manivannan *et al.*, 2008; Hou and Yan, 1998; Sawidis *et al.*, 2001; De Oliveira *et al.*, 2009). It is a common fact that in the same genus of seaweed there are huge distinctions in their composition (Riget *et al.*, 1995; Lares *et al.*, 2002; Martínez and Rico, 2002; Dawczynski *et al.*, 2007; Mišurcová *et al.*, 2010).

## 7.2 Various components of seaweeds

### 7.2.1 Proteins and amino acids

Proteins are important and essential factors establishing the nutritional value of food. Their biological values are based on the adequate amounts of essential amino acids. Seaweeds are known to possess significant amounts of these nitrogenous compounds. Further, they contain a small amount of non-protein nitrogen, which is the source for some compounds such as free amino acids, chlorophyll, nitrate and nitrite nitrogen, ammonium ions, and nucleic acids

(Lourenço *et al.*, 2002). The effort to utilize seaweed as a source of proteins has lasted for several decades because protein deficiency, especially in the countries of the developing world. Nowadays, seaweeds are becoming a cheaper alternative source of proteins.

Protein concentration in seaweeds varies according to the factors mentioned above such as different species, environmental conditions and it also depends on the applied method of protein determination (Fleurence, 1999; Lourenço *et al.*, 2002; Fountoulakis and Lahm, 1998). A seasonal influence on the nitrogen content in red seaweed *Palmaria palmata* from the French Atlantic coast was reported by Galland-Irmouli *et al.* (1999) and Fleurence (1999). The highest nitrogen content was observed in winter and early spring months from February to May and also in November, while the lowest value was determined in summer and autumn from July to October. Similar conclusions of changeable nitrogen content of different seaweed species were reported also by other authors (Denis *et al.*, 2010; Fleurence *et al.*, 1999).

The protein value also differs according to the value of the nitrogen-to-protein conversion factor used. In general, protein content in seaweed is calculated by multiplying seaweed nitrogen value, which is determined by the Kjeldahl method, by a nitrogen-to-protein conversion factor. However, not only protein nitrogen but also non-protein nitrogen was determined by Kjeldahl method. The conventional value of the conversion factor is 6.25 for animal (and also some plant proteins) and is established on the assumption that protein contains 16% of nitrogen. Nevertheless, this requirement is not complete in some seaweed because of the presence of different non-protein nitrogen content. Consequently, protein content results might be overestimated using the factor 6.25 for seaweed containing higher amount of other nitrogen compounds such as nucleic acids, free amino acids, non-protein amino acids, amines, amides, nitrates, nitrites, vitamins, phospholipids, and another non-protein nitrogen compounds. On the other hand, protein content results can be underestimated in seaweed that contains smaller amounts of non-protein nitrogen compounds. The true value of nitrogen-to-protein conversion factor should be determined for each seaweed genus from the total nitrogen content based on amino acid composition and the distribution of nitrogen in protein and in other non-protein nitrogen compounds (Fujihara *et al.*, 2001; Ezeagu *et al.*, 2002; Lourenço *et al.*, 1998; Salo-Väänänen and Koivistoinen, 1996). In different genera of green, brown, and red seaweed the values of nitrogen-to-protein conversion factor have been provided. The average value of the nitrogen-to-protein conversion factor is 5.13 for green algae, 5.38 for brown algae and 4.92 for red algae (Lourenço *et al.*, 2002).

Due to these facts, the comparison of protein contents in different genera of seaweeds is difficult. In spite of this fact, in most studies, a nitrogen-to-protein conversion factor of 6.25 has been applied. The accuracy of these analyses has been justified by the fact that possible overestimation of nitrogen content is corrected by amino acid losses during the analytical process (Darragh, *et al.*, 1996). The same presumption has been accepted in the case of free amino acids (Lourenço *et al.*, 2002).

Generally it has been reported that red and green seaweeds have relatively high protein concentration and the average reaches 10–30% dry matter (Mabeau and Fleurence, 1993; Burtin, 2003; Ramos *et al.*, 2000). In red seaweed *Palmaria palmata* and *Porphyra tenera*, which are known under the commercial names Dulse and Nori, proteins range from 35 to 47% dry matter (Mabeau and Fleurence, 1993; Burtin, 2003; Marsham *et al.*, 2007) and form a large part of their chemical composition. On the other hand, in brown seaweed, the protein content commonly low between 5 and 15% (Burtin, 2003; Dawczynski *et al.*, 2007).

Table 7.1 presents the characteristics of some seaweed genera demonstrating variability of seaweed chemical composition such as crude protein, total amino acids, and a percentage part of the acidic and essential amino acids in

relation to total amino acids (Denis *et al.*, 2010; Galland-Irmouli *et al.*, 1999; Kolb *et al.*, 2004; Marsham *et al.*, 2007; Mišurcová 2008; Mišurcová *et al.*, 2010; Wong and Cheung, 2001). In contrast to generally reported data, lower values of protein were provided in red seaweed *Palmaria palmata* 18.3% by Galland-Irmouli *et al.* (1999); further 26.5% and 18.7% in *Palmaria palmata* and *Porphyra tenera*, respectively by Mišurcová *et al.* (2010). In agreement with published general information, in green seaweed *Ulva lactuca*, 29% of protein was determined (Marsham *et al.*, 2007), which is contrary to the data gained by Wong and Cheung (2001) who established 7.1% of protein in the same green seaweed. Protein content in brown seaweed has a similar changeable pattern. The most often determined protein content in brown seaweed occurs in declared interval of 5 to 15%, apart from *Hypnea charoides* and *H. japonica* with 18.1 and 19.4%, respectively (Wong and Cheung, 2001). Further, in *Fucus serratus* there was high protein content of 44% (Marsham *et al.*, 2007) and in *Undaria pinatifida* it was 21.3% (Mišurcová *et al.*, (2010). Values of protein are changeable depending on different seasons, as published by Denis *et al.* (2010).

Protein quality is based on bioavailability and composition of protein amino acids (Friedman, 1996; Satterlee,

**Table 7.1** Crude protein and amino acids contents in dry matter of seaweed

Author	Seaweed		CP	AA total	AA-acidic	EAA	AA limit
			%	%	%	%	
Galland-Irmouli <i>et al.</i> (1999)	<i>Palmaria palmata</i>	R	18.3		28.5	35.8	Met
Wong and Cheung (2001)	<i>Hypnea charoides</i>	B	18.1	78.7	29.0	42.7	Met
	<i>Hypnea japonica</i>	B	19.4	78.8	36.2	47.1	Met
	<i>Ulva lactuca</i>	G	7.1	73.9	33.7	52.9	
Kolb <i>et al.</i> (2004)	<i>Laminaria digitata japonica</i>	B	6.2			50.7	Trp
	<i>Undaria pinatifida</i>	B	16.3			47.1	Trp
Marsham <i>et al.</i> (2007)	<i>Porphyra</i> sp.	R	15.9				
	<i>Laminaria digitata</i>	B	17.4				
	<i>Fucus serratus</i>	B	44.0				
	<i>Ulva lactuca</i>	G	29.0				
Mišurcová (2008); Mišurcová <i>et al.</i> (2010)	<i>Palmaria palmata</i>	R	26.5	85.5	23.4	35.6	Leu
	<i>Porphyra tenera</i>	R	18.7	87.9	23.6	36.2	Lys
	<i>Hizikia fusiformis</i>	B	7.8	89.3	27.1	39.6	Lys
	<i>Laminaria japonica</i>	B	12.0	73.6	32.4	32.4	Lys
	<i>Undaria pinatifida</i>	B	21.3	70.6	22.5	41.7	Trp
Denis <i>et al.</i> (2010)	<i>Grateloupia turuturu</i>	R	14.1–27.5				

AA, acidic and EAA are expressed as % of total AA.

B, brown seaweed; R, red seaweed; G, green seaweed.

Marshall and Tennyson, 1979; Wolzak *et al.*, 1981; Levesque *et al.*, 2010). Important factors such as amino acid score (AAS) and index of essential amino acid (IEAA), are used for establishing food protein quality. The limiting amino acid of food protein is evaluated according to the amino acid score of each essential amino acid in comparison to the same essential amino acid of high-quality protein, which is a protein with high nutritional value. Reference or standard proteins according to WHO (2002) or egg proteins are considered as high-quality proteins. Thus, a limiting amino acid is indicated as an amino acid in food protein showing the greatest concentration difference in contrast to the same amino acid in high-quality protein. Further, if the food protein contains all essential amino acids in available amounts then it is indicated as a full-value protein.

In general, seaweed proteins, as well as plant proteins, contain all the essential amino acids but they are not full-value proteins, as these amino acids are contained in low amounts in comparison to standard protein or egg protein. Tryptophan (Dawczynski *et al.*, 2007), lysine (Ramos *et al.*, 2000; Mišurcová, 2008) and methionine (Ramos *et al.*, 2000) are the main limiting amino acids of seaweed.

Whereas proteins gained from red seaweed *Palmaria palmata* (Mišurcová, 2008) and brown seaweed *Undaria pinnatifida* approximated the EAAI value of standard protein by their EAAI values of 103.7 and 95.9, respectively (Dawczynski *et al.*, 2007), which enables them to be considered as high-value proteins. Nevertheless, amino acid composition varied a lot not only within different species but also among seaweed of the same species, thus indicating the data is not useful for general evaluation. The assessment of true seaweed protein quality should be performed only in terms of actual results of amino acid composition of each species of seaweed.

Even though some essential amino acids in seaweed proteins are in low concentration, these seaweeds can be added to some cereal food products such as pasta, to improve amino acid composition (Prabhasankar *et al.*, 2009).

Except from their main function as structural units of protein, amino acids also have important roles in many metabolic pathways (Fürst, 2009); for example, tryptophan is a precursor of the vitamin niacin, cysteine is a component of bile acids, and tyrosine takes part in the synthesis of the hormone thyroxine.

As far as utilization of seaweed as food is concerned, their taste is very important and it is created by the presence of free amino acids. Three amino acids, (alanine, glutamic acid, and glycine) are the main components of seaweed flavor. The palatability of seaweed is based on the presence of glutamic acid in particular as one of three components of umami taste, which was discovered by the Japanese researcher Ikeda in 1908. Ikeda isolated glutamic acid from

brown seaweed *Laminaria* sp. (known as kombu; Lölliger, 2000; Yamaguchi and Ninomiya, 2000).

Recently, various biological activities of phycobiliproteins from red seaweed have been well studied. Phycoerythrin and phycocyanin are light-harvesting photosynthetic pigments. Antioxidant properties of phycobiliproteins could be used in the prevention of neurodegenerative diseases caused by oxidative stress as well as in the prevention of cancers and gastric ulcers (Burtin, 2003). Mycosporine-like amino acids such as mycosporine-glycine, which have been extracted from red seaweed *Palmaria palmata*, are able to exhibit antioxidant activity (Yuan *et al.*, 2009; Cardozo *et al.*, 2007). Mycosporine-like amino acids as natural UV screening compounds are used as the components of cosmetic products for the prevention against photoaging (Schmid *et al.*, 2003) and also for the protection from non-biological materials as plastics, paints and varnish (Cardozo *et al.*, 2007).

Many proteins known to be bioactive compounds with health benefit properties have been extracted from different species of seaweed. The enzymatic digests of antihypertensive peptides used as drugs have been produced from the red seaweed *Porphyra yezoensis* and also the brown seaweed *Undaria pinnatifida* and *Hizikia fusiformis* (Suetsuna and Nakano, 2000).

## 7.2.2 Minerals

Minerals are an essential part of human diet due to the inability of the human body to form them. More than 95% of mineral intake originates from food. Thus, the mineral level in human population depends on their concentration in vegetable or meat raw material for food products. Minerals play an important role in the human body as they are structural materials for building tissues and also significant factors in vital reactions, and as cofactors of many metalloenzymes (Hamza and Gitli, 2002; Leary *et al.*, 2007; McCall *et al.*, 2000; Parisi and Vallee, 1969; Tanaka *et al.*, 2000; Yoshioka *et al.*, 2007).

Seaweeds are known as a significant source of minerals due to their capacity to absorb inorganic substances from the environment. This capability is regarded with the presence of polysaccharides in seaweed cell walls and also is able to predestine a place of mineral storage in different parts of seaweed tissue (Küpper *et al.*, 1998; Hashim and Chu, 2004). The distribution and storage of minerals in seaweed may be influenced by several factors such as different environmental conditions (geographic location, wave exposure, seasonal effects) and condition of seaweed such as age (Küpper *et al.*, 1998; Teas *et al.*, 2004; Villares *et al.*, 2002).

In addition, the chemical forms of minerals and background levels of minerals in seawater could affect the sorption of minerals by seaweed (Küpper *et al.*, 1998; Tsui *et al.*, 2006). Thus, the accumulation of some minerals by seaweed could be higher than concentration of the same elements in the surroundings by several orders of magnitude (Saenko *et al.*, 1978). It was reported that Laminariales have the great capacity to accumulate iodine by more than 30 000 times the iodine concentration in the seawater (Küpper *et al.*, 1998).

The presence of different polysaccharides in seaweed cell walls is the reason why diverse groups of seaweed have different ability to uptake minerals. The higher metal sorption capacity of brown over green and red seaweed may be explained due to the high presence of alginic acid, alginate, and salt of alginic acids. These polysaccharides, which are included in seaweed cell walls mostly like calcium, magnesium, sodium and potassium salts, have strong ion-exchange properties (Antunes *et al.*, 2003). The mechanism of accumulation of some minerals in different species of seaweed has been documented. However, adsorption studies have been conducted mostly in the connection with recognition of uptake mechanism of toxic metal ions by different species of seaweed (Ghimire *et al.*, 2008; Hu *et al.*, 1996; Riget *et al.*, 1995). In addition, cell walls of brown seaweeds formed by cellulose and its carboxyl groups can participate in the accumulation of metals (Hu *et al.*, 1996).

The mechanism of an ion-exchange process has been found responsible for cationic metal sorption into brown seaweed *Sargassum hemiphyllum* according to Tsui *et al.* (2006) and also into brown seaweed *Ecklonia maxima* according to Williams and Edyvean (1997).

It is observed that red seaweed possesses more cationic sites than brown seaweed so it could show the low affinity for positively charged metal ions such as cadmium, but higher affinity for hexavalent chromium (Hashim and Chu, 2004). The polysaccharides of red seaweed are represented by sulphate galactans such as agar and carrageenan. According to Vasconcelos and Leal (2001) the level of sulfatation in carrageenan molecules and also the presence of hydroxyl and carboxyl groups might be responsible for binding metals. Further, it was reported that the extent of cadmium accumulation in red seaweed *Gracilaria tenuistipitata* depends also on the light and cadmium uptake in darkness proceeds mainly by passive diffusion across the cell wall along the concentration gradient (Hu *et al.*, 1996).

High metal sorption enables seaweed to be used as bioindicators of the environment pollution and consequently as biosorbents for heavy metals removal from the environment (Aderhold *et al.*, 1996; Basha *et al.*, 2007; Caliceti *et al.*, 2002; Ghimire *et al.*, 2008; Gupta and Chandra, 1998; Muñoz-Barbosa *et al.*, 2000; Phaneuf *et al.*, 1999; Suzuki *et al.*, 2005; Yu *et al.*, 1999; Zhang and Wong, 2007)

Mineral content of few algal genera are well documented as the trace elements (mg/kg) and macroelements (g/kg) in brown, red and green seaweed are shown in Tables 7.2 and 7.4 (Dawczynski *et al.*, 2007; Kolb *et al.*, 2004; Mišurcová *et al.*, 2009; Santoso *et al.*, 2006).

For the evaluation of contribution of mineral contents in seaweed on daily intake, the amounts of minerals were expressed in 8 g sample of seaweed matter. This value was calculated as an average mean from reported seaweed consumption ranging from 5 to 12 g for adults per day in Asian countries (MacArtain *et al.*, 2007; Miyake *et al.*, 2006). In addition, these values were compared with recommended daily intake (RDI) according to Velíšek (2002), and expressed in the percentage proportion to RDI of minerals. The presented results concluded that it is not possible to generalize mineral contribution depending on different seaweed group, but it is necessary to consider individual benefit of specific seaweed.

It appears that seaweed, within different seaweed groups, is a good contributor of iron to RDI. According to Mišurcová *et al.* (2009), the contribution of red seaweed *Porphyra tenera* of iron to RDI was 146.6% in contrast to Dawczynski *et al.* (2007), where that of *Porphyra* sp. was 30.7%. In the case of copper and manganese, the seaweed contribution to RDI ranges from 0.4 to 63.6% and 0.6 to 82.3%, respectively. The highest value of copper was observed in green seaweed *Ulva reticulata* according to Santoso *et al.* (2006) and the highest amount of manganese was established in red seaweed *Porphyra tenera* according to Mišurcová *et al.* (2009). However, seaweed is a weak contributor to RDI in the case of zinc. The highest percentage of 30.7% zinc to RDI was observed in red seaweed *Porphyra* sp. according to Dawczynski *et al.* (2007).

Seaweed has been used for the prevention of goiter for a long time (Norris *et al.*, 1936). Brown seaweed, especially genus *Laminaria*, is a significant source of iodine. The concentration of iodine in different genera of brown, red, and green seaweed from diverse authors is presented in Table 7.3 *Laminaria japonica* had the highest content of iodine 5.6 and 3.04 mg/kg from all investigated seaweed. On the other hand, the content of iodine in red and green seaweed is poor (Saenko *et al.*, 1978; Hou and Yan, 1998; Dawczynski *et al.*, 2007; Teas *et al.*, 2004).

The participation of macroelements on RDI is presented in Table 7.4 and is also different across diverse seaweed genera. But it does not reach the high values as for the contribution of trace elements, except for magnesium in red seaweed *Porphyra tenera* which was 100% (Mišurcová *et al.*, 2009). The contribution of magnesium to RDI ranges from 7.2 to 100%. Calcium and phosphorus intake is lower in comparison to RDI. The extent of the calcium contribution ranged from 2.1 to 28.3%. The highest value of calcium was

**Table 7.2** Trace elements contents in some genera of seaweed

	Fe	Zn	Mn	Cu	Fe	Zn	Mn	Cu	Fe	Zn	Mn	Cu
	mg/kg	mg/kg	mg/kg	mg/kg	mg in 8 g	mg in 8 g	mg in 8 g	mg in 8 g	% RDI	% RDI	% RDI	% RDI
Kolb <i>et al.</i> (2004)												
<i>Laminaria digitata</i>	B 11.9	8.9	2.9	2.5	0.1	0.07	0.20	0.2	1.0	0.7	0.7	0.9
<i>japonica</i>												
<i>Undaria pinnatifida</i>	B 15.4	9.4	3.3	1.9	0.1	0.08	0.03	0.02	1.2	0.8	0.9	0.9
Santoso <i>et al.</i> (2006)												
<i>Padina australis</i>	B 446.0	13.0		5.0	3.57	0.10		0.04	35.7	1.0		1.8
<i>Sargassum</i>	B 277.0	4.0		2.0	2.22	0.03		0.02	22.2	0.3		0.9
<i>polycystum</i>												
<i>Kappaphycus</i>	R 70.0	18.0		5.0	0.56	0.14		0.04	5.6	1.4		1.8
<i>alvarezii</i>												
<i>Caulerpa racemosa</i>	G 813.0	10.0		8.0	6.50	0.08		0.06	65.0	0.8		2.7
<i>Ulva reticulata</i>	G 280.0	17.0		179.0	2.24	0.14		1.43	22.4	1.4		63.6
Mišurcová <i>et al.</i> (2009)												
<i>Eisenia bicyclis</i>	B 63.4	27.2	3.9	4.3	0.51	0.22	0.03	0.03	5.1	2.2	0.9	1.3
<i>Hizikia fusiformis</i>	B 56.4	16.2	6.2	2.0	0.45	0.13	0.05	0.02	4.5	1.3	1.4	0.9
<i>Laminaria japonica</i>	B 73.8	18.2	4.7	1.6	0.59	0.15	0.04	0.01	5.9	1.5	1.1	0.4
<i>Undaria pinnatifida</i>	B 70.9	22.5	6.9	3.4	0.57	0.18	0.06	0.03	5.7	1.8	1.7	1.3
<i>Palmaria palmata</i>	R 717.0	37.0	27.5	4.6	5.74	0.30	0.04	0.04	57.4	3.0	1.1	1.8
<i>Porphyra tenera</i>	R 1833.0	19.4	360.0	15.8	14.66	0.16	2.88	0.13	146.6	1.6	82.3	5.8
Dawczynski <i>et al.</i> (2007)												
<i>Hizikia fusiformis</i>	B 679.0	15.7	24.5	2.6	5.43	0.13	0.20	0.02	54.3	1.3	0.6	0.9
<i>Laminaria</i> sp.	B 264.0	9.71	11.1	1.1	2.11	0.08	0.09	0.01	21.1	0.8	2.6	0.4
<i>Undaria pinnatifida</i>	B 184.0	33.1	7.5	1.5	1.47	0.26	0.06	0.01	14.7	2.6	1.7	0.4
<i>Porphyra</i> sp.	R 384.0	37.2	38.1	9.5	3.07	3.07	0.30	0.08	30.7	30.7	8.6	3.6
RDI*									10	10	3.5	2.3

RDI\* (reference daily intake) for adult in mg per day (Velišek, 2002).

B, brown seaweed; R, red seaweed; G, green seaweed.

observed in brown seaweed *Padina australis* by Santoso *et al.* (2006). The contribution of phosphorus was determined as the lowest of other macroelements and it ranged from 0.5 to 4.0%.

In addition, the bioavailability of these seaweed minerals in human is significantly different and depends on chemical forms of elements. Generally, the extent of mineral bioavailability expresses the proportion of minerals that can be utilized for essential body functions. The chemical forms of minerals determine their distribution in seaweed tissue and consequently also their utilization by human. The inorganic form of iodine is almost completely absorbed by

the gastrointestinal human tract, but organic iodine is excreted in the feces (Fairweather-Tait and Hurrell, 1996). The water-soluble form of minerals is mostly required for their utilization by humans. However, the water-soluble parts of mineral content in seaweed may decrease by food processing (Santoso *et al.*, 2006; Teas *et al.*, 2004).

The bioavailability of metals by humans depends on the presence of many dietary factors that are able to decrease their uptake. In the case of iron bioavailability, it is important if the source is non-heme or heme iron. The heme form of iron is relatively well absorbed by the human body whereas the bioavailability of the non-heme form of

**Table 7.3** Iodine contents in some genera of seaweed

	Authors <sup>a-e</sup>			
Seaweed	g/kg dry matter			
<b>Brown seaweed</b>				
<i>Ascophylum nodosum</i>		0.65 <sup>c</sup>		
<i>Cymathaere japonica</i>	2.0–4.5 <sup>a</sup>			
<i>Ecklonia maxima</i>		2.12 <sup>c</sup>		
<i>Eisenia bicyclis</i>		0.59 <sup>c</sup>		
<i>Fucus evanescens</i>	0.2–0.61 <sup>a</sup>			
<i>Hizikia fusiformis</i>				0.26 <sup>e</sup>
<i>Laminaria angustata</i>		2.35 <sup>c</sup>		
<i>Laminaria digitata</i>		2.00 <sup>c</sup>		
<i>Laminaria digitata japonica</i>	1.7 <sup>b</sup>			
<i>Laminaria japonica</i>	5.6 <sup>a</sup>		3.04 <sup>d</sup>	
<i>Laminaria</i> sp.	1.1–3.4 <sup>a</sup>			2.93 <sup>e</sup>
<i>Sargassum myabei</i>	0.43 <sup>a</sup>			
<i>Undaria pinnatifida</i>		0.26 <sup>b</sup>	0.02 <sup>c</sup>	0.16 <sup>e</sup>
<b>Red seaweed</b>				
<i>Palmaria palmata</i>		0.07 <sup>c</sup>		
<i>Porphyra</i> sp.	0.03–0.34 <sup>a</sup>			0.04 <sup>e</sup>
<b>Green seaweed</b>				
<i>Ulva fenestrata</i>	0.04 <sup>a</sup>			
<i>Ulva pertusa</i>				0.013 <sup>d</sup>

<sup>a</sup>Saenko *et al.* (1978)<sup>b</sup>Kolb *et al.* (2004)<sup>c</sup>Teas *et al.* (2004)<sup>d</sup>Hou and Yan (1998)<sup>e</sup>Dawczynski *et al.* (2007)

iron is strongly influenced by many seaweed components such as phytic acid, presence of polyphenolic compounds and proteins (Fairweather-Tait and Hurrell, 1996; Reddy *et al.*, 2000). The uptake of magnesium, which is derived from green vegetables in the form of chlorophyll, may be better utilized due to the tetrapyrrole ring. The tetrapyrrole ring can protect magnesium from inhibitors such as phytate and prevent reduction of magnesium bioavailability (Fairweather-Tait and Hurrell, 1996). Zinc absorption by humans from seaweed can be impaired by presence of phytic acid due to the formation of insoluble complexes (Likuski and Forbes, 1964; Walsh *et al.*, 1994).

Finally, the interactions between elements can influence their uptake and utilization. The iron uptake and consequently its utilization are impaired by abundant levels of

zinc that could cause anemia. The bioavailability of copper is negatively affected by zinc, iron, and molybdenum, but negative interaction with zinc is significant. Abundance of zinc can lead to a reduction of copper (Chernoff, 2005). On the other hand, copper and iron have minimal negative effect on the absorption of zinc, whereas excess of calcium in the presence of dietary phytate decreases zinc absorption (O'Dell, 1989). The effect of interaction between toxic metals and macroelements in seaweed has also been observed. The bioabsorption of cadmium considerably decreases with growing concentration of calcium ions. The correlation between cadmium and copper, cadmium and zinc as well as between lead and iron has been established (Caliceti *et al.*, 2002).

### 7.2.3 Vitamins

Vitamins are organic macronutrients with significant catalytic effects on many metabolic reactions in humans. However, autotrophic organisms can synthesize vitamins, but heterotrophic organisms, including humans, are only able to synthesize vitamins in a restricted extent. For that reason vitamins must be obtained from the diet and they are called endogenous essential catalysts. Seaweed is a significant source of some water-soluble and lipid-soluble vitamins.

#### Water-soluble vitamins

Water-soluble vitamins in seaweed are represented by vitamin C and vitamins of the B group, especially B<sub>1</sub>, B<sub>2</sub> and B<sub>12</sub>. Because of the abundant amount of L-ascorbic, a biologically active form of vitamin C, seaweeds have been used for a long time for in the prevention of scurvy (Norris *et al.*, 1936). L-ascorbic acid participates in many significant reactions in the human body such as biosynthesis of mucopolysaccharides, prostaglandins, absorption of iron forms and iron transport (Wienk *et al.*, 1997). The antioxidant activity of vitamin C is related to reactions with free radicals and further it participates on the protection of vitamin E by reacting with oxidized forms of vitamin E (Chen and Tappel, 1995). Vitamin C has been also studied in relation to the influence on blood pressure in hypertensive humans (Houston, 2005). L-ascorbic acid can exert the significant effects upon the central adrenergic neural processes by its influence on catecholamine metabolism, in which the rate of oxidation of tyramine, dopamine and adrenalin increases and the inhibition of monoamine oxidase by inhibitors caffeine and ephedrine declines (Kerxhali *et al.*, 1975). According to previous literature, some green and brown seaweeds contain high amounts of vitamin C – on average between 500 and 3000 mg/kg dry matter. This level of vitamin C is comparable with the content of this

**Table 7.4** Macroelements contents in some genera of seaweed

		Ca	Mg	P	Ca	Mg	P	Ca	Mg	P
		g/kg	g/kg	g/kg	mg in 8 g	mg in 8 g	mg in 8 g	% RDI	% RDI	% RDI
Kolb <i>et al.</i> (2004)										
<i>Laminaria digitata japonica</i>	B	8.8	5.5	3.0	70.4	44.0	24.0	8.8	13.6	2.0
<i>Undaria pinnatifida</i>	B	9.5	4.1	4.5	76.0	32.4	36.0	9.5	10.0	3.0
Santoso <i>et al.</i> (2006)										
<i>Padina australis</i>	B	28.3	4.0		226.4	32.0		28.3	9.9	
<i>Sargassum polycystum</i>	B	18.7	5.7		149.6	45.6		18.7	14.0	
<i>Kappaphycus alvarezii</i>	R	2.8	2.9		22.4	23.2		2.8	7.2	
<i>Caulerpa racemosa</i>	G	18.5	3.8		148.0	30.4		18.5	9.4	
<i>Ulva reticulata</i>	G	17.9	21.5		143.2	172.0		17.9	53.0	
Mišurcová <i>et al.</i> (2009)										
<i>Eisenia bicyclis</i>	B	6.8	6.6	0.8	54.3	52.4	6.2	6.8	16.1	0.5
<i>Hizikia fusiformis</i>	B	6.5	6.9	1.0	51.9	54.8	8.2	6.5	16.9	0.7
<i>Laminaria japonica</i>	B	5.7	6.7	4.8	45.9	53.8	38.1	5.7	16.6	3.2
<i>Undaria pinnatifida</i>	B	4.9	12.0	6.0	39.5	96.0	48.3	4.9	29.6	4.0
<i>Palmaria palmata</i>	R	2.1	3.5	5.0	16.6	27.7	39.8	2.1	8.5	3.3
<i>Porphyra tenera</i>	R	5.7	40.6	2.0	45.8	324.8	16.2	5.7	100.0	1.3
Dawczynski <i>et al.</i> (2007)										
<i>Hizikia fusiformis</i>	B	13.3	6.7	2.9	106.4	53.4	23.4	13.3	16.5	1.9
<i>Laminaria</i> sp.	B	7.4	5.7	5.7	59.4	45.6	45.6	7.4	14.0	3.8
<i>Undaria pinnatifida</i>	B	9.0	8.7	2.7	71.9	69.4	21.3	9.0	21.4	1.8
<i>Porphyra</i> sp.	R	3.3	3.5	5.2	26.4	27.9	41.8	3.3	8.6	3.5
RDI*								800	325	1200

RDI\* (reference daily intake) for adult in mg per day (Velišek, 2002).

B, brown seaweed; R, red seaweed; G, green seaweed.

vitamin in significant vegetable sources of vitamin C such as parsley and red pepper (Burtin, 2003).

In green seaweeds *Enteromorpha* sp. and *Ulva lactuca* levels of 150 and 460 mg/kg wet matter, respectively have been measured. Further, in red seaweed *Porphyra nereocystis* and *P. perforata* there a higher content of vitamin C of 530 and 600 mg/kg wet matter, respectively was determined. Similarly, 530 mg/kg wet matter was observed in brown seaweed *Alaria valida* (Norris *et al.*, 1936). However, according to other authors, vitamin C was determined in abundant amounts in green seaweeds *Enteromorpha flexuosa* and *Ulva fasciata* of 3000 and 2200 mg/kg dry matter and in red seaweed *Euchema denticulatum* of 2000 mg/kg dry matter (McDermid and Stuercke, 2003).

The majority of red and brown seaweeds contain other water-soluble vitamins of the B group, especially thiamine and riboflavin. Vitamin B<sub>1</sub> or thiamine is a sulfur-containing vitamin, whose free form is esterified in its active form to thiamine pyrophosphate, which is a coenzyme of

many significant enzymes (decarboxylases, pyruvate dehydrogenases, transketolases, carboligases and other) involved in catabolism of saccharides and branched-chain amino acids (Smith *et al.*, 2007; Croft *et al.*, 2006). Cereal products are the most significant source of thiamine due to their high consumption among people. Brown seaweed *Undaria pinnatifida* and *Ascophylum nodosum* with 5.0 and 2.7 mg/100 g dry matter, respectively (MacArtain *et al.*, 2007), and contain the comparable amount of this vitamin as in cereal flours. For instance, wheat flour has 5.8, barley flour 5.6 and rough rice 2.8 mg/100 g dry matter (Hegedüs *et al.*, 1985).

Vitamin B<sub>2</sub> (riboflavin) is the main component of the cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These active forms of riboflavin are redox cofactors of many enzymes that catalyze oxidation-reduction reactions in energy metabolism, and the metabolism of fats, saccharides, and proteins (Harrill *et al.*, 1959; Vermilion *et al.*, 1981). The riboflavin content in seaweed has

been documented as similar to the thiamine content. In red seaweed *Porphyra umbilicalis* a content of 3.4 mg/100 g dry matter of riboflavin was determined (MacArtain *et al.*, 2007). Nevertheless, in brown seaweed *Undaria pinnatifida* different contents of this vitamin were observed. The higher concentration of 11.7 mg/100 g dry matter was determined by MacArtain *et al.* (2007) in contrast to 1.35 mg/100 g dry matter according to Kolb *et al.* (2004).

Vitamin B<sub>12</sub> (or cobalamin) is a cobalt-containing tetrapyrrole related to chlorophyll and heme. Cobalamin is a cofactor of enzymes (methionine synthase and methylmalonyl CoA mutase) in radical and methyl transfer reactions (Croft *et al.*, 2006; Smith *et al.*, 2007).

Higher plants do not contain cobalamin-dependent methionine synthase and therefore they do not synthesize B<sub>12</sub>. Thus, the main source of vitamin B<sub>12</sub> is animal-derived products and strict vegetarians or vegans are a risk group for deficiency of this vitamin (Herbert, 1988). Low serum levels of vitamin B<sub>12</sub> were also observed in elderly people (Lindenbaum *et al.*, 1994). Cobalamin deficiency may cause serious health disorders such as megaloblastic anemia, neuropsychiatric disorders, subacute combined degeneration of the spinal cord, and gastrointestinal problems such as malabsorption (Stott *et al.*, 1997). However, it was observed that more than a half of the algal kingdom contains methionine synthase and they are cobalamin auxotrophs. In particular, the red seaweed *Porphyra yezoensis* is a rich source of this vitamin (Croft *et al.*, 2005). The high cobalamin amount of 78.8 mg/100 g dry matter was established also in green seaweed *Ulva* sp. (MacArtain *et al.*, 2007).

### Fat-soluble vitamins

Seaweed is a significant source of fat-soluble vitamin E and carotenoids (as provitamins of vitamin A). Vitamin E is one of the most important fat-soluble vitamins with a strong antioxidant activity. Its special function is lipid membrane protection from peroxidation. It exists in eight forms:  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -tocopherols and  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -tocotrienols. The  $\alpha$ -forms show the highest antioxidant effect (Yamamoto *et al.*, 2001). Further, the connection of vitamin E and decrease of blood pressure was reported (Houston, 2005).

Brown seaweed contains higher amounts of vitamin E than red and green seaweed. The extent of vitamin E content in seaweed is formed due to the antioxidant protection from the environmental conditions and varies between the immersion and emersion periods. In *Eisenia arborea*, an annual average vitamin E content of 8.3 mg/100 g dry matter was determined during 10 months from March to December, whereas the lowest content of 6.2 mg/100 g of dry matter was observed in May. On the other hand, the highest content of 9.6 mg/100 g dry matter was determined in

September (Hernández-Carmona *et al.*, 2009). Distribution of tocopherol and tocotrienols of brown seaweed was reported by Ortiz *et al.* (2006). The high total tocol contents of 1112.9 and 1071.4 mg/kg of lipid were observed in *Durvillaea antarctica* and *Ulva lactuca*, respectively. The highest level of tocopherol was of  $\delta$ -tocopherol in *D. antarctica* (245.9 mg/kg of lipid) and  $\gamma$ -tocopherols (25.8 mg/kg of lipid) in *Ulva lactuca*.

Carotenoids are represented by different pigments which form the resulting seaweed color together with chlorophyll. Carotenoids are also very strong antioxidants. The antioxidant effect of  $\beta$ -carotene is based on the ability to quench singlet oxygen and thus to participate on the inhibition of lipid oxidation (Böhm *et al.*, 1993). Further, the correlation between diet rich in  $\beta$ -carotene and lycopene and reduced risk of cardiovascular disease and cancers as well as the influence of lutein and zeaxanthin on ophthalmological diseases has been reported earlier (Burtin, 2003).

Fucoxanthin,  $\beta$ -carotene and violaxanthin are found in brown seaweed. The main carotenoids of red seaweed are  $\alpha$ - and  $\beta$ -carotene and their derivatives such as zeaxanthin and lutein. Green algae have a similar composition of carotenoids in comparison to higher plants. The main part of carotenoids of brown seaweed is formed by  $\beta$ -carotene, lutein, violaxanthin, anteraxanthin, zeaxanthin and neoxanthin (Burtin, 2003).

Fucoxanthin is the main carotenoid of brown seaweed and it was proved to be a compound with a lot of health benefit effects. It was established that it has the chemopreventive effect and antitumor activity of fucoxanthin in human leukemia cells (HL-60) via the induction of apoptosis (Kim *et al.*, 2010b). Further, fucoxanthin was described as an anti-obesity and anti-diabetic agent (Maeda *et al.*, 2008; Matsumoto *et al.*, 2010; Miyashita, 2009). Fucoxanthin from *Undaria pinnatifida* forms 9.6% of seaweed lipid (Maeda *et al.*, 2008).

Red seaweed *Gracilaria changi* is a good potential source of  $\beta$ -carotene due to established high content of 5.2 mg/100 g, which is in comparison with 6.8 mg/100 g in carrots. Vitamin A activity of *Gracilaria changi* was 865  $\mu$ g/100 g expressed as a retinol equivalent (RE) (Norziah and Ching, 2000).

Seaweed carotenoids belong among bioactive compounds with diverse health benefits and because of this reason, they are focused in recent scientific surveys.

### 7.2.4 Lipids

Seaweed lipids are present in very small amounts ranging between 1 and 5% of dry seaweed matter. However, the majority of lipids are polyunsaturated  $\omega$ -3 and  $\omega$ -6 fatty acids,

which effectively reduce the risk of cardiovascular diseases, osteoporosis, and diabetes (Maeda *et al.*, 2008). Fatty acids are precursors in the eicosanoid biosynthesis, which are important bioregulators of many cellular processes (Gressler *et al.*, 2010). Eicosapentaenoic (EPA) and docosahexaenoic (DHA) polyunsaturated  $\omega$ -3 fatty acids have been reported to show cardioprotection activities, by the reduction of triacylglycerol and cholesterol and have anti-inflammatory and anticancer effects (Maeda *et al.*, 2008).

Red and brown seaweed predominantly contain the polyunsaturated 20 carbon-fatty acids eicosapentaenoic acid (EPA,  $\omega$ -3, C 20:5) and arachidonic acid (AA,  $\omega$ -6, C 20:4) (Norziah and Ching, 2000; Ortiz *et al.*, 2006). In red seaweed *Gracilaria changgi* polyunsaturated fatty acids formed 74% with 33.1% of EPA and 12.9% of docosahexaenoic acid ( $\omega$ -6, C 22:3); palmitic acid was the main saturated fatty acid with 26% (Norziah and Ching, 2000).

In brown seaweed *Durvillaea antarctica* and green seaweed *Ulva lactuca*, higher amounts of unsaturated fatty acids (72.5% and 51.9%, respectively) and the most abundant saturated fatty acid palmitic acid (12.1% and 14.0%, respectively) were observed. *D. antarctica* had higher content of polyunsaturated  $\omega$ -6 arachidonic acid of 11.2% and the highest content of 25.4% of monounsaturated oleic acid, whereas in *U. lactuca*, 27.4% of oleic acid was also observed (Ortiz *et al.*, 2006).

The lipid level and composition of seaweed fatty acids are influenced by different environmental conditions such as different light intensity and salinity of seawater. According to Floreto and Teshima (1998), different changes of total fatty acid content were observed after diverse exposition of light and salinities to green seaweed *Ulva pertusa*, red seaweed *Grateloupia sparsa* and brown seaweed *Sargassum piluliferum*. The exposure of high salinity to *U. pertusa* and *S. piluliferum* and low and high salinities to *G. sparsa* resulted in increased levels of total fatty acids. The combination of high light intensity and low salinity resulted in decrease of total fatty acid content in *U. pertusa*. Further, the changes of fatty acid composition in dependence on different exposure of light and diverse salinity were observed. At high light intensity the content of most saturated fatty acids was raised in *U. pertusa*, in contrast to decrease of almost all fatty acids in *S. piluliferum* (Floreto and Teshima, 1998). Further, the influence of low temperature on the content of fatty acids in different species of seaweed was reported (Ortiz *et al.*, 2006).

Recently, the importance of the  $\omega$ -6/ $\omega$ -3 ratio has been discussed in scientific reports. The origin value 1 of the ratio  $\omega$ -6/ $\omega$ -3 involved the balance of intake of both polyunsaturated  $\omega$ -6 and  $\omega$ -3 fatty acids. The high dietary intake of  $\omega$ -6 fatty acids from a diet rich in vegetable oils causes detrimental turnover of the balance ratio ( $\omega$ -6/ $\omega$ -3) to  $\omega$ -3 fatty acids.

This ratio is 50 in Europe and the United States, and 12 in Japan, which is compared to 1 for Greenland Eskimos due to their higher consumption of fish fatty acids (Simopoulos, 2002a). The adverse effect of trans-polyunsaturated fatty acids participates either on the growth of, for example, low-density lipoprotein (LDL), body weight or an decrease of high-density lipoprotein (HDL) and inhibition of incorporation of other fatty acids into cell membranes. They interfere with elongation and desaturation of essential fatty acids. Arachidonic and eicosapentaenoic acids are the main contributors to eicosanoid production. Eicosanoids are produced in large amounts due to high dietary intake of arachidonic acid. This could induce formation of thrombus and atheromas and may cause allergic and inflammatory disorders (Simopoulos, 2002a). Further, the connection of fatty acid composition of immune cells and their function has been reported. The change of the nature of fatty acid nutrition can modify immune cell behavior and consequently also the immune response, including inflammatory component (Calder, 2008; Simopoulos, 2002b). In summary, huge intake of  $\omega$ -6 fatty acids and excessively high  $\omega$ -6/ $\omega$ -3 ratio promotes the pathogenesis of cardiovascular, inflammatory, and autoimmune diseases and cancer (Simopoulos, 2008).

The low values of  $\omega$ -6/ $\omega$ -3 fatty acid ratios were determined in different species of seaweed and they ranged in brown seaweed from 0.49 *Undaria pinnatifida* (MacArtain *et al.*, 2007) to 4.1 in a stem of *Durvillaea antarctica* (Ortiz *et al.*, 2006). In green seaweed *Ulva lactuca* this ratio was 1.31 (Ortiz *et al.*, 2006). Red seaweed *Palmaria palmata* contained higher amount of  $\omega$ -3 fatty acid and its  $\omega$ -6/ $\omega$ -3 ratio was 0.13, where as in *Porphyra tenera*, it was 1.21 (MacArtain *et al.*, 2007). Other red seaweeds *Laurencia filiformis* and *L. intricata* were balanced at the same  $\omega$ -6/ $\omega$ -3 fatty acid ratio of 1.1 (Gressler *et al.*, 2010). From the results of  $\omega$ -6/ $\omega$ -3 fatty acid ratio, it could be concluded that it is highly recommended to use seaweed in a diet to decrease this ratio in the diet.

### 7.2.5 Dietary fiber

Seaweed polysaccharides are a wide group of compounds with numerous biological functions. From the nutritional point of view, seaweed polysaccharides play an important role as dietary fiber (DF), which is defined as the skeletal remains of plant cells in the diet that are resistant to hydrolysis by the human digestive enzymes. This definition was later modified to include "all polysaccharides and lignin, which are not digested by the endogenous secretions of the human digestive tract" (Bach Knudsen, 2001). The definition of DF has been continuously discussed and

establishing it is very difficult due to the presence of complex compounds of various compositions and structures. Consequently, it is complicated because of limited capabilities of analytical methods (AACC Report, 2001; DeVries, 2003; DeVries *et al.*, 1999; Prosky, 1999; Slavin, 2003; McCleary, 2003). The most commonly used method is the crude fiber (CF) method, which is based on the subsequent hydrolysis of samples with diluted acid and alkali followed by gravimetric determination of the residue after drying. Only a small and variable fraction of fiber components (structural polysaccharides and lignin) is measured by this method. Further, a detergent method has been developed by Van Soest (1963) and Van Soest and Wine (1967). It is based on the determination of fiber fractions that are insoluble in neutral detergents as neutral detergent fiber (NDF – hemicelluloses, cellulose and lignin) and in acid detergents as acid detergent fiber (ADF – cellulose and lignin). However, the water-soluble non-starch polysaccharides and water-insoluble pectic substances are lost during the NDF method because starch and protein may contaminate the NDF residue and hemicellulose may be left in the ADF fraction (Bach Knudsen, 2001). Dietary fiber could be measured as soluble as well as insoluble fiber. The soluble fractions of fiber include pectin, xyloglucans, and galactomannan hemicelluloses, gums and waxes, while the insoluble fractions involve celluloses, arabinoxylan hemicelluloses, and lignin (Bach Knudsen, 2001; Jiménez-Escrig and Sánchez-Muniz, 2000). Recently, dietary fiber is mostly determined as total dietary fiber (TDF) that includes both soluble (SF) and insoluble (IF) fractions. Recently, TDF has been measured after enzyme hydrolysis by  $\alpha$ -amylase, protease, and amyloglucosidase. It is possible to distinguish also soluble and insoluble dietary fiber by this method.

In Tables 7.5 and 7.6 there are presented dietary fiber values determined by different methods according to some authors. Values of CF have a small predictive ability of seaweed dietary fiber content as it is shown in Table 7.5 (Marshall *et al.*, 2007; Mišurcová *et al.*, 2010). NDF represents the structural fiber, which is only partially digestible and lignin forms the indigestible part, because of which the values of NDF are higher than the values of CF. In red seaweed *Porphyra* sp., NDF was 33.5% (Marshall *et al.*, 2007) in comparison to the SF values of 17.9% and 14.6% in *Porphyra tenera*, according to Mabeau and Fleurence (1993) and Rupérez and Saura-Calixto (2001), respectively. A similar trend was observed in green seaweed *Ulva lactuca*, there was NDF 32.9% (Marshall *et al.*, 2007) in contrast to SF 21.3% (Mabeau and Fleurence, 1993) and 27.2% (Ortiz *et al.*, 2006). The ADF method has replaced CF method. However, it is not suitable for total fiber analysis since it is possible to establish only insoluble cellulose and lignin. Moreover, higher values of ADF than NDF were observed in

**Table 7.5** Crude (CF), neutral (NDF) and acido detergent (ADF) fiber contents in some seaweed

Seaweed	CF % <sup>a</sup>	CF % <sup>b</sup>	NDF % <sup>a</sup>	NDF % <sup>b</sup>	ADF % <sup>b</sup>
<b>Brown</b>					
<i>Eisenia bicyclis</i>		7.3		14.6	19.3
<i>Fucus serratus</i>	16.0		26.2		
<i>Hizikia fusiformis</i>	12.6			20.7	29.4
<i>Laminaria digitata</i>	7.7		16.6		
<i>Laminaria japonica</i>		5.5		22.1	13.8
<i>Undaria pinnatifida</i>		3.1		13.9	16.2
<b>Red</b>					
<i>Palmaria palmata</i>		1.5		15.1	3.1
<i>Porphyra tenera</i>		3.2		28.2	12.4
<i>Porphyra</i> sp.	1.1		33.5		
<b>Green</b>					
<i>Ulva lactuca</i>	2.8		32.9		

<sup>a</sup>Marshall *et al.* (2007)

<sup>b</sup>Mišurcová *et al.* (2010)

brown seaweed *Eisenia bicyclis*, *Hizikia fusiformis* and *Undaria pinnatifida* (Mišurcová *et al.*, 2010). This fact could be explained by Bach Knudsen (2001) as it was mentioned above. The values of TDF with SF and IF in some brown, red and green seaweed according to some authors are expressed in Table 7.6 (Dawczynski *et al.*, 2007; Mabeau and Fleurence, 1993; Ortiz *et al.*, 2006; Rupérez and Saura-Calixto, 2001; Sánchez-Machado *et al.*, 2004; Santoso *et al.*, 2006). General conclusions about levels of total, soluble and insoluble fibers in different groups of seaweed cannot be deduced from these results. Great differences of TDF values have been established across all seaweed groups. TDF in brown seaweed ranged from 35.3% to 71.4%. In red seaweed TDF formed 31.4% up to 69.3% and in green seaweed TDF ranged from 33.4% to 65.7%. As far as the distribution of SF and IF is concerned, general conclusions also could not be made. The contribution of SF and IF was different depending on individual seaweed species and even within the same species, according to different authors. Differences of TDF, SF, and IF were established depending also on various placement in seaweed tissue. The content of all types of dietary fiber in brown seaweed *Durvillaea antarctica* was analyzed to be higher in leaves than in the stem (Sánchez-Machado *et al.*, 2004).

Different seaweed groups contain a wide group of polysaccharides whose chemical composition and amount vary within various seaweed species. Seaweed polysaccharides can be divided into storage and structural

**Table 7.6** Total, soluble and insoluble dietary fiber contents in some seaweed

Seaweed	TDF %	SF %	IF %
<b>Brown</b>			
<sup>e</sup> <i>Durvillaea antarctica</i> leaves	71.4	27.7	43.7
<sup>e</sup> <i>Durvillaea antarctica</i> stem	56.4	24.2	32.2
<sup>f</sup> <i>Fucus vesiculosus</i>	50.1	9.8	40.3
<sup>b</sup> <i>Hizikia fusiformis</i>	<sup>a</sup> 62.3 <sup>b</sup> 49.2	<sup>b</sup> 32.9	<sup>b</sup> 16.3
<sup>a</sup> <i>Laminaria</i> sp.	<sup>a</sup> 36.0		
<sup>b,d</sup> <i>Laminaria digitata</i>	<sup>b</sup> 37.3 <sup>d</sup> 36.1	<sup>b</sup> 32.6 <sup>d</sup> 9.1	<sup>b</sup> 4.7 <sup>d</sup> 27.0
<sup>e</sup> <i>Laminaria ochroleuca</i>	37.0		
<sup>b,d</sup> <i>Undaria pinnatifida</i>	<sup>b</sup> 35.3 <sup>d</sup> 33.6	<sup>b</sup> 30.0 <sup>d</sup> 17.3	<sup>b</sup> 5.3 <sup>d</sup> 16.3
<b>Red</b>			
<sup>f</sup> <i>Kappaphycus alvarezii</i>	69.3	10.7	58.6
<sup>e</sup> <i>Palmaria</i> sp.	<sup>e</sup> 31.4		
<sup>a,e</sup> <i>Porphyra</i> sp.	<sup>a</sup> 48.6 <sup>e</sup> 40.5		
<sup>b,d</sup> <i>Porphyra tenera</i>	<sup>b</sup> 34.7 <sup>d</sup> 33.8	<sup>b</sup> 17.9 <sup>d</sup> 14.6	<sup>b</sup> 6.8 <sup>d</sup> 19.2
<b>Green</b>			
<sup>f</sup> <i>Caulerpa racemosa</i>	64.9	0.9	64.1
<sup>b</sup> <i>Enteromorpha</i> spp.	33.4	17.2	16.2
<sup>b,c</sup> <i>Ulva lactuca</i>	<sup>b</sup> 38.1 <sup>c</sup> 60.5	<sup>b</sup> 21.3 <sup>c</sup> 27.2	<sup>b</sup> 16.8 <sup>c</sup> 33.3
<sup>f</sup> <i>Ulva reticulata</i>	65.7	0.9	64.8
<sup>a</sup> Dawczynskiet al. (2007)			
<sup>b</sup> Mabeu and Fleurence (1993)			
<sup>c</sup> Ortiz et al. (2006)			
<sup>d</sup> Rupérez and Saura-Calixto (2001)			
<sup>e</sup> Sánchez-Machado et al. (2004)			
<sup>f</sup> Santoso et al. (2006)			

polysaccharides based on their chemical structures and functions. These are presented in Table 7.7.

The main storage polysaccharides of vast majority of living organisms are  $\alpha$ -glucans such as starch and glycogen. Starch is the main storage polysaccharide of green seaweed. Starch is formed by a mixture of soluble amylase ( $\alpha$ -1,4-linked D-glucose polymer) and insoluble amylopectin (highly branched polymer with the same basic structure

and frequent  $\alpha$ -1,6 branch point). Starch is deposited in the chloroplasts of green algae and higher plants as semi-crystalline granules (Viola *et al.*, 2001). Floridean starch is the main storage polysaccharide of red seaweed. It has a similar structure as starch without amylose. However, it was confirmed that some species of red algae form also amylose units. Another difference is the imposition of granules of floridean starch outside the plastids (Shimonaga *et al.*, 2007).

Laminaran is the main storage polysaccharide of brown seaweed. Its chemical structure is formed by (1,3)- $\beta$ -D-glucan with  $\beta$ -(1,6) branching. Laminaran can have different reducing endings with either mannitol or glucose residues. The different solubility of laminarans depends on the level of branching. Highly branched laminaran is soluble in cold water while lower levels of branching enables solubility only in warm water (Rioux *et al.*, 2007; Rupérez *et al.*, 2002). The chemical composition and content of polysaccharides in seaweed matter changed depending on the season, age of population, particular species, and geographic location. Partly, seasonal changes of laminaran and mannitol in different species of brown seaweed have been reported. Laminaran content was variable regarding especially to different stage of the life cycle. It was present in the bladelet of *Ecklonia cava* particularly in summer as the energy source to produce zoosporangia in bladelets in the maturation season (Iwao *et al.*, 2008). On the other hand, the molecular mass of the laminaran isolated from young seaweed *Fucus evanescens* was higher than that from mature seaweed (Zvyagintseva *et al.*, 2005). Surprisingly, these authors observed absence of laminaran and fucoidan in *Alaria* species, which is known as a rich source of these polysaccharides.

Cell walls of different groups of seaweed vary with regard to the presence of large extent of structural polysaccharides. Green seaweed cell walls are formed of ulvans, which are constructed by main units made from sulfated rhamnose residues linked to uronic acids (Jaulneau *et al.*, 2010). Further components of green seaweed cell walls are xylan, mannan, and cellulose. The main part of red seaweed cell walls are represented by sulfated galactans, which are known as carrageenans and agars, and their main saccharide units are shown in Table 7.7. Carrageenans and agars are important phycocolloids, which are extracted from different genera of red seaweed, particularly *Chondrus*, *Gelidium*, and *Gracilaria*. They have a wide utilization in the food and cosmetics industries, and agar also as a cultivation medium in microbiology. Xylans and mannans are also in the cell walls of red seaweed (Rupérez and Toledano, 2003). Cellulose is the main polysaccharide of the majority of red and brown seaweed cell walls in lower levels than in higher plants, though. What is more, in red seaweed *Palmaria palmata* cellulose is found in a small amount and in the genus

**Table 7.7** Polysaccharides in some green, red and brown seaweed

Seaweed	Polysaccharides		Main unit
Green	Starch	<b>Storage</b>	$\alpha$ -D-glucose 20% amylose, 80–90% amylopectin
Red	Floridean starch		$\alpha$ -D-glucose amylopectin
Brown	Laminaran		(1,3)- $\beta$ -D-glucose and (1,6)- $\beta$ -D-glucose branch unit mannitol
Green	Ulvan-sulfated Xylan Mannan	<b>Structural</b>	$\beta$ -D-glucuronosyl-(1,4)- $\alpha$ -L-rhamnose 3-sulfate $\beta$ (1,4)-D-xylose mannose (C-2 epimer of glucose)
Red	Carrageenans – sulfated Agar – sulfated Xylan Mannan		$\alpha$ (1,3)-galactose acid and $\beta$ -(1,4,3,6)-anhydro-D-galactose D-galactose acid and (3,6)-anhydro-L-galactose $\beta$ (1,4)-D-xylose mannose (C-2 epimer of glucose)
Brown	Alginate Fucoidan – sulfated fucans		$\beta$ -(1,4)-D-mannuronic acid and $\alpha$ -(1,4)-L-guluronic acid (1,2)- $\alpha$ -L-fucose-1-sulfate $\beta$ -(1,4)-D-mannuronic acid and 3-D-xylosyl-L-fucose-4-sulfate (1,4)-D-galactose and L-fucosyl-3-sulphate branch units – D-xylose, D-galactose and D-mannose $\beta$ -(1,4)-glucose
Green, red, brown	Cellulose		

*Pophyra* is even replaced by insoluble mannan and xylan (Deniaud *et al.*, 2003; Rupérez and Toledano, 2003).

Cell walls of several orders of brown seaweed, particularly of Fucales and Laminariales, consist mainly of fucoidans, which are composed from variable amounts of saccharide units with different degree of sulfation (Berteau and Mulloy, 2003). Primarily, fucoidans consist of a main unit of  $\alpha$ -L-fucose and a branch unit of D-galactose, D-mannose and D-xylose. According to their chemical composition fucoidans could be divided further into xylofucoglycuronans and glycuronogalactofucans (Jiménez-Escrig and Sánchez-Muniz, 2000). Different structures of fucoidans from diverse seaweed species were confirmed and their molecular weights were determined in great ranges from 43 to 1600 kDa (Rioux *et al.*, 2007). The content and composition of fucans is season changeable as well as laminarans in *Laminaria japonica*. In October, when the kelp blades were decaying and sporangia were maturing, the fucan content was more than doubled than in young seaweed (Honya *et al.*, 1999). Fucoidans create a substantial part of brown seaweed cell walls, but they are important factors in seaweed morphogenesis of their embryos (Berteau and Mulloy, 2003).

Dietary fiber is not a source of energy for humans and thus cannot be included into nutritional factors. However, SF and IF used to be connected with many physiological effects with health benefits for human. SF is considered to

be a considerable factor in the prevention of obesity and serious diseases such as cancer and cardiovascular diseases. Whereas IF shows a water-holding capacity, which results in increasing the bulk of the colon content and reduces its transit time (Schneeman, 1999).

The health benefit of DF such as protection from colorectal cancer was demonstrated although the mechanism is still not clear. It is assumed that carcinogens are bound by IF and the interaction of carcinogens with the colonic mucosal cells is reduced thanks to their excretion in feces (Harris and Ferguson, 1993). Fucoidans from brown seaweed induce apoptosis in human colon cancer cells but efficiency of that varies among different types of colon cancer cells (Kim *et al.*, 2010a, b). Also a relationship between molecular size and anticancer activity has been observed. Fucoidans from *Undaria pinnatifida* with the smaller molecular size and loosed conformation have appeared to be more efficient with regard to anticancer activity (You *et al.*, 2010). Moreover, fucoidans from *Undaria pinnatifida* can be used as a bone health supplement because it has been observed that it induces osteoblastic cell differentiation due to their positive effect on the activity of alkaline phosphatase and the ability to increase osteocalcin level (Cho *et al.*, 2009). Further, the antitumor activity of alginates has also been studied, which is triggered by immunomodulatory action (Alves de Sousa *et al.*, 2007).

Recently, polysaccharides of seaweed have been widely investigated due to their chemical properties and important biological effects. Sulfated polysaccharides, such as alginic acids, carrageenans, and agars are able to reduce cholesterol absorption in gut, in the case of acidic polysaccharides (alginic acids, carrageenans) due to their production of indigestible ionic colloid and in the case of neutral polysaccharides (agars) thanks to their water dispersibility (Jiménez-Escrig and Sánchez-Muniz, 2000). Further, anticoagulant, antithrombotic, and antiviral activities including anti-human immunodeficiency virus (HIV) infection, herpes and hepatitis viruses have been deeply studied as the properties of seaweed sulfated polysaccharides (Athucorala *et al.*, 2007; Costa *et al.*, 2009; Damonte *et al.*, 1994; De Zoysa *et al.*, 2008). Generally, their biological activity is related to different composition and extent of sulfation. It was reported that sulfated homopolysaccharides are more potent anti-HIV agents than heteropolysaccharides (Melo *et al.*, 2002). Also the effect of oversulfated fucoidans from *Undaria pinnatifida* has been studied on postprandial glycemia. It has been confirmed that oversulfated fucoidans have the inhibition effect on the activity of amyloglucosidase. They could be used as therapeutic agents to reduce the severity of Type 1 diabetes due to the decrease in postprandial hyperglycemia thanks to glucose absorption retardation (Cho *et al.*, 2010).

The definition of DF includes the affirmation that DF is not digested by the enzymes of gastrointestinal human tract. However, some parts of SF such as pectin and hemicelluloses are degraded or metabolized in the large intestine (Holloway *et al.*, 1980, 1983). In addition to that, cellulose is probably partially fermented by human intestinal bacteria into short-chains fatty acids (SCFs) such as acetate, propionate, and butyrate. Butyrate is the primary energy substrate for colonic mucosa in human (Holloway *et al.*, 1978; Titgemeyer *et al.*, 1991).

Moreover, fucoidan research is focused on the significant antioxidant activity as a strong tool for the prevention of free-radical-mediated diseases. It has been documented that fucoidans from *Laminaria japonica* are able to prevent the increase of lipid peroxide and effectively protect biological membranes (Li *et al.*, 2008).

### 7.3 Conclusion

Seaweed is considered as a significant source of many nutritional factors such as proteins, vitamins, and minerals. The huge diversity of seaweed species is the reason for its different chemical composition. In fact, the great extent of secondary seaweed metabolites is formed as an ecological response. Seaweeds are water-living organisms, which

are exposed to ultraviolet radiation and should have effective protection from the effect of free radicals. Seaweed polyphenols are formed also as defense mechanism against herbivores and to reinforce seaweed tissue against wave exposure.

Bioactivity of diverse secondary metabolites and other compounds extracted from different seaweed species is an important topic of numerous scientific studies. Seaweed contribution to prevention of different serious diseases including cancer and cardiovascular diseases has been confirmed. The antioxidant activity of different seaweed extract and possibility of its utilization as effective protective agents against harmful effects of free radicals have been studied extensively.

Algae could be used to raise low DF intake, especially in European countries. Seaweed as a rich source of DF is associated with the reduction of LDL-cholesterol in plasma and may favourably influence the glycemic response. In addition, seaweed has a potential to become widely used as health-promoting food but also as important pharmaceutical and medicinal products.

### References

- AACC Report (2001) The definition of dietary fiber. *Cereal Foods World*, **46**, 112–126.
- Aderhold, D., Williams, C.J., Edyvean, R.G.J. (1996) The removal of heavy-metal ions by seaweeds and their derivatives. *Biores. Technol.*, **58**, 1–6.
- Alves de Sousa, A.P., Torres, M.R., Pessoa, C. *et al.* (2007) *In vivo* growth-inhibition of Sarcoma 180 tumor by alginates from brown seaweed *Sargassum vulgare*. *Carbohydr. Polym.*, **69**, 7–13.
- Antunes, W.M., Luna, A.S., Henriques, C.A. and Costa, A.C.A. (2003) An evaluation of biosorption by a brown seaweed under optimized conditions. *Electron. J. Biotechnol.*, **6**, 174–184.
- Athucorala, Y., Lee, K.W., Kim, S.K. and Jeon, Y.J. (2007) Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. *Biores. Technol.*, **98**, 1711–1716.
- Artan, M., Li, Y., Karadeniz, F., Lee, S.H., Kim, M.M. and Kim, S.K. (2008) Anti-HIV-1 activity of phloroglucinol derivative, 6,6'-bieckol, from *Ecklonia cava*. *Bioorg. Med. Chem.*, **16**, 7921–7926.
- Bach Knudsen, K.E. (2001) The nutritional significance of "dietary fibre" analysis. *Anim. Feed Sci. Technol.*, **90**, 3–20.
- Basha, S., Murthy, Z.V.P. and Jha, B. (2007) Biosorption of hexavalent chromium by chemically modified seaweed, *Cystoseira indica*. *Chem. Eng. J.*, **137**, 480–488.

- Berteau, O. and Mulloy, B. (2003) Sulphated fucans, fresh perspectives: structures, functions, and biological properties of sulphated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology*, **13**, 29R–40R.
- Böhm, F., Haley, J., Truscott, T.G. and Schalch, W. (1993) Cellular bound beta-carotene quenches singlet oxygen in man. *J. Photochem. Photobiol. B*, **21**, 219–221.
- Burtin, P. (2003) Nutritional value of seaweeds. *Electron. J. Env. Agric. Food Chem.*, **2**, 498–503.
- Calder, P.C. (2008) The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **79**, 101–108.
- Caliceti, M., Argese, E., Sfriso, A. and Pavoni, B. (2002) Heavy metal contamination in the seaweeds of the Venice lagoon. *Chemosphere*, **47**, 443–454.
- Cardozo, K.H.M., Guaratini, T., Barros, M.P. *et al.* (2007) Metabolites from algae with economical impact. *Comp. Biochem. Physiol., Part C*, **146**, 60–78.
- Chen, H. and Tappel, A.L. (1995) Protection of vitamin E, selenium, trolox C, ascorbic acid palmitate, acetyl-cysteine, coenzyme Q0, coenzyme Q10, beta-carotene, canthaxanthin, and (+)-catechin against oxidative damage to rat blood and tissues *in vivo*. *Free Rad. Biol. Med.*, **18**, 949–953.
- Chernoff, R. (2005) Micronutrients requirements in older women. *Am. J. Clin. Nutr.*, **81**, 1240S–1245S.
- Cho, M.L., Han, J.H. and You, S.G. (2011) Inhibitory effects of fucans sulfates on enzymatic hydrolysis of starch. *LWT – Food Sci. Technol.*, **44**, 1164–1171.
- Cho, Y.S., Jung, W.K., Kim, J.A., Choi, I.W. and Kim, S.K. (2009) Beneficial effects of fucoidan on osteoblastic MG-63 cell differentiation. *Food Chem.*, **116**, 990–994.
- Choi, E.Y., Hwang, H.J., Kim, I.H. and Nam, T.J. (2009) Protective effects of a polysaccharide from *Hizikia fusiformis* against ethanol toxicity in rats. *Food Chem. Toxicol.*, **47**, 134–139.
- Costa, L.S., Fidelis, G.P., Cordeiro, S.L. *et al.* (2010) Biological activities of sulphated polysaccharides from tropical seaweeds. *Biomed. Pharmacother.*, **64**, 21–28.
- Croft, M.T., Lawrence, A.D., Raux-Deery, E., Warren, M.J. and Smith, A.G. (2005) Algae acquire vitamin B12 through a symbiotic relationship with bacteria. *Nature*, **438**, 90–93.
- Croft, M.T., Warren, M.J. and Smith, A.G. (2006) Algae need their vitamins. *Eukaryot. Cell*, **5**, 1175–1183.
- Damonte, E., Neyts, J., Pujol, C.A. *et al.* (1994) Antiviral activity of a sulphated polysaccharide from the red seaweed *Nothogenia fastigiata*. *Biochem. Pharmacol.*, **47**, 2187–2192.
- Darragh, A., Garrick, D.J., Moughan, P.J. and Hendriks, W.H. (1996) Correction for amino acid loss during acid hydrolysis of a purified protein. *Anal. Biochem.*, **236**, 199–207.
- Dawczynski, CH., Schäfer, U., Leiterer, M. and Jahreis, G. (2007) Nutritional and toxicological importance of macro, trace, and ultra-trace elements in algae food products. *J. Agric. Food Chem.*, **55**, 10470–10475.
- Dawczynski, CH., Schubert, R. and Jahreis, G. (2007) Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chem.*, **103**, 891–899.
- Deniaud, E., Quemener, B., Fleurence, J. and Lahaye, M. (2003) Structural studies of the mix-linked  $\beta$ -(1→3)/ $\beta$ -(1→4)-D-xylans from the cell wall of *Palmaria palmata* (Rhodophyta). *Int. J. Biol. Macromol.*, **33**, 9–18.
- Denis, C., Moranças, M., Li, M. *et al.* (2010) Study of the chemical composition of edible red macroalgae *Grateloupia turuturu* from Brittany (France). *Food Chem.*, **119**, 913–917.
- De Oliveira, M.N., Freitas, A.L.P., Carvalho, A.F.U. *et al.* (2009) Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. *Food Chem.*, **115**, 254–259.
- DeVries, J.W. (2003) On defining dietary fibre. *Proc. Nutr. Soc.*, **62**, 37–43.
- DeVries, J.W., Prosky, L., Li, B. and Cho, S. (1999) A historical perspective on defining dietary fiber. *Cereal Foods World*, **44**, 367–369.
- De Zoysa, M., Nikapitiya, Ch., Jeon, Y.J., Jee, Y. and Lee, J. (2008) Anticoagulant activity of sulphated polysaccharide isolated from fermented brown seaweed *Sargassum fulvellum*. *J. Appl. Phycol.*, **20**, 67–74.
- Díez, I., Santolaria, A. and Gorostiaga, J.M. (2003) The relationship of environmental factors to the structure and distribution of subtidal seaweed vegetation of the western Basque coast (N Spain). *Estuarine, Coastal and Shelf Science*, **56**, 1041–1054.
- Ezeagu, I.E., Petzke, J.K., Metges, C.C., Akinsoyinu, A.O. and Ologhobo, A.D. (2002) Seed protein contents and nitrogen-to-protein conversion factors for some uncultivated tropical plant seeds. *Food Chem.*, **78**, 105–109.
- Fairweather-Tait, S. and Hurrell, R.F. (1996) Bioavailability of minerals and trace elements. *Nutr. Res. Rev.*, **9**, 295–324.
- FAO (2006) *The State of World Fisheries and Aquaculture 2006*. World Aquaculture Production by Species Groups. FAO Fisheries Department, Rome.
- Fleurence, J. (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.*, **10**, 25–28.

- Fleurence, J., Chenard, E. and Luçon, M. (1999) Determination of the nutritional value of proteins obtained from *Ulva armoricana*. *J. Appl. Phycol.*, **11**, 231–239.
- Floreto, E.A.T. and Teshima, S. (1998) The fatty acid composition of seaweeds exposed to different levels of light intensity and salinity. *Bot. Mar.*, **41**, 467–484.
- Fountoulakis, M. and Lahm, H.W. (1998) Hydrolysis and amino acid composition analysis of proteins. *J. Chromatogr. A*, **826**, 109–134.
- Friedman, M. (1996) Nutritional value of proteins from different food sources. A review. *J. Agric. Food Chem.*, **44**, 6–29.
- Fujihara, S., Kasuga, A. and Aoyagi, Y. (2001) Nitrogen-to-protein conversion factors for common vegetables in Japan. *J. Food Sci.*, **66**, 412–415.
- Fürst, P. (2009) Basics in clinical nutrition: Proteins and amino acids. (2009) *e-SPEN, Eur. e-J. Clin. Nutr. Metab.*, **4**, e62–e65.
- Galland-Irmouli, A.V., Fleurence, J., Lamghari, R. *et al.* (1999) Nutritional value of proteins from edible seaweed *Palmaria palmata* (Dulse). *J. Nutr. Biochem.*, **10**, 353–359.
- Ghimire, K.N., Inoue, K., Ohto, K. and Hayashida, T. (2008) Adsorption study of metal ions onto crosslinked seaweed *Laminaria japonica*. *Biores. Technol.*, **99**, 32–37.
- Gressler, V., Yokoya, N.S., Fujii, M.T. *et al.* (2010) Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. *Food Chem.*, **120**, 585–590.
- Gupta, M. and Chandra, P. (1998) Bioaccumulation and toxicity of mercury in rooted-submerged macrophyte *Vallisneria spiralis*. *Env. Poll.*, **103**, 327–332.
- Hamza, I. and Gitli, J.G. (2002) Chaperones for cytochrome c oxidase and human disease. *J. Bioenerg. Biomembr.*, **34**, 381–388.
- Harrill, I., Kylen, A.M., Weis, A. and Dyar, E. (1959) Relation of dietary fat and supplementary riboflavin to tissue levels of cholesterol, riboflavin and total lipids in rats. *J. Nutr.*, **69**, 356–364.
- Harris, P.J. and Ferguson, L.R. (1993) Dietary fibre: its composition and role in protection against colorectal cancer. *Mutat. Res.*, **290**, 97–110.
- Hashim, M.A. and Chu, K.H. (2004) Biosorption of cadmium by brown, green, and red seaweeds. *Chem. Eng. J.*, **97**, 249–255.
- Hegedüs, M., Pedersen, B. and Eggum, B.O. (1985) The influence of milling on the nutritive value of flour from cereal grains. 7. Vitamins and tryptophan. *Plant Foods Hum. Nutr.*, **35**, 175–180.
- Herbert, V. (1988) Vitamin B-12: plant sources, requirements, and assay. *Am. J. Clin. Nutr.*, **48**, 852–858.
- Hernández-Carmona, G., Carrillo-Domínguez, S., Arvizu-Higuera, D.L. *et al.* (2009) Monthly variation in the chemical composition of *Eisenia arborea* J.E. Areschoug. *J. Appl. Phycol.*, **21**, 607–616.
- Holloway, W.D., Tasman-Jones, C. and Bell, E. (1980) The hemicellulose component of dietary fiber. *Am. J. Clin. Nutr.*, **33**, 260–263.
- Holloway, W.D., Tasman-Jones, C. and Lee, S.P. (1978) Digestion of certain fractions of dietary fiber in humans. *Am. J. Clin. Nutr.*, **31**, 927–930.
- Holloway, W.D., Tasman-Jones, C. and Maher, K. (1983) Pectin digestion in humans. *Am. J. Clin. Nutr.*, **37**, 253–255.
- Honya, M., Mori, H., Anzai, M., Araki, Y. and Nisizawa, K. (1999) Monthly changes in the content of fucans, their constituent sugars and sulphate in culture *Laminaria japonica*. *Hydrobiologia*, **398/399**, 411–416.
- Hou, X. and Yan, X. (1998) Study on the concentration and seasonal variation of inorganic elements in 35 species of marine algae. *Sci. Total Environ.*, **222**, 141–156.
- Houston, M.C. (2005) Nutraceuticals, vitamins, antioxidants, and minerals in the prevention and treatment of hypertension. *Progr. Cardiovasc. Dis.*, **47**, 396–449.
- Hu, S., Tang, C.H.H. and Wu, M. (1996) Cadmium accumulation by several seaweeds. *Sci. Total Environ.*, **81**, 1240S–1245S.
- Iwao, T., Kurashima, A. and Maegawa, M. (2008) Effect of seasonal changes in the photosynthates mannitol and laminaran on maturation of *Ecklonia cava* (Phaeophyceae, Laminariales) in Nishiki Bay, central Japan. *Phycol. Res.*, **56**, 1–6.
- Jaulneau, V., Lafitte, C., Jacquet, Ch. *et al.* (2010) Ulvan, a sulphate polysaccharide from green algae, activates plant immunity through the jasmonic acid signaling pathway. *J. Biomed. Biotechnol.*, **2010**, 1–11.
- Jiménez-Escrig, A. and Sánchez-Muniz, F.J. (2000) Dietary fibre from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res.*, **4**, 585–598.
- Kerxhali, J.S., Vogel, W., Broverman, D.M., Klaiber, E.L. (1975) Effect of ascorbic acid on the human electroencephalogram. *J. Nutr.*, **105**, 1356–1358.
- Kim, E.J., Park, S.Y., Lee, J.Y. and Park, J.H.Y. (2010a) Fucoidan present in brown algae induces apoptosis of human colon cancer cells. *BMC Gastroenterol.*, **10**, 1–11.
- Kim, K.N., Heo, S.J., Kang, S.M., Ahn, G. and Jeon, Y.J. (2010b) Fucoxanthin induces apoptosis in human leukemia HL-60 cells through a ROS-mediated Bcl-xL pathway. *Toxicol. in Vitro*, **24**, 1648–1654.
- Kolb, N., Vallorani, L., Milanovic, N. and Stocchi, V. (2004) Evaluation of marine algae Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*) as food supplements. *Food Technol. Biotechnol.*, **42**, 57–61.

- Kumar, V. and Kaladharan, P. (2007) Amino acids in the seaweeds as an alternative source of protein for animal feed. *J. Mar. Biol. Ass. India*, **49**, 35–40.
- Küpfer, F.C., Schweigert, N., Gall, E.A., Legendre, J.M., Vilter, H. and Kloareg, B. (1998) Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodine. *Planta*, **207**, 163–171.
- Lares, M.L., Flores-Muñoz, G. and Lara-Lara, R. (2002) Temporal variability of bioavailable Cd, Hg, Zn, M and Al in an upwelling regime. *Env. Poll.*, **120**, 595–608.
- Leary, S.C., Cobine, P.A., Kaufman, B.A. *et al.* (2007) The human cytochrome c oxidase assembly factors SCO1 and SCO2 have regulatory roles in the maintenance of cellular homeostasis. *Cell Metab.*, **5**, 9–20.
- Levesque, C.L., Moehn, S., Pencharz, P.B. and Ball, R.O. (2010) Review of advances in metabolic bioavailability of amino acids. *Livest. Sci.*, **133**, 4–9.
- Li, B., Lu, F., Wei, X. and Zhao, R. (2008) Fucoidan: structure and bioactivity. *Molecules*, **13**, 1671–1695.
- Likuski, H.J.A. and Forbes, R.M. (1964) Effect of phytic acid on the availability of zinc in amino acid and casein diets fed to chicks. *J. Nutr.*, **84**, 145–148.
- Lindenbaum, J., Rosenberg, I.H., Wilson, P.W.F., Stabler, S.P. and Allen, R.H. (1994) Prevalence of cobalamin deficiency in the Framingham elderly population. *Am. J. Clin. Nutr.*, **60**, 2–11.
- Lourenço, S.O., Barbarino, E., Marquez, U.M.L. and Aidar, E. (1998) Distribution of intracellular nitrogen in marine microalgae: basis for the calculation of specific nitrogen-to-protein conversion factors. *J. Phycol.*, **34**, 798–811.
- Lourenço, S.O., Barbarino, E., De-Paula, J.C., Otávio da Pereira, L.S. and Marquez, U.M.L. (2002) Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. *Phycol. Res.*, **50**, 233–241.
- Lölliger, J. (2000) The use and utility of glutamates as flavoring agents in foods. Function and importance of glutamate for savory foods. *J. Nutr.*, **130**, 915S–920S.
- Mabeau, S. and Fleurence, J. (1993) Seaweed in food products: biochemical and nutritional aspects. *Trends Food Sci. Technol.*, **4**, 103–107.
- MacArtain, P., Gill, C.H.I.R., Brooks, M., Campbell, R. and Rowland, I.R. (2007) Nutritional Value of Edible Seaweed. *Nutr. Rev.*, **65**, 535–543.
- Maeda, H., Tsukui, T., Sashima, T., Hosokawa, M. and Miyashita, K. (2008) Seaweed carotenoid, fucoxanthin, as multi-functional nutrient. *Asia Pac. J. Clin. Nutr.*, **17**, 196–199.
- Manivannan, K., Thirumaran, G., Devi, G.K., Hemalatha, A. and Anantharaman, P. (2008) Biochemical Composition of Seaweeds from Mandapam Coastal Regions along Southwest Coast of India. *Am.-Eur. J. Bot.*, **1**, 32–37.
- Marinho-Soriano, E., Fonseca, P.C., Carneiro, M.A.A. and Moreira, W.S.C. (2006) Seasonal variation in the chemical composition of two tropical seaweeds. *Biores. Technol.*, **97**, 2402–2406.
- Marshall, S., Scott, G.W. and Tobin, M.L. (2007) Comparison of nutritive chemistry of a range of temperate seaweeds. *Food Chem.*, **100**, 1331–1336.
- Martínez, B. and Rico, J.M. (2002) Seasonal variation of P content and major N pools in *Palmaria palmata* (Rhodophyta). *J. Phycol.*, **38**, 1082–1089.
- Matsumoto, M., Hosokawa, M., Matsukawa, N. *et al.* (2010) Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. *Eur. J. Nutr.*, **49**, 243–249.
- McCall, K.A., Huang, C.H., Fierke, C.A. (2000) Function and mechanism of zinc metalloenzymes. *J. Nutr.*, **130**, 1437S–1446S.
- McCleary, B.V. (2003). Dietary fibre analysis. *Proc. Nutr. Soc.*, **62**, 3–9.
- McDermid, K.J. and Stuercke, B. (2003) Nutritional composition of edible Hawaiian seaweeds. *J. Appl. Phycol.*, **15**, 513–524.
- McHugh, D.J. (2003) *A guide to the seaweed industry*, FAO Fisheries technical paper 441. Food and Agriculture Organization of the United Nations, Rome.
- Melo, M.R.S., Feitosa, J.P.A., Freitas, A.L.P. and de Paula, R.C.M. (2002) Isolation and characterization of soluble sulphated polysaccharide from the seaweed *Gracilaria cornea*. *Carbohydr. Polym.*, **49**, 491–498.
- Mihrianyan, A., Edsman, K. and Strømme, M. (2007) Rheological properties of cellulose hydrogels prepared from Cladophora cellulose powder. *Food Hydrocoll.*, **21**, 267–272.
- Mišurcová, L. (2008) New nutritional aspects and utilization of seaweed and freshwater algae in human diet. Thesis. TBU Zlín (in Czech).
- Mišurcová, L., Stratilová, I. and Kráčmar, S. (2009) The mineral content of products from freshwater algae and seaweed (in Czech). *Chem. listy*, **103**, 1027–1033.
- Mišurcová, L., Kráčmar, S., Klejdus, B. and Vacek, J. (2010) Nitrogen content, dietary fiber, and digestibility in algal food products. *Czech J. Food Sci.*, **28**, 27–35.
- Miyake, Y., Sasaki, S., Ohya, Y. *et al.* (2006) Dietary intake of seaweed and minerals and prevalence of allergic rhinitis in Japanese pregnant females: baseline data from the Osaka Maternal and Child Health Study. *Ann. Epidemiol.*, **16**, 614–621.
- Miyashita, K. (2009) The carotenoid fucoxanthin from brown seaweed affects obesity. *Lipid Technol.*, **21**, 186–190.
- Mozzachiodi, R., Scuri, R., Roberto, M. and Brunelli, M. (2001) Caulerpyne, a toxin from the seaweed *Caulerpa*

- taxifolia*, depresses afterhyperpolarization in invertebrate neurons. *Neuroscience*, **107**, 519–526.
- Muñoz-Barbosa, A., Gutierrez-Galindo, E.A. and Flores-Muñoz, G. (2000) *Mytilus californianus* as an indicator of heavy metals on the northwest coast of Baja California, Mexico. *Mar. Env. Res.*, **49**, 123–144.
- Norris, E.R., Simeon, M.K. and Williams, H.B. (1936) The vitamin B and vitamin C content of marine algae. *J. Nutr.*, **13**, 425–433.
- Norziah, M.H. and Ching, CH.Y. (2000) Nutritional composition of edible seaweed *Gracilaria changgi*. *Food Chem.*, **68**, 69–76.
- O'Dell, B.L. (1989) Mineral interactions relevant to nutrient requirements. *J. Nutr.*, **119**, 1832–1838.
- Ortiz, J., Romero, N., Robert, P. *et al.* (2006) Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chem.*, **99**, 98–104.
- Parisi, A.F. and Vallee, B.L. (1969) Zinc metalloenzymes: characteristics and significance in biology and medicine. *Am. J. Clin. Nutr.*, **22**, 1222–1239.
- Phaneuf, D., Cote, I., Dumas, P., Ferron, L.A. and Leblanc, A. (1999) Evaluation of the contamination of marine algae (seaweed) from the St. Lawrence River and likely to be consumed by humans. *Env. Res. Sect. A*, **80**, 175–182.
- Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N. and Gowda, L.R. (2009) Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chem.*, **115**, 501–508.
- Prosky, L. (1999) What is fibre? Current controversies. *Trends Food Sci. Technol.*, **30**, 271–275.
- Ramos, M.V., Monteiro, A.C.O., Moreira, R.A., Carvalho, A.F.F.U. (2000). Amino acid composition of some Brazilian seaweed species. *J. Food Biochem.*, **24**, 33–39.
- Reddy, M.B., Hurrell, R.F. and Cook, J.D. (2000) Estimation on nonheme-Iron bioavailability from meal composition. *Am. J. Clin. Nutr.*, **71**, 937–943.
- Reitz, S.R. and Trumble, J.T. (1996a) Effects of cytokinin-containing seaweed extract on *Phaseolus lunatus* L.: influence of nutrient availability and apex removal. *Bot. Mar.*, **39**, 33–38.
- Reitz, S.R. and Trumble, J.T. (1996b) Cytokinin-containing seaweed extract does not reduce damage by an insect herbivore. *HortScience*, **31**, 102–105.
- Riget, F., Johansen, P. and Asmud, G. (1995) Natural seasonal variation of cadmium, lead and zinc in brown seaweed (*Fucus vesiculosus*). *Mar. Poll. Bull.*, **30**, 409–413.
- Rioux, L.E., Turgeon, S.L. and Beaulieu, M. (2007) Characterization of polysaccharides extracted from brown seaweeds. *Carbohydr. Polym.*, **69**, 530–537.
- Rupérez, P. and Saura-Calixto, F. (2001) Dietary fibre and physicochemical properties of edible Spanish seaweed. *Eur. Food Res. Technol.*, **212**, 349–354.
- Rupérez, P., Ahrazem, O. and Leal, J.A. (2002) Potential antioxidant capacity of sulphated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *J. Agric. Food Chem.*, **50**, 840–845.
- Rupérez, P. and Toledano, G. (2003) Indigestible fraction of edible marine seaweeds. *J. Sci. Food Agric.*, **83**, 1267–1272.
- Saenko, G.N., Kravtsova, Y.Y., Ivanenko, V.V. and Sheludko, S.I. (1978) Concentration of iodine and bromine by plants in the Seas of Japan and Okhotsk. *Mar. Biol.*, **47**, 243–250.
- Salo-Väänänen, P.P. and Koivistoinen, P.E. (1996) Determination of protein in foods: comparison of net protein and crude protein ( $N \times 6.25$ ) values. *Food Chem.*, **57**, 27–31.
- Sánchez-Machado, D.I., López-Cervantes, J., López-Hernández, J., Paseiro-Losada, P. and Simal-Lozano, J. (2004) Determination of the uronic acid composition of seaweed dietary fibre by HPLC. *Biomed. Chromatogr.*, **18**, 90–97.
- Santoso, J., Gunji, S., Yosire-Stark, Y. and Suzuki, T. (2006) Mineral contents of Indonesian seaweeds and mineral solubility affected by basic cooking. *Food Sci. Technol. Res.*, **12**, 59–66.
- Satterlee, L.D., Marshall, H.F. and Tennyson, J.M. (1979) Measuring protein quality. *J. Am. Oil Chem. Soc.*, **56**, 103–109.
- Sawidis, T., Brown, M.T., Zachariadis, G. and Srtatis, I. (2001) Trace metal concentrations in marine microalgae from different biotopes in the Aegean Sea. *Env. Int.*, **27**, 43–47.
- Shimonaga, T., Fujiwara, S., Kaneko, M. *et al.* (2007) Variation in storage  $\alpha$ -polyglucans of red algae: amylose and semi-amylopectin types in Porphyridium and glycogen type in Cyanidium. *Mar. Biotechnol.*, **9**, 192–202.
- Schmid, D., Schürch, C., Züllig, F., Nissen, H.P. and Prieur, H. (2003) Mycosporine-like amino acids: natural UV-screening compounds from red algae to protect the skin against photoaging. *SÖFW-Journal*, **129**, 1–5.
- Schneeman, B.O. (1999) Fiber, inulin and oligofructose: similarities and differences. *J. Nutr.*, **129**, 1424S–1427S.
- Simopoulos, A.P. (2002a) The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.*, **56**, 365–379.
- Simopoulos, A.P. (2002b) Omega-3 fatty acids in inflammation and autoimmune diseases. *J. Am. Coll. Nutr.*, **21**, 495–505.
- Simopoulos, A.P. (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular diseases and other chronic diseases. *Exp. Biol. Med.*, **233**, 674–688.

- Slavin, J. (2003) Impact of the proposed definition of dietary fiber on nutrient databases. *J. Food Comp. Anal.*, **16**, 287–291.
- Smith, A.G., Croft, M.T., Moulin, M. and Webb, M.E. (2007) Plants need their vitamins too. *Curr. Opin. Plant Biol.*, **10**, 266–275.
- Stirk, W.A. and Staden, J.V. (1997) Comparison of cytokinin- and auxin-like activity in some commercially used seaweed extracts. *J. Appl. Phycol.*, **8**, 503–508.
- Stott, D.J., Langhorne, P., Hendry, A. *et al.* (1997) Prevalence and haemopoietic effects of low serum vitamin B<sub>12</sub> levels in geriatric medical patients. *Br. J. Nutr.*, **78**, 57–63.
- Suetsuna, K. and Nakano, T. (2000) Identification of an antihypertensive peptide from peptic digest of Wakame (*Undaria pinnatifida*). *J. Nutr. Biochem.*, **11**, 450–454.
- Suzuki, Y., Kametani, T. and Maruyama, T. (2005) Removal of heavy metals from aqueous solution by nonliving Ulva seaweed as biosorbent. *Water Res.*, **39**, 1803–1808.
- Tanaka, T., Kurabayashi, M., Aihara, Y., Ohyama, Y. and Nagai, R. (2000) Inducible expression of manganese superoxide dismutase by phorbol 12-myristate 13-acetate is mediated by Sp1 in endothelial cells. *Arterioscler., Thromb., Vasc. Biol.*, **20**, 392–401.
- Teas, J., Pino, S., Critchley, A. and Braverman, L.E. (2004) Variability of iodine content in common commercially available edible seaweeds. *Thyroid*, **14**, 836–841.
- Titgemeyer, E.C., Bourquin, L.D., Fahey, G.C., Jr. and Garleb, K.A. (1991) Fermentability of various fiber sources by human fecal bacteria in vitro. *Am. J. Clin. Nutr.*, **53**, 1418–1424.
- Tsui, M.T.K., Cheung, K.C., Tam, N.F.Y. and Wong, M.H. (2006) A comparative study on metal sorption by brown seaweed. *Chemosphere*, **65**, 51–56.
- Valls, R., Artaud, J., Amade, P., Vincente, N. and Piovetti, L. (1994) Determination of caulerpyne, a toxin from the green alga *Caulerpa taxifolia* (Caulerpaceae). *J. Chromatogr. A*, **663**, 114–118.
- Van Soest, P.J. (1963) Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *J. Ass. Off. Anal. Chem.*, **46**, 829–835.
- Van Soest, P.J. and Wine, R.H. (1967) Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituent. *J. Ass. Off. Anal. Chem.*, **50**, 50–55.
- Vasconcelos, M.T.S.D. and Leal, M.F.C. (2001) Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. *Mar. Chem.*, **74**, 65–85.
- Velíšek, J. (2002) *Food Chemistry 2*. (in Czech), OSSIS, Tábor.
- Vermilion, J.L., Ballou, D.P., Massey, V. and Coon, M.J. (1981) Separate roles for FMN and FAD in catalysis by liver microsomal NADPH-cytochrome P-450 reductase. *J. Biol. Chem.*, **256**, 266–277.
- Villares, R., Puente, X. and Carballeira, A. (2002) Seasonal variation and background levels of heavy metals in two green seaweeds. *Env. Poll.*, **119**, 79–90.
- Viola, R., Nyvall, P. and Pedersén, M. (2001) The unique features of starch metabolism in red algae. *Proc. Roy. Soc. Lond. B*, **268**, 1471–1422.
- Walsh, C.T., Sandstead, H.H., Prasad, A.S., Newberne, P.M. and Fraker, P.J. (1994) Zinc: Health Effects and Research Priorities for the 1990s. *Env. Health Persp.*, **102**, 5–46.
- Werner, T., Motyka, V., Strnad, M. and Schmölling, T. (2001) Regulation of plant growth by cytokinin. *Proc. Natl. Acad. Sci. USA*, **98**, 10487–10492.
- WHO (2002) Report of a Joint WHO/FAO/UNU Expert Consultation, WHO Technical Report Series 935. Protein and Amino Acid requirements in Human Nutrition. WHO Press, Geneva.
- Wienk, K.J.H., Marx, J.J.M., Santos, M. *et al.* (1997) Dietary ascorbic acid raises Iron absorption in anaemic rats through enhancing mucosal iron uptake independent of iron solubility in the digesta. *Br. J. Nutr.*, **77**, 123–131.
- Williams, C.J. and Edyvean, R.G.J. (1997) Ion exchange in nickel biosorption by seaweed materials. *Biotechnol. Progr.*, **13**, 424–428.
- Wolzak, A., Elías, L.G. and Bressani, R. (1981) Protein quality of vegetable proteins as determined by traditional biological methods and rapid chemical assay. *J. Agric. Food Chem.*, **29**, 1063–1068.
- Wong, K.H. and Cheung, C.K. (2001) Nutritional evaluation of some subtropical red and green seaweeds Part II. *In vitro* protein digestibility and amino acid profiles of protein concentrates. *Food Chem.*, **72**, 11–17.
- Wong, C.K., Ooi, V.E.C. and Ang, P.O. (2000) Protective effects of seaweeds against liver injury caused by carbon tetrachloride in rats. *Chemosphere*, **41**, 173–176.
- Yamaguchi, S. and Ninomiya, K. (2000) The use and utility of glutamates as flavoring agents in food. Umami and food palatability. *J. Nutr.*, **130**, 921S–926S.
- Yamamoto, Y., Fujisawa, A., Hara, A. and Dunlap, W.C. (2001) An unusual vitamin E constituent ( $\alpha$ -tocomonoenol) provides enhanced antioxidant protection in marine organisms adapted to cold-water environments. *Proc. Natl. Acad. Sci. USA*, **98**, 13144–13148.
- Yoshioka, Y., Satoh, H. and Mitani, M. (2007) Theoretical study on electronic structures of FeOO, FeOOH, FeO(H<sub>2</sub>O), and FeO in hemes: As intermediate models of dioxygen reduction in cytochrome c oxidase. *J. Inorg. Biochem.*, **101**, 1410–1427.
- You, S.G., Yang, Ch., Lee, H.Y. and Lee, B.Y. (2010) Molecular characteristic of partially hydrolyzed fucoidans from sporophyll of *Undaria pinnatifida* and their *in vitro* anti-cancer activity. *Food Chem.*, **119**, 554–559.

- Yu, Q., Matheickal, J.T., Yin, P. and Kaewsarn, P. (1999) Heavy metal uptake capacities of common marine macro algal biomass. *Water Res.*, **33**, 1534–1537.
- Yuan, Y.V., Westcott, N.D., Hu, Ch. and Kitts, D.D. (2009) Mycosporine-like amino acid composition of the edible red alga, *Palmaria palmata* (dulse) harvested from the west and east coasts of Grand Manan Island, New Brunswick. *Food Chem.*, **112**, 321–328.
- Zhang, L. and Wong, M.H. (2007) Environmental mercury contamination in China: Sources and impacts. *Env. Int.*, **33**, 108–121.
- Zvyagintseva, T.N., Shevchenko, N.M., Nazarenko, E.L. *et al.* (2005) Water-soluble polysaccharides of some brown algae of the Russian Far-East. Structure and biological action of low-molecular mass polyuronans. *J. Exp. Mar. Biol. Ecol.*, **320**, 123–131.

# 8

## Structural Peculiarities of Sulfated Polysaccharides from Red Algae *Tichocarpus crinitus* (Tichocarpaceae) and *Chondrus pinnulatus* (Gigartinaceae) Collected at the Russian Pacific Coast

**Anna O. Barabanova and Irina M. Yermak**

*Pacific Institute of Bioorganic Chemistry Far-East Branch of Russian Academy of Sciences, Vladivostok, Russia*

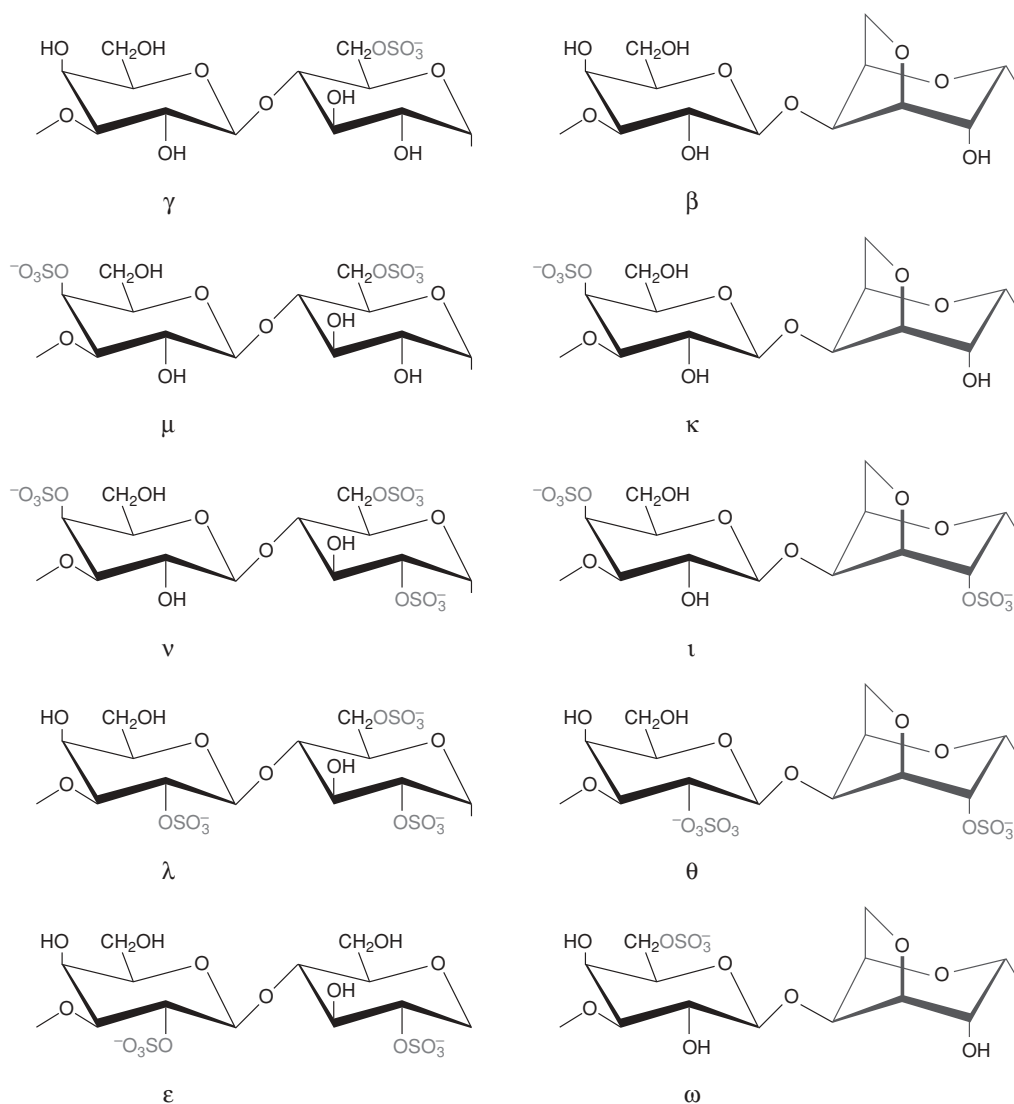
### 8.1 Introduction

Marine algae (seaweeds) have been used in the food industry and medical practice for more than 600 years. The cell walls of red algae contain considerable amounts of peculiar sulfated polysaccharides, in particular, carrageenan and agar, which find a wide application in practice and have no analogues among other plant polysaccharides. These galactans possess several functions in seaweed. Polysaccharides are the energy resources, which take part in the formation of cell wall, outer capsules and internal matrices; and provide selective cation absorption (Craigie, 1990).

In the food industry, carrageenans are widely utilized due to their excellent physical functional properties, such as thickening, gelling and stabilizing abilities (Yermak and Khotimchenko, 2003). These polysaccharides have been

safely consumed as constituents of food products for many years. The safety of carrageenans for use in foods was confirmed at the 57th meeting of the JECFA-Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee in Food Additives (JECFA 2001). According to the JECFA, only degraded carrageenans were associated with adverse effects and should not be used as food additives.

Recently, carrageenans have been found to be of importance in pharmaceutical development. Carrageenans were used to reduce amount of polymorphic transformation in tableting (Schmidt *et al.*, 2003) to produce controlled release matrix tablets (Keppeler *et al.*, 2009), to achieve interactions with drugs for modified release systems (Thommes and Kleinebudde, 2006). Carrageenans have been found to exhibit various biological activities, including antiviral,



**Figure 8.1** The disaccharide units of the main carrageenan types.

antitumoral (Yermak and Khotimchenko, 2003, Zhou *et al.*, 2004) antihyperlipidemic (Panlasigui *et al.*, 2003) and anticoagulant activities (Caceres *et al.*, 2000). Carrageenans also possess immunomodulatory properties, and they can exert both immunopotentiative and immunosuppressive actions (Hanazawa *et al.*, 1982, Mancino and Minucci, 1983, Yermak and Khotimchenko, 2003).

Carrageenans can thus be defined as a group of galactan polysaccharides extracted from red seaweeds of the Gigartinales, Solieriales, Hypniales, and Phyllophorales families that have an ester sulfate content of 18% or more (Chapman and Chapman, 1980). Carrageenans are sulfated linear galactans, whose basic structural units are disaccharide, carrabiose, consisting of alternating  $\beta$ -1,3- and  $\alpha$ -1,4-linked galactose residues. The 1,4-linked residues

are commonly, but not invariably, present as the 3,6-anhydrogalactose. Some repeating disaccharide units of that general structure are shown in Figure 8.1. Variation on this basic structure result from the content of 3,6-anhydrogalactose, location, and number of sulfate groups (Rees 1963, Dolan and Rees, 1966, Anderson *et al.*, 1968, Penman and Rees, 1973). The 1,3-linked D-galactose residues occur as the 2- and 4-sulphate, or are occasionally unsulfated, while the 1,4-linked residues occur as the 2-sulfate, the 6-sulfate, the 2,6-disulfate, the 3,6-anhydride and 3,6-anhydride 2-sulfate. Sulfate at C-3 apparently never occurs. The hydroxyl groups on the carrabioses can be further substituted by pyruvate ketal, branched glycosides or occasionally be methyl ethers (Chiovitti *et al.*, 1997, 2001). There are about 20 different structures recognized, and

referred to by Greek letters, that are grouped in families:  $\kappa$ ,  $\beta$ , and  $\lambda$ .

The chemical composition of a carrageenan sample varies with the algae source and even between samples prepared in the same way from different batches of a given species of algae. However, it is usually possible to obtain more homogeneous fractions by salt precipitation. In 1953, Smith and Cook fractionated the carrageenan with potassium chloride and isolated two fractions, which they named  $\kappa$ - and  $\lambda$ -carrageenan (Smith and Cook, 1954).  $\kappa$ -Carrageenan can be separated from  $\lambda$ -carrageenan because it precipitates by potassium ions whereas the other does not. Chemical studies of these fractions revealed that nearly half of the sugar units in  $\kappa$ -carrageenan were 3,6-anhydrogalactose, while  $\lambda$ -carrageenan contained little or none of this residue. Later, a third carrageenan type, gelling with calcium ions, was defined as  $\iota$ -carrageenan.

$\mu$ , $\nu$ -Carrageenans are believed to be the precursors in the biosynthesis of  $\kappa$ - and  $\iota$ -carrageenans, respectively and can be transformed, correspondingly, into carrageenans by alkaline or enzymatic modification, which affecting the 3,6-anhydro-region (Chapman and Chapman 1980). In 1996, the letter  $\alpha$  was assigned to the carrageenan with alternating 3-linked  $\beta$ -D-galactopyranosyl and 4-linked 3,6-anhydro- $\alpha$ -D-galactopyranosyl-2-sulfate units, that had been isolated from a Burmese sample of *Catenella nipae* (Falshaw *et al.*, 1996).  $\alpha$ -Carrageenan differs from the more commonly occurring  $\iota$ -carrageenan by absence sulfate ester group on the 4-position of the 3-linked units.

A new type of carrageenan in which the 1,3-linked residues are not sulfated and 1,4-linked units lack sulfate at C-2 was first reported by Greer and Yaphe (1984). This type was called  $\beta$ -carrageenan. Later other researchers have shown that ion-independent  $\beta$ -carrageenan may also be isolated from *Euclima speciosa* and *Endocladia muricatum* (Renn *et al.*, 1993).

Most of the algae species contain two or more recognizable carrageenan types; it is unusual for an alga to be reported as containing a single carrageenan. Native carrageenan from different algae may be regarded as varying mixtures of the limit polysaccharides and intermediate hybrids ranging in degree of anhydridation and 2-sulfation of the 1,4-linked residues (Greer and Yaphe 1984; Craigie 1990). It is often difficult to determine either the various carrageenans occur as separate chemical entities in a mixture of cell wall polysaccharides or they are glycosidically linked in a single hybrid macromolecule.

$\beta$ -Carrageenans typically occur together with  $\kappa$ -units. Even the first reported source for  $\beta$ -carrageenan, *E. gelatinosa* (Greer and Yaphe 1984), contains molecules with a hybrid structure of both  $\beta$ - and  $\kappa$ -types. Evidence for a  $\kappa/\beta$ -hybrid carrageenan in *Tichocarpus crinitus* is derived from  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy and

methylation analyses (Usov and Arkhipova, 1981). Investigations of polysaccharides from five species of the Dictyoneleaceae show that they are composed mainly of hybrid (or mixed)  $\beta/\kappa$  carrageenans (Liao *et al.*, 1993). Furcellaran from *Furcellaria lumbricalis* containing  $\kappa$ - and  $\beta$ -segments is a low-sulfated carrageenan (Knutsen and Grasdalen, 1987). The distribution of the  $\kappa$ - and  $\beta$ -segments along the furcellaran polymer is unknown.

To describe complex carrageenan structure, a uniform letter code nomenclature has been developed (Knutsen *et al.*, 1994). Based on this nomenclature the 3-linked  $\beta$ -D-galactopyranose is G, while 4-linked  $\beta$ -D-galactopyranose and 4-linked  $\alpha$ -D-galactopyranose have D- and DA-units, respectively. The hemi-ester sulfate, methyl and pyruvate ketal are denoted by single letters S, M, and P, respectively. If agarose, the 4-linked  $\alpha$ -D-galactopyranose, has the L-configuration then the corresponding letter is L. For example, commercial  $\iota$ -,  $\kappa$ -, and  $\lambda$ -carrageenans can be described as G4S-DA2S, G4S-DA and G2S-D2S,6S.

The formation of these "molecular hybrids" is usually explained in terms of irregular or incomplete modification of a preformed galactan backbone at the polymeric level. The first biosynthetic step is considered to be the formation of a regular backbone from galactose residues. The second is sulfation of several hydroxyls (some other substitutions may also be introduced at this stage), and the final step is the enzyme elimination of sulfate from position 6 of some 4-linked  $\alpha$ -galactose residues, termed precursors, to give 3,6-anhydro- $\alpha$ -galactose residues. Since modification reactions of polymer molecules can run to completion, the biosynthesis may end in a "molecular hybrid" containing links of several carrageenan types at the same time (Craigie and Wong 1979; Chapman and Chapman 1980). Enzymatic, infrared, and NMR studies suggest that carrageenan structural hybrids (the main repeating disaccharide units are interrupted by segments of other carrageenan types) are common (Bellion *et al.* 1982; Knutsen and Grasdalen 1987; Stortz and Cerezo 1993; Falshaw *et al.* 1996). The interrupting segments may be closely spaced or widely distributed in the macromolecule. Nothing is known of the mechanisms that may control the insertion or number of such segments in polysaccharide.

It has been found that "carrageenophytes" of related families as Gigartinaceae, Phylophoraceae and Solieriaceae produce small amount of agaran, which is a diastereomer of carrageenan (Ciancia *et al.*, 1997; Estevez *et al.* 2000, 2001, 2008). Agarans are sulfated galactans, in which the  $\alpha$ -galactose or 3,6-anhydro- $\alpha$ -galactose units have the an L configuration. The sulfated galactans obtained from *Gymnogongrus turulosus* were shown composed not only by carrageenan, but also by DL-hybrid galactans or mixtures of carrageenan- and agaran-structures (Stortz and Cerezo, 2000). The same hybrid with highly complex polysaccharide

structure was also observed in *Cryptonemia crenulata* (Halymenaceae) (Zibetti *et al.*, 2005).

Carrageenan biosynthesis depends on many factors, which can be divided into exogenous, determined by the conditions of growing algae – light, temperature and salinity, and endogenous, related to the physiology of algae, in particular the species affiliation and stage of development (Chapman and Chapman, 1980; Craigie, 1990; Chopin *et al.*, 1990; Reani *et al.*, 1998). The latter is particularly important because red algae have a complex life cycle involving the alternation of vegetative, sexual and asexual reproduction (Lahaye and Kaeffer, 1997). The first studies on carrageenan structure were performed mainly on samples of polysaccharides isolated from the mixture of different forms of the alga, this greatly hamper the interpretation of the results. The detailed analysis of the carrageenans from the algae *Gigartina skottsbergii* and *Gymnogongrus torulosus* demonstrated that the cystocarps produced  $\kappa/\iota$ -carrageenans (Estevez *et al.*, 2002). The gametophytic phase of *Gigartina pistillata* synthesizes complex  $\kappa/\iota$ -type carrageenan with varying proportions of  $\nu$ -carrabiose units, which are precursors of  $\iota$ -type (Amimi *et al.*, 2001). The tetrasporic life stage of Gigartinacean seaweeds contains different types of carrageenan to those found in the gametophytes. These carrageenans are characterized by having a sulfate ester substituent on the 2-position of the 3-linked units, most commonly  $\lambda$ -family of carrageenan. The extract obtained from the tetrasporic alga *Gigartina atropurpurea* appears to be a complex mixture or hybrid containing, not only  $\lambda$ -,  $\xi$ -, and  $\pi$ -carrageenans but also some carrageenan structures containing unsulfated 3,6-anhydrogalactose units (Falshaw *et al.*, 2003). Small amounts of  $\theta$ -carrageenan have been found in extract of tetrasporic *Gigartina* species. Such distribution of carrageenans on the life history stage of the alga is characteristic for most species of Gigartinaceae and Phylloporaceae (Craigie, 1990; McCandless *et al.*, 1982), while the representatives of Solieriaceae and Hypneaceae produce only  $\kappa$ - and  $\theta$ -carrageenans independently of the form of the alga (Craigie, 1990). This suggests that taxonomic position of algae does not provide full information on the type of polysaccharide they contain. Therefore, the establishment of the polysaccharide composition of an alga in relation to the phase of its life cycle remains an important research objective.

Variation in carrageenan structures occur not only between polysaccharides from different species of algae. Environmental factors are known to influence phycocolloid yield and quality (Pereira *et al.*, 2009; Chopin *et al.*, 1990; Bulboa and Macchiavello, 2001), and different physiological and environmental tolerances may also influence variation in carrageenan content. This has been reported for different algae species, between different life stages of the same species (Piriz and Cerezo 1991), and even between individuals of

the same species growing under different environmental conditions.

In the early 1990s, world manufacturing of carrageenan topped 15 500 tonnes a year (Bixler, 1996), but now according to Guiry (2008), carrageenan production exceeded 50 000 tonnes in 2007/2008 with a value of over US\$600 million (not including China). An increasing demand for seaweeds and the development of new applications for hydrocolloids, have lead to the identification of alternative sources of raw material.

The main algal species that are used for carrageenan production, comprise *Chondrus crispus*, *C. ocellatus*, *Gigartina stellata*, *G. acicularis*, *G. pistillata*, *G. canaliculatus*, *G. radula*, *G. skottsbergii*, *Eucheuma cottonii*, *E. spinosum*, *E. gelatinae*, *Furcellaria fastigata*, and *Hypnea musciformis* (Bixler, 1996; Lewis *et al.*, 1988). Large reserves of *C. crispus* are found near the shores of Canada, and together with *G. stellata*, they form thick growths on the coast of France and Spain. *G. acicularis* and *G. pistillata* are widespread along the southern shore of France, north Portugal, and Morocco. The basic commercial reserves of red algae in Chile are represented by *G. radula* and *G. skottsbergii* (Buschmann *et al.*, 2001), while in Indonesia and Philippines, *E. cottonii* and *E. spinosum* are produced. In Australia and New Zealand, several species of *Gigartina* were introduced and *Eucheuma* sp. from Philippines was cultivated for their prevalence (Pickmer *et al.*, 1975). This chapter focuses on structural aspects of carrageenan from red seaweeds of Gigartinaceae and Tichocarpaceae families from the Russian Pacific coast.

## 8.2 Carrageenan sources in the Russian Far East

Carrageenan-yielding species (carrageenophytes) are abundant in the Far Eastern seas and more than 10 potential sources of hydrocolloids have been identified among the algae from the Sea of Japan (Usov, 1974; Yermak *et al.*, 1999). A high carrageenan content was found in representatives of the families Phylloporaceae (species of the *Gymnogongrus*) and Gigartinaceae (*Mastocarpus pacificus* and *Irideae cornicopiae*, as well as in three species of *Chondrus* (Yermak *et al.*, 1995). Representatives of the latter are common to all the Far Eastern seas, but they do not form pure growth and intermingle with other red algae.

At present, the potential sources of hydrocolloids for commercial utilization are algae of the Gigartinaceae (*Chondrus armatus* and *C. pinnulatus*) and Tichocarpaceae (*Tichocarpus crinitus*) families. Although it was shown that *T. crinitus*, and *C. pinnulatus* are carrageenanophytes (Usov, 1974; Yermak *et al.*, 1999), so far it has not been differentiated whether the structure of carrageenan is in transition from one developmental stage to the other and whether

both were affected by the habitat of macrophytes. Addressing these issues is important both in terms of biosynthesis of polysaccharides during ontogeny of algae, and when choosing the conditions of carrageenanophyte cultivation; and their rational use in the industrial production of carrageenan.

### 8.3 The polysaccharide composition of algae in relation to the phase of its life cycle

#### 8.3.1 The polysaccharides of *Chondrus pinnulatus* (Gigartineaceae)

*Chondrus* is one of the economically important red algae worldwide. It can be used as raw material for the phycocolloid carrageenan (Anderson and Rees, 1966). Several species of this genus – *Chondrus crispus*, *C. ocellatus* and *C. canaliculatus* – are known to exist. It should be noted that the binomial *C. canaliculatus* is under recent revision, since the morphology and phylogenetic analysis based on the rbcL sequence demonstrated that the species does not belong to *Chondrus*. The original source of carrageenan was from the red seaweed *C. crispus* (Irish moss). The first chemical analysis indicated that major components of carrageenan were of kappa and lambda types present in *Chondrus* species (Stancioff and Stanley, 1969). *C. ocellatus* could represent an alternative to a declining Irish moss fishery (Chopin and Yarish, 1998) and source of high quality carrageenan of the  $\kappa$ -family (Chopin and Wagey, 1999).

*C. pinnulatus* is an endemic representative of the Gigartineaceae family in the Far East region. This species of marine alga grows mostly on lower eulittoral and upper sublittoral rocks and stones, on open coasts with low wave action. The red alga *C. pinnulatus* (Harvey) Okamura (Gigartineaceae, Gigartinales) was first described by Harvey (1857) as *Gymnogonops pinnulatus*, collected at Hakodate, Hokkaido, Japan. Later, it was reported as *Chondrus*, largely on the basis of vegetative structures. The cells in cross-section are not regularly parenchymatous, as in *Gymnogongrus*, but loosely set as in *Chondrus*. Study of longitudinal sections revealed that the thallus was also indicative for this genus (Masuda *et al.* 1995). Among the other species of *Chondrus*, *C. pinnulatus* is quite distinct in gross morphology, and proposals have been made to include a plethora of intraspecific taxa (Brodie *et al.*, 1997).

We investigated the chemical structure of the carrageenans isolated from cystocarpic and sterile plants of *C. pinnulatus*, collected on the Russian Pacific coast (Yermak *et al.*, 2006). The total carrageenan content of the sterile plant was observed to be twice of that of the cystocarpic plants. According to data obtained by  $^{13}\text{C}$ -NMR

and Fourier transform infrared spectroscopy (FT-IR), the gelling polysaccharides from cystocarpic and sterile plants of *C. pinnulatus* have similar structures and were identified as  $\kappa/\iota$ -carrageenans. The difference between those polysaccharides was in the ratio of the  $\kappa$ - and  $\iota$ -segments, with a predominant content of  $\kappa$ -segments in the cystocarpic plants (80%). Moreover, KCl-insoluble fractions possibly contain hetero-disperse  $\mu/\nu$ -precursors: amounts of which in the polysaccharide from sterile plants were more than that extracted from the cystocarpic plants.

According to the data obtained by  $^{13}\text{C}$ -NMR and FT-IR, the KCl-soluble fractions (non gelling) from *C. pinnulatus* were  $\lambda$ -carrageenans with another carrageenan type that had a low amount of 3,6-anhydrogalactose.

Using sedimentation analysis apparent molecular weights of the carrageenans were determined. The values of molecular weights appeared to be 420 to 220 kDa. The highest values of apparent molecular weight were obtained for fractions of carrageenans from cystocarpic plants.

Non-gelling-carrageenans from both types of *C. pinnulatus* plants showed high macrophage-phosphatase activity and  $\kappa/\iota$ -carrageenan from cystocarpic plants possessed a potent anticoagulant activity, which was extremely strong at a low concentration of the polysaccharide (Yermak *et al.*, 2006).

#### 8.3.2 The polysaccharides of *Tichocarpus crinitus* (Tichocarpaceae)

The alga *Tichocarpus crinitus* is unique specimen of the family Tichocarpaceae which is widely spread in the seas of the Far East. Due to its rapid growth, large size, and characteristic features of its development, *T. crinitus* can be considered as a promising species for industrial production of polysaccharides and for introduction into mariculture. There are three forms of this alga (A, B and C) that differ by their morphology and habitats under different conditions in the south of Russian Primorye (Yakovleva and Scriptsova, 2002). The form A grows in open well illuminated areas of a upper subtidal zone in an area of non-permanent surf. This form characterizes by small relative number of branches with rounded tips, and thick cuticle. The content of carrageenan produced by this form of *T. crinitus* is 17%. The form B forming large, densely branching thalli that grows at low light (8–10% photosynthetically active radiation, PAR) and biosynthesizes the great concentration of carrageenan, which is almost twice as high compared with other forms. The development of form C is significantly influenced by wave effects in its habitats. It has slender branches and numerous proliferations arranged throughout the surface of branches of the last two orders (Yakovleva and Scriptsova, 2002).

The first information concerning *T. crinitus* as the source of carrageenan polysaccharides was published in 1969 (Usov *et al.*, 1969). Chemical analysis (Usov *et al.*, 1970; Usov, and Arkhipova, 1981) and  $^{13}\text{C}$ -NMR spectroscopy (Yarotsky, *et al.*, 1978) revealed that the fraction of polysaccharide-forming gels at presence of potassium chloride corresponded to not completely sulfated  $\kappa$ -carrageenan. The fraction soluble at presence of potassium chloride exhibited the usual for  $\lambda$ -carrageenan structure of the carbohydrate chain with alternating  $\alpha$ -1,3 and  $\beta$ -1,4 bonds between the residues of D-galactopyranose (Kochetkov *et al.*, 1971). Whether the polysaccharide composition of *T. crinitus* when change one stage to another and how seaweed habitat influence on the characteristics of polysaccharide remains unknown. For this reason the comparative analysis of the structure and properties of carrageenans isolated from the vegetative and reproductive forms of *T. crinitus* was carried out.

According to the data of  $^{13}\text{C}$ -NMR and IR spectroscopy, the polysaccharides of the gelling fractions isolated from the two forms of the alga *T. crinitus* are  $\kappa/\beta$ -carrageenans (Barabanova *et al.*, 2005). These fractions differ by the ratio of the disaccharide residues of types of carrageenans, which results from the intensity of the corresponding signals in  $^{13}\text{C}$ -NMR spectra. In the reproductive form, their contents are close (60:40), while in the vegetative form the disaccharide units of the  $\kappa$ -type predominate (80%). Currently, it is impossible to determine precisely whether these polysaccharides have a block of  $\kappa/\beta$ -structure or there is a mixture of  $\kappa$ - and  $\beta$ -carrageenans. Nevertheless, the results of analytical centrifugation and gel filtration, as well as the identity of the IR spectra of the polysaccharide fractions obtained by the fractionation using different concentrations of KCl, suggested the block structure. Another difference between the gelling fractions from the different forms of the alga consist in polysaccharides from the reproductive form containing a small amount of disaccharide residues corresponding to the precursors of  $\kappa$ -carrageenan. It is in accordance with the data from  $^{13}\text{C}$ -NMR studies and IR spectroscopy and the results of chemical analysis indicating the irregular structure of the investigated polysaccharides. The application of the negative-ion electrospray ionization mass-spectrometry (ESIMS) and tandem matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) allowed fast estimation of the compositional and structural features of the oligosaccharides mixtures, obtained from the  $\kappa/\beta$ -carrageenan by different methods of its degradation. According to our preliminary results, the mass-spectroscopy investigation of the oligosaccharide products obtained by recombinant  $\kappa$ -carrageenase digestion of  $\kappa/\beta$ -carrageenan of *T. crinitus* revealed that the mixture contained  $\kappa$ -

carrabiose and  $\kappa$ -carratetraose, as its main components, and a set of hybrid oligosaccharides as less abundant components:  $\beta \rightarrow \kappa$ ,  $\kappa \rightarrow \beta \rightarrow \kappa$ ,  $\beta \rightarrow \kappa \rightarrow \kappa$ , and minor  $\beta \rightarrow \iota \rightarrow \kappa$ ,  $\beta \rightarrow \beta \rightarrow \kappa \rightarrow \kappa$ ,  $\beta \rightarrow \kappa \rightarrow \beta \rightarrow \kappa$ ,  $\kappa \rightarrow \beta \rightarrow \beta \rightarrow \kappa$ ,  $\kappa \rightarrow \beta \rightarrow \kappa \rightarrow \kappa$ ,  $\kappa \rightarrow \beta \rightarrow \beta \rightarrow \beta \rightarrow \kappa$ ,  $\beta \rightarrow \beta \rightarrow \kappa \rightarrow \beta \rightarrow \kappa$ ,  $\kappa \rightarrow \beta \rightarrow \kappa \rightarrow \beta \rightarrow \kappa$ ,  $\kappa \rightarrow \beta \rightarrow \beta \rightarrow \kappa \rightarrow \kappa$ . The obtained results have proven of hybridity of the  $\kappa/\beta$ -carrageenan from *T. crinitus* (in press).

A gelling polysaccharide with the same structure was found from *Furcellaria lumrinalis* (Phylophoraceae), which is widely distributed in both eastern and western North Atlantic, from the Barents Sea to the Bay of Biscay, and in the Baltic region. This polysaccharide has been characterized as a hybrid  $\kappa/\beta$ -carrageenan complex containing a small amount of  $\omega$ - and  $\alpha$ -carrageenans (Craigie, 1990), but with methyl groups and small amounts of  $\iota$ -carrageenans, content of which was depended on origin (Usov and Arkhipova 1981; Bird *et al.*, 1991; Yang *et al.*, 2010). For example, the major components of galactan from *F. lumrinalis* of the Baltic Sea are  $\beta$ -D-galactose-4-sulfate, unsubstituted  $\beta$ -D-galactose, and 3,6-anhydro- $\alpha$ -D-galactose, indicating the  $\kappa/\beta$ -carrageenan backbone. Also, the  $\alpha$ -D-galactose-6-sulfate from  $\gamma$ -carrageenan,  $\omega$ - and  $\alpha$ -carrageenan segments presented in low amounts (Tuvikene *et al.*, 2010).

The KCl-soluble fraction from *T. crinitus* was also subjected to  $^{13}\text{C}$ -NMR analysis (Barabanova *et al.*, 2008). Although the NMR spectrum presented was not well resolved and difficult to assign, it revealed that the structure is definitely different from  $\kappa/\beta$ -carrageenan recovered in the KCl-insoluble fraction. We have found that the KCl-soluble fraction of *T. crinitus* is a carrageenan unrelated to any previously observed structure. The structure of this fraction was determined based on two lines of evidence. First, chemical analyses have revealed occurrence of galactose, anhydrogalactose and sulfated ester groups. Second, by using  $^1\text{H}$ , two-dimensional and  $^{13}\text{C}$  NMR, the structure of the alkali-treated polysaccharide was solved, which resulted in consisting a unique carrabiose unit: G2S4S-DA (Barabanova *et al.*, 2008). This structure is consistent with the chemical shift previously recorded for the DA unit of  $\kappa$ - and  $\omega$ -carrageenan, and for the G2S4S unit observed in the fraction of *G. skottsbergii*  $\lambda$ -carrageenan (Guibert *et al.*, 2006). Consequently, it was concluded that the non-gelling fraction extracted from *T. crinitus* vegetative plants represented a novel type carrageenan-like polysaccharide. The backbone of this polysaccharide was composed of alternating 1,3-linked  $\beta$ -D-galactopyranosyl-2,4-disulfates and 1,4-linked 3,6-anhydro- $\alpha$ -D-galactopyranosyl residues. Furthermore, it seems that a large amount of the biosynthetic precursor of this polysaccharide seems to present also in native fraction.

A novel carrageenan structure consisting of 3-linked  $\beta$ -D-galactopyranosyl 2-sulfate units alternating with

3,6-anhydro- $\alpha$ -D-galactopyranosyl units from *Callophyllis hombroniana* has been established (Miller, 2003; Falshaw *et al.*, 2005). Sulfation in the 2- and 4-position of the 3-linked unit has also been suggested for the polysaccharide of *Kallymenia berggrenii* (Miller and Furneaux, 1996).

It is shown that  $\kappa/\beta$ -carrageenan obtained from algae *T. crinitus* possesses antiviral activity against Tobacco mosaic virus (TMV). Our observation indicated that the  $\kappa/\beta$ -carrageenan inhibits TMV infection in detached tobacco leaves at early stages (Reunov *et al.*, 2004). These data allows to consider it as potential antiviral plant protectant.

### 8.3.3 Influence of environmental conditions on polysaccharide composition of *T. crinitus*

The alga *T. crinitus* differs from numerous representatives of the family Gigartinales, by sterile state for a long time (from April to October). Investigation of algal physiology and the chemical structure of carrageenan during this period allows to separate the influence of the reproductive status of the plant from the effects of environmental factors.

A comparative analysis of polysaccharides isolated from *T. crinitus* alga inhabiting at three areas of the Great Peter Bay (the Sea of Japan) that differing by their biotopic characteristics and the degree of anthropogenic pressure was done. According to data of IR-spectroscopy, the KCl-insoluble polysaccharides of algae collected at different sites were identical and had the structure of  $\kappa/\beta$ -carrageenans, while the KCl-soluble polysaccharide fractions had differences in their structures (Barabanova *et al.*, 2010).

Because the carrageenans have sulfate groups and, therefore, can undergo autohydrolysis, the influence of raw material storage duration on the structure and properties of the polysaccharides isolated from *T. crinitus* was studied (Barabanova *et al.*, 2010). It was found that the amount of extracted polysaccharides and their molecular weights had been decreased upon storage of dried alga for 3 years. The yield of polysaccharides of freshly collected alga was shown to consist 40–44% and less than 25% in dried material. The molecular weight (MW) of the polysaccharides from dried alga stored for more than three years was approximately halved whereas their MW did not change after storage for a month (Barabanova *et al.*, 2010).

The relationship between growth rate, anatomical changes and carrageenan accumulation were studied in the non-reproductive (sterile) forms of *T. crinitus* grown under variant irradiances from July to October. Variation in growth rates of algae cultured in the field from June to October was showing a correlation with seasonal patterns in

water temperature. A growth increase at modified PAR had been observed for period from June to August, when sea water temperatures were from 14 to 24 °C, except for the algae cultured at 90% of incident PAR. The highest growth rate was observed in algae growing at 10–15% of PAR in August. It was found that *T. crinitus* adapts to the lighting conditions of habitat through changes in both morphological and anatomical features, and through biochemical processes associated with the biosynthesis of polysaccharides (Yakovleva *et al.*, 2001; Barabanova *et al.*, 2004). From July to September the polysaccharide content was showing a constant negative correlation with photon irradiance ( $p < 0.05$ ). The highest carrageenan yield (35.5%) found to have been observed during August at 10–15% of PAR, and it showed a positive correlation with the thickness of the internal cortical cell walls ( $p < 0.05$ ). At 90% of PAR irradiance, alga showed a marked decrease in polysaccharide content. From August to October, the polysaccharide yield was gradually decreasing for a minimum in October for lower irradiance treatment. Linear correlation coefficient between carrageenan content and the thickness of medullary cell walls were insignificant throughout the experimental period. Our results agreed to data observed for *Hypnea musciformis* (Reis *et al.*, 2008) and *Gigartina pistillata* (Amimi *et al.*, 2007). The maximum carrageenan content in *G. pistillata* occurred in June and September (about 37%) and the minimum carrageenan content occurred in February (19.0%). As shown for the *H. musciformis* a decrease in epilithic biomass occurred at the end of spring and summer (Reis and Yoneshigue-Valentin, 1998) that could be related to the increase in the carrageenan viscosity in spring, when the abiotic factors in this period (low waves and high temperature) induced desiccation. High temperatures were considered negative to the growth of *H. musciformis* (Reis and Yoneshigue-Valentin 1998), and some authors suggested that carrageenan was very important for the survival of a algae in saline sites and that it was responsible for the ionic equilibrium of cells (Percival 1979), due to the cation–anion balance of some negatively charged polysaccharides (Mariani *et al.* 1990).

The analysis of literature did not give any information about chemical analysis of polysaccharide under light condition. Our results of chemical analysis showed that polysaccharides contained some protein. It should be noted that the protein content in the algae grown at depth (10–15% PAR), was less than that in the algae taken from a water surface. The high content of 3,6-anhydrogalactose (30%) was found in the gelling polysaccharide fractions from algae grown under different light condition. However, the non-gelling fraction of polysaccharides also contained 3,6-anhydrogalactose, the amount of which decreased from 8 to 5% with decrease in the intensity of incident light.

The relative proportions of the gelling and non-gelling polysaccharide fractions from the sterile form of *T. crinitus* were strongly associated with decreasing irradiance. *T. crinitus* cultured at 10–15% of PAR, showed significant differences ( $p < 0.05$ ) in fraction distribution with the highest contribution of KCl-soluble polysaccharides found during August and September. In contrast, there were no significant differences between the relative amounts of KCl-soluble and KCl-insoluble polysaccharides for the algae cultured at 30–35% of the PAR throughout the experimental period, except for October. From September to October, the content of KCl-insoluble carrageenans was constant, whereas the amount of KCl-soluble polysaccharides decreased for both irradiance conditions, especially for the algae cultured at 10–15% of PAR.

The IR spectra of the polysaccharide gelling fractions of algae grown under different light conditions were similar and identical and corresponded to  $\kappa/\beta$ -carrageenan isolated from the vegetative form of *T. crinitus*. The decomposition of IR-spectra allowed us to estimate the ratio of  $\kappa$ - and  $\beta$ -segments in the polysaccharide chain depending on light conditions (unpublished data). We calculated the ratios of square of absorbance bands, which are characteristic for non-sulfated galactose and 3,6-anhydrogalactose residues – ( $S_{893}/S_{933}$ ). The volume of this ratio for polysaccharide from algae growing at 10–15% of PAR is 0.66, while for the plants growing under high irradiance level (30–35% PAR) it is 0.27. Hence, with decrease in irradiance from 30–35% to 10–15% of PAR the content of  $\beta$ -units in the polymer chain of carrageenan increased in 2.4-fold times. We had suggested that low light conditions promote additional desulfation of polysaccharides in cell walls of algae. The analysis of IR-spectra of non-gelling polysaccharides also showed some differences. The seaweeds growing under low irradiance level (10–15% PAR) and biosynthesize the non-gelling polysaccharides along with 3,6-anhydrogalactose of sulfate content.

Positive correlation of carrageenan yield with the thickness of the internal cortical cell walls and absence of a correlation with the thickness of medullary cell walls indicates that the carrageenans are localized mostly in the internal cortex of a *T. crinitus* thalli. This is not in agreement with the data reported by Zablackis *et al.*, (1988) for carrageenan-containing alga *Euchema alvarezii* var. *tambalang* Doty, which has the same anatomical structure (Doty 1985, Perestenko, 1994). In contrast to our findings, carrageenans from *E. alvarezii* are localized mostly in medullary cell walls. These differences might result from the different polysaccharide composition. *E. alvarezii* produces  $\kappa$ -carrageenan (Doty, 1985) in contrast to *T. crinitus*, which accumulates mainly non-gelling polysaccharides at low irradiances. Zablackis *et al.* (1988) indicated that large medullary cells could be better to accumulate the gelling

fractions of carrageenan because they had less matrix material than the thick cell walls of cortical cells.

Variation in carrageenan yield and composition have been shown to be considerably influenced by irradiance conditions in natural beds of *T. crinitus* and thus to reflect limiting steps in the biosynthesis of these cell wall constituents. Shade-requiring *T. crinitus* had optimum values of carrageenan content only at 10–15% of PAR. Thus, irradiance is an important factor affecting not only the growth of *T. crinitus*, but also the content and chemical composition of carrageenan in the algal thalli.

## 8.4 The rheological and viscosity properties of carrageenan from *C. pinnulatus* and *T. crinitus*

Gel-forming process is highly sensitive to structural and compositional characteristics of galactans. The rheological properties of  $\kappa/\iota$ - and  $\kappa/\beta$ -carrageenans from the *C. pinnulatus* and *T. crinitus* were investigated. Since gelling properties of carrageenans are strongly dependent on the type and concentration of cations present in a solution (Morris and Belton, 1982), the influence of potassium concentrations in 1% carrageenan solutions was determined. The optimal potassium concentration distinguished for the different type carrageenans. The gel strength of the  $\kappa/\iota$ -carrageenans from *C. pinnulatus* rose rapidly, reaching a maximum with 1.0–1.5% of KCl, but that of the  $\kappa/\beta$ -carrageenans from *T. crinitus* was maximum at lower KCl concentrations (0.75%).

The highest gel strength was obtained with the gelling carrageenans from *C. pinnulatus* (1232.7 Pa) at 2.5% of polymer concentration, whereas the gelling carrageenans from *T. crinitus* formed the weakest gel (77.4 Pa) at the same concentration (Yermak *et al.*, 1999). These different gelling behaviors may be connected with the chemical structure and in particular for  $\kappa/\beta$ -carrageenan from *T. crinitus* where unsulfated residues are presented. Unlike the ion-dependent gelling  $\kappa$ - and  $\iota$ -carrageenans,  $\beta$ -carrageenan apparently does not require a sulfate ester on C-4 of the 3-linked galactose residue for gel formation, and gelling  $\beta$ -carrageenan has been isolated from *Eucheuma gelatinae*, *E. speciosa* and *Endocladia muricata* (Reen *et al.*, 1993). The water-extracted furcellaran, from *Furcellaria lumbricalis*, which had a  $\kappa/\beta$ -carrageenan structure also showed low gelling properties, but hot alkaline treatment of seaweeds increased the stiffness of furcellaran gels more than 11 times (Tuvikene *et al.*, 2010). Although the distribution of the  $\beta$ - and  $\kappa$ -segments along the  $\kappa/\beta$ -carrageenan from *T. crinitus* and *F. lumbricalis* differs, furcellaran forms gels at a distinctly lower potassium-ion

concentration than  $\kappa$ -carrageenan (Glicksmann, 1983) as does  $\kappa/\beta$ -carrageenan from *T. crinitus*.

The mechanical spectra of non-gelling carrageenans from *C. pinnulatus* had a broad range of shear-rates indicating that this system had classical rheo-thinning behavior (Yermak *et al.*, 1999). Double logarithmic plots of shear viscosity against shear-rate of those polysaccharides in the range of temperature between 20 and 65°C were essentially identical to those of the conformationally disordered 'random coil' polysaccharides in concentrated solutions (Morris, 1988). These plots showed horizontal Newtonian plateaus at low shear-rates and then a drastic reduction in viscosity from this maximum value at high shear-rates. This behavior is consistent with the formation of a dynamic entangled structure in concentrated solutions of 'random coil' polymer. At low shear-rates, the disruption of polysaccharide chain entanglement, by the imposed deformation and the formation of new interactions between different chains, equilibrated, and thus no reduction of viscosity was observed (Newtonian plateau). The beginning of shear-thinning occurred at high shear-rates when the rate of disruption of existing entanglements became greater than the rate of formation of new ones, and thus the cross line density of the network was depleted and the viscosity was reduced (Morris, 1988).

The non-gelling carrageenans from *T. crinitus* displayed the properties of 'random coil' polymers only at high temperature. Below 65°C, that polysaccharide demonstrated a behavior that, we suggest, is of polysaccharides forming 'weak gels' in solution (Morris, 1988), although the shear-thinning of that carrageenan in solution might begin at lower shear-rates than those we used. The different behaviors of the non-gelling fractions from *C. pinnulatus* and those from *T. crinitus* may be due to the presence of 3,6-anhydro-galactose in the latter sample.

A large number of scientific publications devoted to the search of new sources of carrageenan, problems with its chemical modification, and correlation of chemical and physical structures attests to the constant scientific interest of different groups of researchers in this polysaccharide.

## References

- Amimi, A.A., Mouradi, T. and Givernaud, *et al.* (2001) Structural analysis of *Gigartina pistillata* carrageenans (Gigartinales, Rhodophyta). *Carbohydr. Res.*, **333**, 271–279.
- Amimi, A., Mouradi, A., Bennasser, L. and Givernaud, T. (2007) Seasonal variations in thalli and carrageenan composition of *Gigartina pistillata* (Gmelin) Stackhouse (Rhodophyta, Gigartinales) harvested along the Atlantic coast of Morocco. *Phycol. Res.*, **55**, 143–149.
- Anderson, N.S. and Rees, D.A. (1966) The repeating structure of polysaccharide sulfate from red seaweeds. In: *Proceedings of the 5th International Symposium* (eds E.G. Yong and J. McLachlan). Pergamon Press, London, pp. 243–279.
- Anderson, N.S., Dolan, T.C.S., Lawson, C.J. and Rees D.A.V. (1968) Carrageenans. V. The masked repeating structures of carrageenans. *Carbohydr. Res.*, **7**, 468–473.
- Barabanova, A.O., Yermak, I.M., Glazunov, V.P., *et al.* (2004) Influence of life-history stage and photon irradiance on yield and quality of carrageenan in *Tichocarpus crinitus* (Rhodophyta, Tichocarpaceae). *Jap. J. Phycol.*, **52** (Supplement), 61–65.
- Barabanova, A.O., Yermak, I.M., Glazunov, V.P. *et al.* (2005) Comparative study of carrageenan from reproductive and sterile forms of *Tichocarpus crinitus* (Gmel.) Rupr. (Rhodophyta, Tichocarpaceae). *Biochemistry* (Moscow), **70**, 430–437.
- Barabanova, A.O., Shashkov, A.S., Glazunov V.P. *et al.* (2008) Structure and properties of carrageenan-like polysaccharide from the red alga *Tichocarpus crinitus* (Gmel.) Rupr. (Rhodophyta, Tichocarpaceae). *J. Appl. Phycol.*, **20**, 1013–1020.
- Barabanova, A.O., Tischenko, I.P., Glazunov, V.P. *et al.* (2010) Chemical composition of polysaccharides of the red alga *Tichocarpus crinitus* (Tichocarpaceae) from different sites of Peter the Great Bay, Sea of Japan. *Russ. J. Mar. Biol.*, **36**, 195–200.
- Bellion, C., Hamer, G.K. and Yaphe, W. (1982) The degradation of *Euchema spinosum* and *Euchema cottonii* carrageenans by  $\iota$ -carrageenases and  $\kappa$ -carrageenases from marine bacteria. *Can. J. Microbiol.*, **28**, 874–880.
- Bird, C. J., Saunders, G. W. and McLachlan, J. (1991). Biology of *Furcellaria lumbricalis* (Hudson) Lamouroux (Rhodophyta: Gigartinales), a commercial carrageenophyte. *J. Appl. Phycol.*, **3**, 61–82.
- Bixler, H.J. (1996) Recent developments in manufacturing and marketing carrageenan. *Hydrobiologia*, **326/327**, 35–37.
- Brodie, J., Masuda M., Mine, I. and Guiry, M.D. (1997) Two morphologically similar biological species: *Chondrus pinnulatus* and *C. armatus* (Gigartinales, Rhodophyta). *J. Phycol.*, **33**, 682–698.
- Bulboa, C.R. and Macchiavello, J.E. (2001) The effects of light and temperature on different phases of the life cycle in the carrageenan producing alga *Chondracanthus chamosoi* (Rhodophyta, Gigartinales). *Bot. Mar.*, **44**, 371–374.
- Buschmann, A.H., Correa, J., Westermeier, R., *et al.* (2001) Red algal farming in Chile: a review. *Aquaculture*. **194**, 203–220.
- Caceres, P.J., Carlucci, M.J., Damonte, E.B., *et al.* (2000) Carrageenan from Chilean samples of *Stenogramme*

- interrupta* (Phyllophoraceae): structural analysis and biological activity. *Phytochemistry*, **53**, 81–86.
- Chapman, V.J. and Chapman, D.J. (1980) *Seaweeds and Their Uses*. Chapman & Hall. New York.
- Ciancia, M., Matulewicz, M.C. and Cerezo, A.S. (1997) A L-galactose-containing carrageenan from cystocarpic *Gigartina skottsbergii*. *Phytochemistry*, **45**, 1009–1013.
- Chiovitti, A., Bacic, A., Craik, D.J. *et al.* (1997) Cell-wall polysaccharides from Australian red algae of the family Solieriaceae (Gigartinales, Rhodophyta): novel highly pyruvated carrageenans from the genus *Callophycus*. *Carbohydr. Res.*, **299**, 229–243.
- Chiovitti, A., Kraft, G.T. and Bacic, A. (2001) Chemistry and phylogenetic implications of the methylated carrageenans from red algae of the genus *Areschougia* (Areschougaceae, Gigartinales, Rhodophyta). *J. Phycol.*, **37**, 1127–1137.
- Chopin, T. and Yarish, C. (1998) Nutrients or not nutrients? That is the question in seaweed aquaculture . . . and the answer depends on the type and purpose of the aquaculture system. *World Aquacult.*, **29**, 31–3, 60–1.
- Chopin, T., and Wagey, B.T. (1999) Factorial study of the effects of phosphorus and nitrogen enrichments on nutrient and carrageenan content in *Chondrus crispus* (Rhodophyceae) and residual nutrient concentration in seawater. *Bot. Mar.*, **42**, 23–31.
- Chopin, T., Hanisak, M.D., Koehn, F.E. *et al.* (1990) Studies on carrageenan and effects of seawater phosphorus concentration on carrageenan content and growth of *Agardhiella subutata* (C. Agardh) Kraft and Wynne (Rhodophyceae, Solieriaceae). *J. Appl. Phycol.*, **2**, 3–16.
- Craigie, J.S., and Wong, K.F. (1979) Carrageenan biosynthesis. In: *Proceedings of International Seaweeds Symposium*. pp. 369–377.
- Craigie, J.S. (1990) Cell walls. In: *Biology of the Red Algae* (eds K.M. Cole and R.Y. Sheath). Cambridge University Press, Cambridge, pp. 221–257.
- Dolan, T. and Rees, D. (1965) The carrageenans. II. The positions of the glycosidic linkages and sulfate esters in  $\lambda$ -carrageenans. *J. Chem. Soc.*, **1**, 3534–3539.
- Doty, M.S. (1985) *Euchema alvarezii* sp. Nov. (Gigartinales, Rhodophyta) from Malaysia. In: *Taxonomy of Economic Seaweeds With Reference to Some Pacific and Caribbean Species* (eds I.A. Abbott and J.N. Norris). California University Press, California, pp. 37–45.
- Estevez, J.M., Ciancia, M. and Cerezo, A.S. (2000). The system of low-molecular-weight carrageenans and agaroids from the room-temperature extracted fraction of *Kappaphycus alvarezii*. *Carbohydr. Res.*, **325**, 287–299.
- Estevez, J.M., Ciancia, M. and Cerezo, A.S. (2001) DL-Galactan hybrids and agaran from gametophytes of the red seaweed *Gymnogongrus torulosus*. *Carbohydr. Res.*, **331**, 27–41.
- Estevez, J.M., Ciancia, M. and Cerezo, A.S. (2002) Carrageenans biosynthesized by carposporophytes of red seaweeds *Gigartina skottsbergii* (Gigartinales) and *Gymnogongrus torulosus* (Phyllophoraceae). *J. Phycol.*, **38**, 344–350.
- Estevez, J.M., Ciancia, M. and Cerezo, A.S. (2008) The system of sulfated galactans from the red seaweeds *Gymnogongrus torulosus* (Phyllophoraceae, Rhodophyta): location and structural analysis. *Carbohydr. Polym.*, **73**, 594–605.
- Falshaw, R., Furneaux R., Wong, H. *et al.* (1996) Structural analysis of carrageenan from Burmese and Thai samples of *Catenella nipae* Zanardini. *Carbohydr. Res.*, **285**, 81–98.
- Falshaw, R., Bixler H.J. and Johndro, K. (2003) Structure and performance of commercial [kappa]-2 carrageenan extract. Part III. Structure analysis and performance in two dairy applications of extracts from the New Zealand red seaweed, *Gigartina atropurpurea*. *Food Hydrocolloids*, **17**, 129–139.
- Falshaw, R., Furneaux, R. H. and Stevenson, D. E. (2005) Structural analysis of carrageenans from the red alga, *Callophyllis hombroniana* Mont. K (Kallymeniaceae, Rhodophyta). *Carbohydr. Res.*, **340**, 1149–1158.
- Glicksman, M. (1983) Red seaweed extracts. In: *Food Hydrocolloids*, Vol. 2 (ed. M. Glicksman). CRC Press, Baton Rouge, pp. 73–113.
- Greer, C.W. and Yaphe, W. (1984) Characterization of hybrid (beta-kappa-gamma) carrageenan from *Euchema gelatinae* J. Agardh (Rhodophyta, Solieriaceae). Using carrageenans, infrared and  $^{13}\text{C}$ -nuclear magnetic resonance spectroscopy. *Bot. Mar.*, **27**, 473–478.
- Guiry, M.D. (2008) Seaweed site. World-wide electronic publication, National University of Ireland, Galway. <http://www.seaweed.ie/> (accessed 5 April 2011).
- Guibert, M., Kervarec, N., Genicot, S. *et al.* (2006) Complete assignment of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of *Gigartina skottsbergii*  $\lambda$ -carrageenan using carrabiose oligosaccharides prepared by enzymatic hydrolysis. *Carbohydr. Res.*, **341**, 1859–1869.
- Hanazawa, S., Ishikawa, T. and Yamaura, K. (1982) Comparison of the adjuvant effect of antibody response of three types of carrageenan and the cellular events in the induction of the effect. *International J. Immunopharmacol.*, **4**, 521–527.
- Harvey, W. H. (1857) Algae. In: *Narrative of the Expedition of an American Squadron to the China Seas and Japan, Performed in the years 1852, 1853, and 1854, under the Command of Commodore M. C. Perry, United States Navy*. 2. Senate of the Thirty third Congress, Second Session, Executive Document 79. 331–2.
- Joint FAO/WHO Expert Committee on Food Additives (2001) *Compendium of Food Additives Specifications, Addendum 9: FAO food and nutrition paper*. 52, 192–194.

- Keppeler, S., Ellis, A. and Jacquier, J.C. (2009) Cross-linked carrageenan beads for controlled release delivery systems. *Carbohydr. Polym.*, **78**, 937–977.
- Knutsen, S. and Grasdalen, H. (1987) Characterization of water-extractable polysaccharides from Norwegian *Furcellaria lubricallis* (Huds.) Lamour (Gigartinales, Rhodophyceae) by IR and NMR spectroscopy. *Bot. Mar.*, **30**, 497–505.
- Knutsen, S.H., Myslabodski, D.E., Larsen B. and Usov, A.I. (1994) A modified system of nomenclature for red algal galactans. *Bot. Mar.*, **37**, 163–169.
- Kochetkov, N.K., Usov, A.I., and Rekhter, M.A. (1971) Polysaccharides of algae. VIII. Acetolys of  $\lambda$ -polysaccharide of *Tichocarpus crinitus* (Gmel.) Rupr. *Zh. Obshch. Khim.*, **41**, 1160–1165.
- Lahaye, M. and Kaeffer, B. (1997) Seaweed dietary fibres: Structure, physico-chemical and biological properties relevant to intestinal physiology. *Sciences des Aliments*, **17**, 563–584.
- Lewis, G., Stanley, N. and Guist, G. (1988) Commercial production and application of algal hydrocolloids. In: *Algae and Human Affairs*, (ed. C. Lembi). University of Washington, Seattle, pp. 206–232.
- Liao, M.L., Kraft, G.T., Munro, S. and Craik, D.J. (1993) Beta/kappa-carrageenans as evidence for continued separation of the families Didranemataceae and Sarcodiaceae (Gigartinales, Rhodophyta). *J. Phycol.*, **29**, 933–844.
- Mancino, D. and Minucci, M. (1983) Adjuvant effects of  $\iota$ ,  $\kappa$  and  $\lambda$  carrageenans on antibody production in BALB/c mice. *Int. Arch. Allergy Appl. Immunol.*, **7**, 359–361.
- Mariani, P., Tolomio, C., Baldan, B. and Braghetta, P. (1990) Cell wall ultrastructure and cation localization in some benthic algae. *Phycologia*, **29**, 253–256.
- Masuda, M., Kudo, T., Kawaguchi, S. and Guiry, M.D. (1995) Lectotypification of some marine red algae described by W. H. Harvey from Japan. *Phycol. Res.*, **43**, 191–202.
- McCandless, E. L., West, J. A., and Guiry, M. D. (1982) Carrageenan patterns in the Phyllophoraceae. *Biochem. Syst. Ecol.* **10**, 275–284.
- Miller, I.J. (2003) The chemical structure of galactans from some New Zealand red algae. *Bot. Mar.*, **46**, 572–577.
- Miller, I.J. and Furneaux, R.H. (1996) A structural analysis of the polysaccharides from *Kallymenia berggrenii* J. Ag. *Bot. Mar.*, **39**, 141–147.
- Morris, E.R. (1988) Polysaccharide solution properties: origin, rheological characterisation and implications for food systems. In: *Frontiers in Carbohydrate Research-1. Food Applications* (ed. R.P. Millane). Elsevier Science Publishers, London, New York, pp. 132–163.
- Morris, V.J. and Belton, P.S. (1982) The influence of the cations sodium, potassium and calcium on the gelation of iota-carrageenan. *Prog. Food Nutr. Sci.*, **6**, 55–66.
- Panlasigui, L.N., Baello, O.Q., Dimatungal, J.M., and Dumelod, B.D. (2003) Blood cholesterol and lipid-lowering effects of carrageenan on human volunteers. *Asia Pacif. J. Clin. Nutr.*, **12**, 209–214.
- Penman, A. and Rees, D.R. (1973) Carrageenan. X. Synthesis of 3,6-di-O-methyl-D-galactose, a new sugar from the methylation analysis of polysaccharides related to  $\iota$ -carrageenan. *J. Chem. Soc. Perkin Trans.*, **1**, 2188–2191.
- Perestenko, L.P. (1994) *Red Algae of the Far-Eastern Seas of Russia*. Nauka, St.-Petersburg.
- Pereira, L., Critchley, A.T., Amado A.M., and Ribeiro-Claro, P.J.A. (2009) A comparative analysis of phycocolloids produced by underutilized versus industrially utilizes carrageenophytes (Gigartinales, Rhodophyta). *J. Appl. Phycol.*, **21**, 599–605.
- Percival, E. (1979) The polysaccharides of green, red and brown seaweeds: their basic structure, biosynthesis and function. *Br. Phycol. J.*, **14**, 103–117.
- Pickmer, S.E., Parsons, M.J. and Balley, R.M. (1975) Variation carrageenans levels and composition in three New Zealand species of Gigartina. *N. Z. J. Sci.*, **18**, 585–590.
- Piriz, M.L. and Cerezo, A.S. (1991) Seasonal variation of carrageenans in tetrasporic, cystocarpic and 'sterile' stages of Gigartina skottsbergii S. et G. (Rhodophyta, Gigartinales). *Hydrobiology*, **226**, 65–69.
- Reani, A., Cosson, J., Parker, A. and Zaoui, D. (1998) Influence of culture conditions on growth and rheological properties of carrageenans in *Cystoclonium purpureum* (Huds.) Batters. *Bot. Mar.*, **41**, 299–304.
- Reen, D.W., Santos, G.A., Dumont, L.E. et al. (1993)  $\beta$ -carrageenan: isolation and characterization. *Carbohydr. Polym.*, **22**, 247–252.
- Rees, D.A. (1963) The carrageenans system of polysaccharides. 1. The relation between the  $\kappa$ - and  $\lambda$ -components. *J. Chem. Soc.*, **1**, 1821–1832.
- Reis, R.P. and Yoneshigue-Valentin, Y. (1998) Variação espaço-temporal de populações de *H. musciformis* (Rhodophyta, Gigartinales) na Baía de Sepetiba e Armação dos Búzios, Rio de Janeiro, Brasil. *Acta Bot Bras.*, **13**, 465–483.
- Reis, R.P., Yoneshigue-Valentin, Y. and Pereira dos Santos, C. (2008) Spatial and temporal variation of *Hypnea musciformis* carrageenan (Rhodophyta – Gigartinales) from natural beds in Rio de Janeiro State, Brazil. *J. Appl. Phycol.*, **20**, 1–8.
- Reunov, A., Nagorskaya, V., Lapshina, L. et al. (2004) Effect of  $\kappa/\beta$ -carrageenan from red alga *Tichocarpus crinitus* (Tichocarpaceae) on infection of detached tobacco leaves with tobacco mosaic virus. *J. Plant Dis. Protection*, **111**, 165–172.
- Schmidt, A.G., Wartewig, S. and Picker, K.M. (2003) Potential of carrageenans to protect drugs from polymorphic transformation. *Eur. J. Pharm. Biopharm.*, **56**, 101–110.

- Smith, D.B. and Cook, W.H. (1954) Fractionation of carrageenan. *Arch. Biochem. Biophys.*, **45**, 232–233.
- Stancioff, D. and Stanley, N.F. (1969) Infrared and chemical studies on algal polysaccharides. In: *Proceedings of 9th International Seaweeds Symposium* (ed. R. Margalef), Santiago de Compostela, Spain. Subsecretaría de la Marina Mercante, Madrid, pp. 595–609.
- Stortz, C. and Cerezo, A. (1993) The system of carrageenans from cystocarpic and tetrasporic stages from *Iridaea undulosa*. Fraction with potassium chloride and methylation analysis of the fractions. *Carbohydr. Res.*, **242**, 217–227.
- Stortz, A.A. and Cerezo, S. (2000) Novel finding in carrageenans, agaroids and 'hybrids' red seaweed galactans. *Curr. Top. Phytochem.*, **4**, 121–134.
- Thommes, M. and Kleinebudde, P. (2006) Use of k-carrageenan as alternative pelletisation aid to microcrystalline cellulose in extrusion/spheronisation. I. Influence of type and fraction of filler. *Eur. J. Pharm. Biopharm.*, **63**, 59–67.
- Tuvikene, R., Truus, K., Robal, M. *et al.* (2010) The extraction, structure, and gelling properties of hybrid galactan from the red alga *Furcellaria lumbricalis* (Baltic Sea, Estonia). *J. Appl. Phycol.*, **22**, 51–63.
- Usov, A.I., Rekhter, M.A. and Kochetkov, N.K. (1969) Polysaccharides of algae. III. Isolation and preliminary investigation of  $\lambda$ -polysaccharide from *Tichocarpus crinitus* (Gmel.) Rupr. *Zh. Obshch. Khim.* (in Russian), **39**, 905–911.
- Usov, A.I., Rekhter, M.A. and Kochetkov, N.K. (1970) Polysaccharides of algae. III. The investigation of  $\chi$ -polysaccharide from *Tichocarpus crinitus* (Gmel.) Rupr. *Zh. Obshch. Khim.* (in Russian), **40**, 2732–2737.
- Usov, A.I. (1974) Polysaccharides of algae. 13. Monosaccharide compositions of polysaccharides of some red algae from Sea of Japan. *Zh. Obshch. Khim.* (in Russian), **44**, 191–196.
- Usov, A.I. and Arkhipova, V.S. (1981) Polysaccharides of algae. XXX. Methylation of  $\alpha$ -types polysaccharides of the red seaweeds *Tichocarpus crinitus* (Gmel.) Rupr., *Furcellaria fastigiata* (Nuds.) Lam. and *Phyllophosa nervosa* (De Cand.) Grev. *Bioorg. Khim.* (Russian Journal of Bioorganic Chemistry), **7**, 385–390.
- Yakovleva, I.M. and Scriptsova, A.V. (2002) II. Polymorphism of the carrageenan-containing red alga *Tichocarpus crinitus* in Sivuch'ya Bay, Sea of Japan. *Russ. J. Mar. Biol.*, **25**, 65–69.
- Yakovleva I.M., Yermak I.M., Titlyanov E.A. *et al.* (2001) Changes in growth rates, anatomy and polysaccharide content of a sterile form of *Tichocarpus crinitus* under differing photon irradiance in the Sea of Japan (Russia). *Bot. Mar.*, **44**, 491–49.
- Yang, B., Yu, G., Zhao, X., *et al.* (2011) Structural characterisation and bioactivities of hybrid carrageenan from red alga *Furcellaria lubricalis*. *Food Chem.*, **124**, 50–57.
- Yarotsky, S.V., Shashkov, A.S. and Usov, A.I. (1978) Polysaccharides of algae. XXV. Application of  $^{13}\text{C}$ -NMR spectroscopy for structural analysis of  $\chi$ -carrageenan group polysaccharides. *Bioorg. Khim.*, **4**, 745–751.
- Yermak, I.M., Kim, H.Y., Ribachuk, N.M. and Solov'eva, T.F. (1995) Characterization of carrageenan from seaweeds of the families Gigartinaceae, Tichocarpaceae and Phylloporaceae of Russian Pacific coast. In: *Eighth International Symposium "Natural Marine Products"*, Tenerife, Spain, pp. 165–166.
- Yarotsky, S.V., Shashkov, A.S., and Usov, A.I. (1978) *Bioorg. Khim.* (Russian Journal of Bioorganic Chemistry), **4**, 745–751.
- Yermak, I.M., Yong, Hwan Kim, Titlyanov, E.A., *et al.* (1999) Chemical structure and gel properties of carrageenan from algae belonging to the Gigartinaceae and Tichocarpaceae, collected from the Russian Pacific coast. *J. Appl. Phycol.*, **11**, 41–48.
- Yermak, I.M. and Khotimchenko, Yu.S. (2003) Chemical properties, biological activities and applications of carrageenan from red algae. In: *Recent Advances in Marine Biotechnology* (eds M. Fingerman and R. Nagabhushanam). Science Publishers, USA, UK, pp. 207–255.
- Yermak, I.M., Barabanova, A.O., Glazunov, V.P. *et al.* (2006) Carrageenan from cystocarpic and sterile plants of *Chondrus pinnulatus* (Gigartinaceae, Rhodophyta) collected from the Russian Pacific coast. *J. Appl. Phycol.*, **18**, 361–368.
- Zabackis, E., Vreeland, V., Doboszewski, B. and Laetsch, W.M. (1988) Localization of kappa-carrageenan in cell walls of var. *Tambalang* with in situ hybridization probes. In: *Algal Biotechnology* (eds T. Stadler, J. Mollion, M.C. Verdus, Y. Karamanos, H. Morvan and D. Christian). Elsevier Applied Science, London, pp. 441–450.
- Zibetti, R.G.M., Nosedá, M.D., Cerezo A.S. and Duarte, M.E.R. (2005) The system of galactans from *Cryptoneimia crenulata* (Halymeniaceae, Halymeniales) and the structure of two major fractions. Kinetic studies on the alkaline cyclization of the unusual diad G2S→D(L)6S. *Carbohydr. Res.*, **340**, 711–722.
- Zhou, G., Sun, Y., Xin, H., *et al.* (2004) In vivo antitumor and immunomodulation activities of different molecular weight lambda-carrageenans from *Chondrus ocellatus*. *Pharmacol. Res.*, **50**, 47–53.

# 9

## Extraction and Characterization of Seaweed Nanoparticles for Application on Cotton Fabric

**Sivalingam Thambidurai**

*Department of Industrial Chemistry, School of Chemistry, Alagappa University, Karaikudi, Tamil Nadu, India*

### 9.1 Introduction

Microorganisms such as bacteria and fungi are found almost everywhere in the environment and can multiply quickly when basic requirements, such as moisture, nutrients and temperature are met. Since textiles have long been recognized as media to support the growth of microorganisms (Gao and Cranston, 2008), synthetic fibers are more resistant to microorganism attack due to their high hydrophobicity nature. However, natural fiber constituents, such as proteins in keratinous fibers and carbohydrates in cellulosic fibers act as nutrients and energy sources under certain conditions (Purwar and Joshi, 2004).

Among the microorganisms, bacteria can cause infection, disease, odors, and health concerns along with the problems of deterioration, and staining of textile products. People have not realized until recently that it is very important to protect wearers against the spread of bacteria and diseases. Today, textile materials are used widely in various environments, and antimicrobial treatment is rapidly becoming a prerequisite for textile goods used in hospitals, hotels, sports, and personal care industries (Ye *et al.*, 2006). Most textile materials currently used in hospitals and hotels are susceptible to cross-infection or transmission of

diseases caused by microorganisms. Therefore, there is a great demand for antimicrobial finishes of textiles to control the growth of microorganisms, such as bacteria, fungi, or mildew.

### 9.2 Textile materials

Textile materials are mostly manufactured in the form of woven, knitted, and non-woven forms depending upon the requirements and end use applications (Table 9.1). For this, natural cellulosic fibers like cotton, flax and jute; protein fibers like wool and silk; regenerated fibers like viscose and cupramonium rayon and synthetic fibers like polyester, acrylic and polypropylene fibers are most commonly used. Among the natural fibers, cotton fibers are biodegradable and the most used fiber until the present time.

#### 9.2.1 Cotton fiber

Cotton is widely used in clothing because of its excellent properties such as regeneration, biodegradation, softness, affinity to the skin, and hygroscopic properties. These fibers

**Table 9.1** Various end uses of fabrics

Medicine	Leisure	Outdoor	Technical	Household
Stockings	Shoes	Jacket	Carpets	Curtains
Protectives	Socks	Tents	Roof covers	Covers
Underlays	T-shirts	Uniforms	Facade covers	Dishcloths
Encasings	Cycling dresses	Personal overalls	Air filters	Bathmats
Bedfillings	Team dresses	Umbrellas		Sanitary ware

are structurally differentiated into concentric zones and the hollow central zone is known as the lumen. The outermost layer is known as the cuticle and is a thin film of fats, pectins, and waxes. Beneath this is the primary wall, composed of mainly of cellulose, in which the fibrils are arranged in a criss-cross pattern. Further towards the center is the secondary wall composed of cellulose, which constitutes the bulk of the fiber (Trotman, 1984). Because of surface contaminants, raw cotton is water repellent, exhibiting little water absorbency.

### Structure and chemical reactivity

Cotton cellulose is highly crystalline in nature and well oriented and has a long and rigid molecular structure. The  $\beta$ -1,4-D-glucopyranose molecules are the principal building blocks of cotton cellulose chains and are linked by 1,4-glucosidic bonds (Figure 9.1).

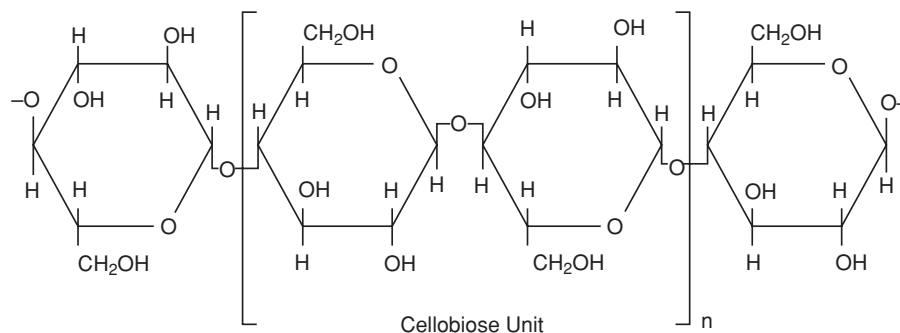
The monomeric unit is represented by cellobiose. The DP varies strongly depending on the cellulose source and processing stage of the cellulosic material. There are three hydroxyl groups attached to each anhydroglucose. One group is attached at C-6 and two at C-2 and C-3. It is well known that the cellulose units repeat themselves throughout the entire length of the cellulose molecule and the only reactive function it possesses is provided by the hydroxyl

groups. The primary and secondary alcohol groups in cellulose react in the same way as in simple substances of similar chemical constitution (Hebeish, 1994).

Cotton cellulose is not soluble in water. However, it can react with certain chemicals under specific conditions. This reaction starts from the amorphous region, which is more accessible and then it reaches to the surface of crystalline region. Etherification and esterification are the two main categories of reactions. Esterification reactions, such as nitration, acetylation, and phosphorylation are usually carried out under acidic conditions. Etherification, on the other hand, favors an alkaline medium. Cellulose is readily attacked by oxidizing agents, such as hypochlorites, chlorous, chloric, and perchloric acids, peroxides, dichromate, permanganates, periodic acid, periodate salts, and nitrogen tetroxide (Bikales and Segal, 1971).

### 9.2.2 Cotton yarn

Cotton fibers, which are naturally in the form of stable fibers, are spun into yarn by spinning. In the spinning mill, the bale forms of materials coming from ginning mill are subjected to various processes, as shown in Table 9.2. With the latest technology, various types of yarn spinning methods like ring spinning, rotor spinning, friction spinning,

**Figure 9.1** Chemical structure of cotton cellulose.

**Table 9.2** Yarn manufacturing process sequence

Feed material	Process	Machines used	Operation involved	Output
Seed cotton	Ginning	Ginning machines	Separation of cotton fiber from its seed	Cotton bale
Cotton bale	Blow room	Bale opener and feeder	Opening and cleaning	Cleaned loose fibers
Cleaned loose fibers	Fiber to fiber separation	Carding	To remove trash, neps and other impurities	Card sliver
Card sliver	Mixing of card slivers	Sliver lap machine	Individualization of fiber and trash removal	Sliver lap
Sliver lap	Mixing of Sliver lap	Ribbon lab machine	Increasing the density of fibers	Final lab
Final lab	Combing	Comber	Increasing the width of lab	Combed sliver
Combed sliver	Drafting	Draw frame	Short fiber removal and parallelization	Draw sliver
Draw sliver	Reduction of sliver size	Fly frame	Doubling and straightening of fibers	Roving
Roving	Spinning	Ring frame	Twist insertion	Spun yarn

and air-jet spinning are available to produce yarns and are named as ring spun, rotor spun, friction-spun, air-jet yarns respectively.

### 9.2.3 Cotton fabric

From the spun yarns fabrics are manufactured by weaving, knitting and non-woven processes. Weaving is the process to interlace the two sets of yarns that are lie at right angles to each other to produce woven fabrics. Different types of weaving machines with shuttle-like hand looms and power looms, without shuttle-like projectiles, rapier, and air jet machines are used. The threads that run along the length of the fabric are known as warp ends whilst the threads that run from selvedge to selvedge, that is from one side to the other side of the fabric, are weft picks. Most of the two-dimensional woven fabrics are constructed using simple plain weaves.

Knitted fabrics are manufactured by warp knitting and weft knitting methods. In warp knitting the loops made from each warp are formed substantially along the length of the fabric, whereas in weft knitting the loops made by each weft thread are formed substantially across the width of the fabric. Non-woven is a textile structure produced by the bonding or interlocking of fibers, or both, accomplished by mechanical, chemical, thermal, or solvent means, and it is generally done in one continuous process directly from the raw material to the finished fabric.

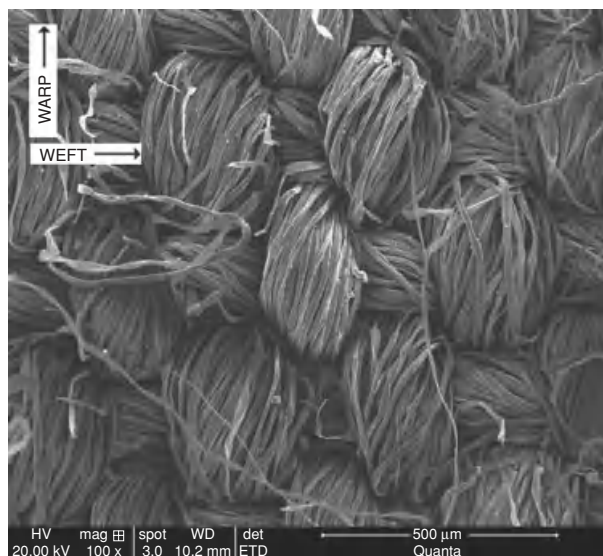
### 9.2.4 Preparatory process

Before any chemical process the preparatory process for cotton fabric is very important because:

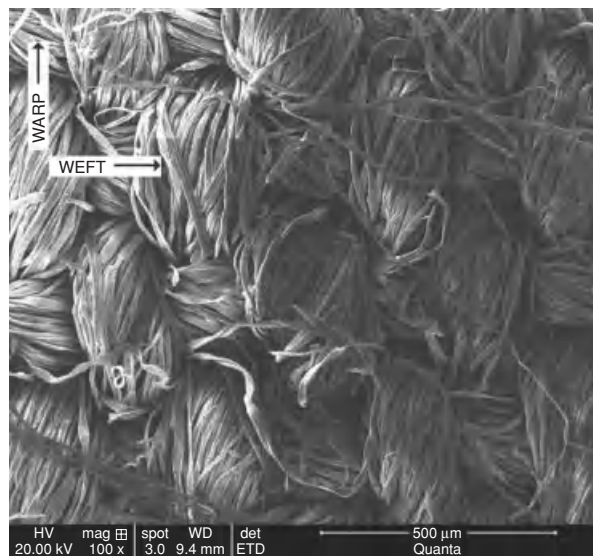
- Starch has been used as the dominant sizing agent applied on the warp yarns during weaving. Hence, presence of starch on the woven fabric hinders water penetration.
- The non-cellulosic materials called as natural and added impurities present on the surface of the cotton fibers should be removed for effective wet processing.
- The natural coloring matter varies from gray to brown and should be removed from the cotton fabric to increase the fabric whiteness.
- To improve the appearance of the fabric and to increase the accessible region it should undergo a mercerization process.

#### Conventional method

The common industrial conventional pretreatments such as desizing, scouring, bleaching, and mercerizing are used to remove sizing impurities with 0.5% hydrochloric acid, natural and added impurities with 4% sodium hydroxide and 2% sodium carbonate, natural coloring material with 0.6% hydrogen peroxide, and convolutions with 18–24% sodium



**Figure 9.2** Conventional method of scoured plain cotton fabric.



**Figure 9.3** Bioscoured plain cotton fabric.

hydroxide, respectively. The structure of simple plain weave cotton fabric scoured by conventional methods is shown in Figure 9.2.

These operation sequences consumes enormous amounts of energy, water, and chemicals. The potential for environmental contamination and depletion of natural resources is also serious. Hence, enzymatic pretreatments are slowly replacing the conventional methods.

### Enzymatic pretreatments

Natural biodegradable sizing compounds, such as starches, can be hydrolyzed by amylases. These enzymes are inexpensive and commercially available with flexible pH and temperature ranges. The scouring process is based on the idea of specifically targeting the non-cellulosic impurities with appropriate enzymes using either with one kind of enzyme, or a combination of proteases, pectinases, lipases, and cellulases. For example, pectinases could be used for the decomposition of pectinic substances, proteases for proteins, lipases for fats. Parts of the natural pigments are associated with the non-cellulosic compounds and could be lifted off the fiber during bioscouring (Buschle-Diller *et al.*, 2001). Literature dealing with enzymatic bleaching of cotton is very limited. Currently peroxide killer enzymes are used after hydrogen peroxide bleaching. Enzymatic-scoured plain weave cotton fabric structure is shown in Figure 9.3.

## 9.3 Antimicrobial agents

An increasing volume of literature demonstrates the survival and growth of harmful microorganisms (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Neisseria*, *Bacillus subtilis*, *diphtheroid bacilli*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Klebsiella*, *Enterobacter aerogenes*, *Citrobacter*, *Candida albicans*) in textiles and the health risks of their dissemination (Baumgartner and Yang, 1997).

In general, antimicrobial properties can be imparted to textile materials by chemically or physically incorporating functional agents onto fibers or fabrics. Techniques used to impart antimicrobial activity generally involve solubilization of active agents onto the fiber, graft polymerization, treatment with resins or cross-linking agents, coating, or microencapsulation (Cohen *et al.*, 2000; Sun and Sun, 2002).

The antimicrobial properties of such textile materials can be grouped into two categories, temporary or durably functional fabrics. Temporary biocidal properties of fabrics are easy to achieve in finishing, but easy to lose in laundering. Durability has generally been accomplished by a common technology, a slow-release method. According to this method, sufficient antibacterial agents are incorporated into fibers or fabrics by means of a wet finishing process. The treated fabrics deactivate bacteria by slowly releasing the biocide from the materials. However, the antibacterial

agents will vanish completely if they are impregnated in materials without covalent bond linkages.

There is an increasing public concern for possible effects of antibacterial finishes on environmental and biological systems. For an ideal textile antibacterial finish, it should not only kill undesirable microorganisms and stop the spread of diseases, but also fulfill three other basic requirements (Mao and Murphy, 2001) such as;

- First, the product should not cause skin allergy and irritations to human.
- Second, the product must not present negative influences to the textile properties or appearance.
- Third, the product should be able to endure laundering, drying, and leaching.

Antimicrobial agents either inhibit the growth (-static) or kill (-cidal) the microorganisms. They can be broadly classified into natural and synthetic products. Natural products consist of plant, leaves, and seaweed extracts. Synthetic agents consist of organic, inorganic, and oxygen bleaching chemicals.

### 9.3.1 Organic chemicals

A number of organic chemicals have been employed to impart antimicrobial activity to textile goods. Those chemicals include Triclosan, polyhexamethylene biguanide, phenolic compounds, *N*-halamine, quaternary ammonium compounds, halogen compounds, organometallics, iodophors, heterocyclics with anionic groups, nitro compounds, ureas and its related compounds, formaldehyde derivatives, amines and cyclodextrins. They damage the cell wall or alter cell membrane permeability, denature proteins, inhibit enzyme activity, or inhibit lipid synthesis, all of which are essential for cell survival. Many of these chemicals, however, are toxic to humans and do not easily degrade in the environment.

Various mechanisms are used to attach these agents to the fabric include the graft polymerization of *N*-halamine monomers onto textile substrate (Liu and Sun, 2006), the addition of *N*-halamine additives into the electrospinning dope of fibers (Obendorf and Sun, 2007), the immobilization of enzymes onto ester-cross-linked cotton fabrics (Ibrahim *et al.*, 2007), the placement of quaternary ammonium salt onto cotton fabrics using a covalently bound adduct (Son *et al.*, 2006), grafting of cellulose with cyclodextrins (Hebeish and El-Hilw, 2001).

### 9.3.2 Inorganic nanoparticles

Many heavy metals are toxic to microbes at very low concentrations either in the free state or in compounds. They kill microbes by binding to intracellular proteins and inactivating them (McDonnell and Russell, 1999). Although some other metals, such as copper, zinc and cobalt, have attracted attention as effective antimicrobial agents for textiles, silver is by far the most widely used in general textiles as well as in wound dressings (Hermans, 2006).

The impact of nanotechnology in the textile industry has made it possible to produce a new generation of antimicrobial textiles by innovative finishes of the fabric surface. Nanosized particles have a larger surface area per unit mass, and hence higher efficiency than bulk materials. And also, because of the developing resistance of bacteria against bactericides and antibodies and the irritant and toxic nature of some antimicrobial agents, biomaterial scientists have focused their research on nanosized metal particles. It is well known that metal nanoparticles of silver, zinc oxide, copper and titanium oxide have been exploited frequently as antibacterial agents in fibers/fabrics. Two distinctive approaches have been made – either applying nanosize entities into or creating nanosize entities in textile materials.

Nano-ZnO acts as an excellent antibacterial agent on cotton fabric with slight reduction on tensile strength (Vigneshwaran *et al.*, 2006). Scanning electron microscope characterization and biocidal tests of bleached cotton fabrics coated with an average size of 40 nm nanoparticles showed that nano-ZnO antibacterial cotton fabric was relatively sensitive to acid artificial sweat, while durable in saline or alkaline solution (Li *et al.*, 2007). In case of nano-ZnO coated fabric, due to its nano-size and uniform distribution, friction was significantly lower than the bulk-ZnO coated fabric (Yadav *et al.*, 2006). Nano-silver-treated garments control bacterial growth even after 50 washes, thereby providing lasting cleanliness and freshness (Parthiban and Gunasekaran, 2007).

### 9.3.3 Oxygen bleach

Durable and rechargeable antimicrobial functions on textile materials have been developed by incorporating halamine structures and using chlorine bleach as an activating and recharging agent. More recently, carboxylic moieties on cellulose also demonstrated rechargeable antimicrobial functions with a commercial active oxygen bleach as a recharging agent (Huang and Sun, 2003a). The carboxylic acid groups can be converted to biocidal peroxyacid structures upon reaction with the oxygen bleach. The cross-linked ester bonds formed between butanetetra-carboxylic acid and cellulose

can also be converted to peroxyacid moieties using sodium perborate bleaching (Huang and Sun, 2003b).

### 9.3.4 Plant products

For the past 10 years, the increase in the number of microbially caused diseases and hospital infections has led to intensive research into new materials and procedures, which would at the same time assure permanent bioactive effects together with complete safety for the customer and an environmentally friendly production process. Therefore, to replace the synthetic chemicals from antibacterial finishing, research is going on to find bioactive agents from natural products such as herbal plants, sea wastes, and seaweeds.

#### Herbal plants

There are many natural plant products that show antibacterial properties. Seeds of neem tree (*Azadirachta indica*) were used for imparting antibacterial properties to blended fabric and tested against Gram-positive and Gram-negative bacteria. The results showed that the antibacterial activity was higher against Gram-positive bacteria (*Bacillus subtilis*) as compared to Gram-negative bacteria (*Proteus vulgaris*) (Joshi *et al.*, 2007). Extracts from leaves of *Azadirachta indica* tree (neem) and aloe vera plant are applied to cotton fabric for an antimicrobial finish (Vyas *et al.*, 2008). Onion (*Allium cepa*) skin liquid and onion pulp juice were extracted using deionized water and a cold press method respectively and applied by grafting onto plasma-treated cotton fabric. Both show good antibacterial activity against *Staphylococcus aureus* (Chen and Chang, 2007). Studies reveal extracts of various natural herbal products such as neem, tulsi, onion skin, and prickly chaff flowers exhibit antibacterial properties.

#### Natural dyes

Recently, there has been a revival of interest in the use of natural dyes in textile coloration. A widespread interest has emerged in the dyeing of textile fibers using natural colorants, on account of their high compatibility with the environment, softer color shades, naturalness, lower toxicity, and antibacterial/antiallergic/deodorizing/anticancer properties, harmonizing natural shades, or just the novelty. It was found that the use of some natural colorants notably enhanced the deodorizing performance. Cotton, silk, and wool fabrics dyed with five kinds of natural colorants (peony, pomegranate, clove, *Coptis chinensis*, and gall-

nut extracts) displayed excellent antibacterial activity (Lee *et al.*, 2009).

### 9.3.5 Chitin and chitosan

Chitosan is the second most abundant natural polymer found on earth next to cellulose. It is processed from crabs, shrimps, and other crustacean waste from chitin (a *N*-acetylglucosamine-polymer) by alkaline treatment. Chitosan is non-toxic, biodegradable, and biocompatible, and has long been used as a biopolymer. It has hemostatic, fungistatic, spermicidal, antimicrobial, and flocculation properties, amongst others (Ye *et al.*, 2006). Because of its polycationic nature, chitosan shows good antibacterial activity against various bacteria and fungi. Its antimicrobial property is related to the interaction of charges between the amino groups of the polycationic form of chitosan with the microbial cell walls (Fang *et al.*, 2001).

Cotton fabric with excellent antibacterial durability was obtained with chitosan-containing core-shell particles without any chemical binders, one with poly (*n*-butylacrylate) soft core and another with a cross-linked poly (*N*-isopropylamide) hard core. Antimicrobial activity was evaluated quantitatively using a Shake Flask Method in which the reduction of the number of *Staphylococcus aureus* cells was counted. The results showed that treated fabric had an excellent antibacterial property with bacterial reduction higher than 99% (Ye *et al.*, 2006).

Non-woven polypropylene and cotton fabrics after plasma pretreatment followed by flash evaporation and radiation crosslinking acrylate polymer coating were dipped into chitosan, carboxymethyl chitosan, and carboxymethyl chitin solution. The inhibition zone of the chitosan-covered samples has increased by a factor of 2–3.1 over the original pretreated samples. The chitosan-modified fabrics showed a good antibacterial activity in killing almost 105 cells/ml within 18–23 h (Abdou *et al.*, 2008).

#### Chitosan-metal oxides

To improve the antibacterial activity, metals such as silver oxide, titanium oxide (Son *et al.*, 2009) and zinc oxide (Li *et al.*, 2010) were incorporated with chitosan polymer using various methods. Recently, a new method of precipitation was followed to prepare a chitosan–ZnO complex by addition of zinc chloride during the chitin deacetylation process (Anandhavelu and Thambidurai, 2011).

A number of published reports are available in the literature about the application of chitosan, modified chitosan and metal complex chitosan on cotton fabrics to obtain good antibacterial property, and discussing these here is beyond the scope of this book.

## 9.4 Seaweeds

Major antibacterial agents for textiles include metals, metal-based compounds, phenolic compounds, and quaternary ammonium salts, etc., which all have toxicity and environmental issues. Hence, it has become increasingly important for antibacterial agents to meet environmental and low toxicity criteria, while retaining their functionality.

Since one of the natural biodegradable polymers chitosan, has certain limitations on application to cotton fabrics such as loss of antimicrobial activity in alkaline conditions, low solubility in water at above pH 6.5, and less durability due to its lack of strong bonding with fabrics, a number of chitosan derivatives with different modifications using organic compounds, transition metals and their oxides have been reported to meet the above limitations. However, these modifications involve complex apparatus, or processing, and in many cases they involve toxic reagents or additives that will be harmful to the environment. Therefore, it is vital to develop ecofriendly antibacterial agents extracted from natural products for textile applications.

The marine world offers an extremely rich resource for important compounds of structurally novel and biologically active metabolites and there is an increasing demand for biomedical applications.

### 9.4.1 Bioactive compounds from seaweed

The chemical compounds responsible for antibacterial activity in seaweed have been variously identified as organic and fatty acids, terpenes, carbonyls, bromophenols, halogenated aliphatic and sulfur-containing heterocyclic compounds, isoprenylated and brominated hydroquinones, as well as phlorotannins (Mtolera and Semesi, 1996).

In several cases, different substances have been found in the same seaweed and it is not surprising that there are differences in the antimicrobial activity of different seaweeds. But variation in antibacterial activity may be due to the method of extraction, the solvent used in extraction, algal species used, season at which samples were collected and differences in assay methods (Bauer *et al.*, 1966). Among these, seaweed collection, solvents used, and method of extraction play a major role in the antibacterial activity.

#### Seaweed collection

Normally seaweeds will be collected at a depth of 1–2 m from the surface of water during low tide. The collected samples have to be cleaned well with seawater to remove all extraneous matter such as epiphytes, sand particles, peb-

bles and shells, and brought to the laboratory in plastic bags. The samples should then thoroughly washed with freshwater, blotted and spread out at room temperature for drying. The shade-dried samples will be ground into fine powder.

#### Solvent selection

The degree of antibiotic property depends upon the suitable solvents used for extraction; as an efficient strategy of investigation, organic solvents have been used to extract the possible lipid-soluble active principles from macroalgae, because this always gives greater efficiency in extracting antimicrobial properties, as compared to water extraction. From the literature, it was found that various organic solvents such as methanol, *n*-hexane, ethyl acetate, chloroform, benzene, dichloromethane, acetone, methanol–toluene, diethyl ether, ethanol, dichloroethane, diethyl methyl formide, and chloroform–methanol were used for extraction.

Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain which kind of solvent is the most effective and suitable for extraction of seaweeds. A few workers tried using different solvents for screening the antimicrobial activity of seaweeds and made comparisons. Parekh *et al.* (1984) reported that extracts obtained with acetone, ethyl alcohol and ether showed higher antibacterial activity than that of extracts obtained with chloroform.

Rosell and Srivastava (1987) found similar antibacterial activity when they screened brown algae from Canada with acetone, chloroform, ethyl-ether, methanol and acetic acid. Sastry and Rao (1994) carried out a successive extraction using benzene, chloroform and methanol and reported the chloroform extract exhibited the strongest antibacterial activity. It can be seen from the above reports that the efficiency of chloroform in the extraction of seaweeds remains uncertain (Zheng *et al.*, 2001). Recently Patra *et al.* (2009) reported that the chloroform and ethyl acetate extracts were most effective than the methanol and ethanol extracts and Demirel *et al.* (2009) reported that dichloromethane extracts exhibited a higher degree of activity as compared to methanol and hexane extracts. Acetone and ethanol extracts of marine algae collected from south-west coast of India in three seasons showed good inhibitory activity (Choudhury *et al.*, 2005).

Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than *n*-hexane and ethyl acetate, whereas others report that chloroform is better than methanol and benzene, diethyl ether caused better halozones than methanol, acetone, and ethanol. It is clear that

using organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activities compared to water-based methods.

The inhibitory activity observed in the extract obtained with one kind of solvent but not in extracts obtained with other solvents may be due to that a particular solvent is required to extract some antimicrobial substances within the algal plant and therefore the percentage of inhibitory activity will go up when several solvents are used in the screening (Zheng *et al.*, 2001).

### Methods of extraction

A number of methods have been reported in the literature to extract the bioactive compound from dry seaweed using various solvents.

- 1 Cho *et al.* (2007) extracted by mixing seaweed powder and solvent in a 1:10 ratio for 24 hours, and centrifuged to get a supernatant solution. The residue was extracted again twice as described above and combined with the previous extract. The extract was filtered with Advantec 5A filter paper and concentrated using a rotary evaporator.
- 2 Manilal *et al.* (2009) reported that dried algal powder and solvent were mixed in Scott Duran flasks and kept for 2 weeks at 35 °C with shaking at 120 rpm. The mixture was filtered using Whatman filter paper No 1 fitted with a Buchner funnel using suction pressure followed by centrifugation. The supernatant was concentrated up to 5–10 ml in a rotary vacuum evaporator.
- 3 Choudhury *et al.* (2005) carried out extraction by soaking the seaweed powdered material with solvent overnight at room temperature (1:3 v/v) three times. The extracts from three consecutive soakings were pooled and freed from solvent by evaporation under reduced pressure. The crude extracts obtained were finally dried under vacuum.
- 4 Cox *et al.* (2010) powdered the frozen seaweed samples in liquid nitrogen using a mortar and pestle, then extracted with solvent under nitrogen atmosphere for 2 hours. The extraction was carried out at 40 °C at 100 rpm in a shaker incubator, centrifuged and evaporated to dryness using vacuum poly evaporator at 60 °C.
- 5 Joseph and Lipton (2004) refluxed finely powdered algal material with solvent in a round-bottom flask. The extract was filtered and concentrated to recover the excess solvents in another distillation system. Finally it was reduced to a thick oily-natured crude extract in a rotary vacuum evaporator at 40 °C.
- 6 The Soxhlet apparatus was also used by many authors with different solvent ratios and time. Demirel *et al.*

(2009) extracted 15 g of freeze-dried sample in 150 ml of solvent for 24 h. Taskin *et al.* (2007) used 1:50, w/v ratio, whereas Duan *et al.* (2006) used 100 g of seaweed for 500 ml of solvent for 6 h. Lima-Filho *et al.* (2002) extracted with solvent 1:15 at 55–60 °C until saturation (24 h).

## 9.5 Extraction and characterization

### 9.5.1 Crude extract

For textile application, crude extracts of the seaweed were prepared using the solvent diethyl ether. Two grams of dried, powdered sample are accurately weighed and mixed with 50 ml of diethyl ether. The extract was stored in a dark room at room temperature for 16 h. This extract is then centrifuged at 2000 rpm for 15 min. The supernatant liquid were transferred separately into sterile screw-cap test tube and stored at room temperature in the dark (Selvasubha *et al.*, 2007).

### 9.5.2 Nanoparticle extraction

For nanoparticle extraction, fresh weeds of *Turbinaria conoides* were collected at Gulf of Mannar in the east seashore of south India and washed with seawater followed by thorough washing with freshwater. These were then dried under shade for 48 h until the moisture was completely removed; they then were ground well in a mortar. The powdered seaweed sample was mixed with acetone at material to liquor ratio of 1:25. The mixture was stored in a dark place for 24 h at room temperature. The supernatant liquid was transferred separately into a sterile screw-cap test tube and stored at room temperature in the dark.

The supernatant liquid (20 ml) was centrifuged at 4000 rpm and 20 °C for 20 min in a Kubota 6800 Refrigerated Ultra Centrifuge machine (Kubota Corp., Tokyo, Japan). Then the sample was taken out, the clear acetone was removed, and the extract residue transferred on a glass plate to get completely dry (Mercy Sheeba and Thambidurai, 2009).

### 9.5.3 Characterization of nanoparticles

#### UV-Visible spectroscopy

This technique is very useful to measure the number of conjugated double bonds and also used to measure the aromatic conjugation within the various molecules. The extract containing the antibacterial substance was examined for functional group identification with UV-visible spectroscopy in the wavelength range of 200–800 nm.

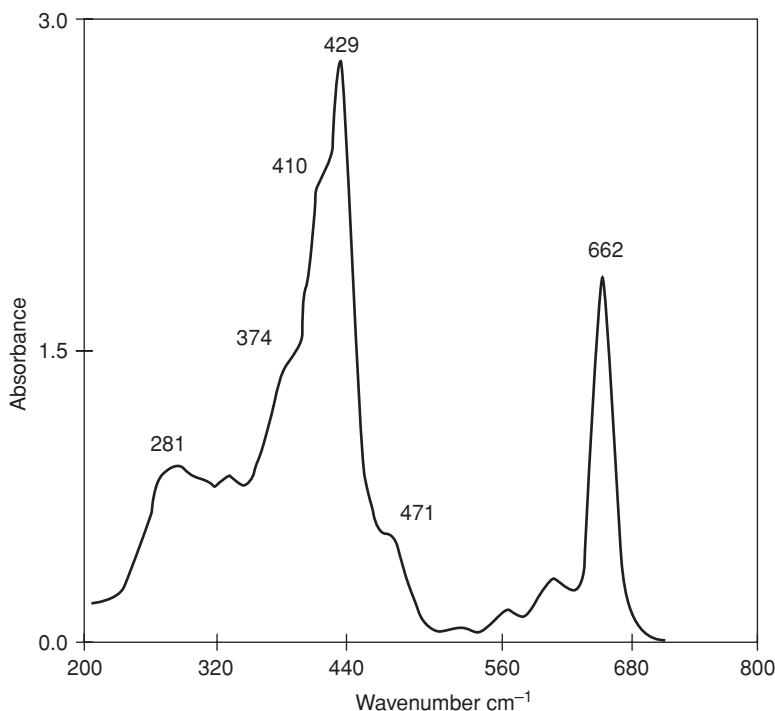


Figure 9.4 UV spectra of seaweed extract.

UV spectra of seaweed extract solution and its transition and absorption maxima are shown in Figure 9.4.

In the UV spectrum the near UV region between 230 and 270 nm shows a broad absorption band. This B bands (Benzenoid bands) are characteristic of aromatic molecules. A band at  $\lambda_{\text{max}}$  281 nm is observed at longer wavelength than the more intense  $\pi-\pi^*$  transition. It occurs when the chromophoric group is attached to an aromatic ring. The absorption band at 281 nm shows the presence of carbonyl group due to this carbonyl group  $\pi-\pi^*$  transition takes place. The  $\pi-\pi^*$  (R bands) occurring in the 350–370 nm region shows the presence of  $\alpha$ -, and  $\beta$ -unsaturated ketones. The band at 471 nm shows the presence of 11 internal double bonds. Because of the lycopene it produces the absorption band at 476 nm. It has 11 external double bonds. The presence of absorption band near 662 nm shows the nitroso group (Kubaneck *et al.*, 2003).

#### Fourier transform infrared (FTIR) spectroscopy

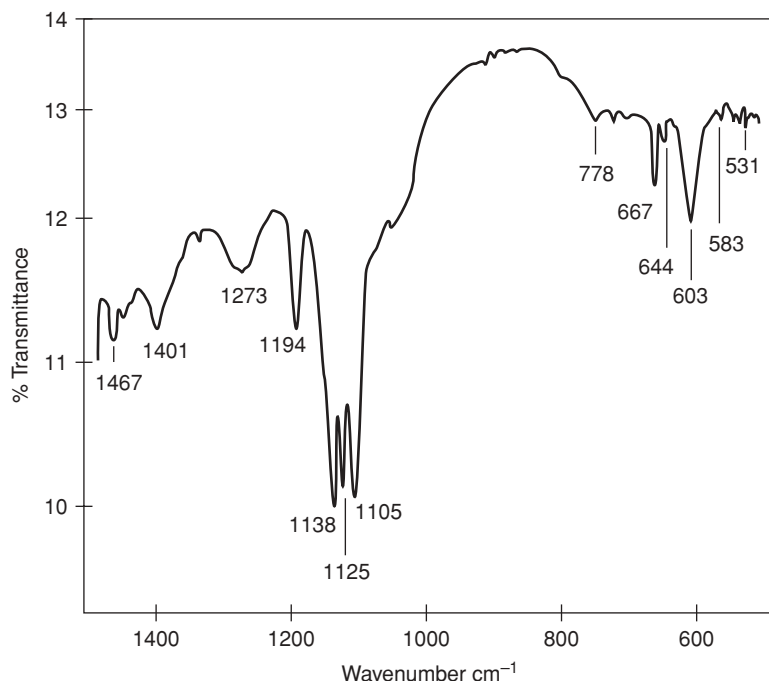
The discovery in 1965 of the fast Fourier transform algorithm allowed the development of a new generation of FTIR spectrometers equipped with software capable of performing the processing of spectral data. FTIR has been exten-

sively applied in carbohydrate chemistry (Mathlouthi and Koenig, 1986). The fact that the even derivatives of IR spectra have sharper peaks at the same frequency values than in the original spectra.

The extract containing the antibacterial substance was examined for functional group identification with FTIR spectroscopy in the spectral range of 4000 to 400  $\text{cm}^{-1}$  and divided into three regions.

Figure 9.5 shows the spectrum of the 1400–600  $\text{cm}^{-1}$  region. The peak appearing between 644 and 531  $\text{cm}^{-1}$  is attributed to the O–C–N bending vibration of the amide group (amide IV & VI bands). The strong band around 1105  $\text{cm}^{-1}$  received due to C–O stretching. It shows the presence of secondary alcohols. The symmetry band at 1125 and 1138  $\text{cm}^{-1}$  attributed to alcoholic C–O–C stretching frequency.

A strong absorbance at 1273  $\text{cm}^{-1}$  is caused by the S=O asymmetric stretching vibration of sulfate groups (Ray and Lahaye, 1995). A band at 583  $\text{cm}^{-1}$ , is due to O–S–O asymmetric deformation of sulfate groups (Nakamoto, 1986). The signal at 788  $\text{cm}^{-1}$  was indicative of the presence of sulfate ester substitutions (Ray and Lahaye, 1995). The sulfate group is considered a highly selective antiherpetic compound, with antiviral effectiveness, particularly against the TK strains of herpes simplex virus (HSV)-1 (Matsuihiro



**Figure 9.5** FTIR spectra of seaweed extract (1400–600  $\text{cm}^{-1}$ ).

*et al.*, 2005). The sharp moderate intensity band at  $1138\text{ cm}^{-1}$  is caused by asymmetric C–N–C stretching vibration with respect of the amide group.

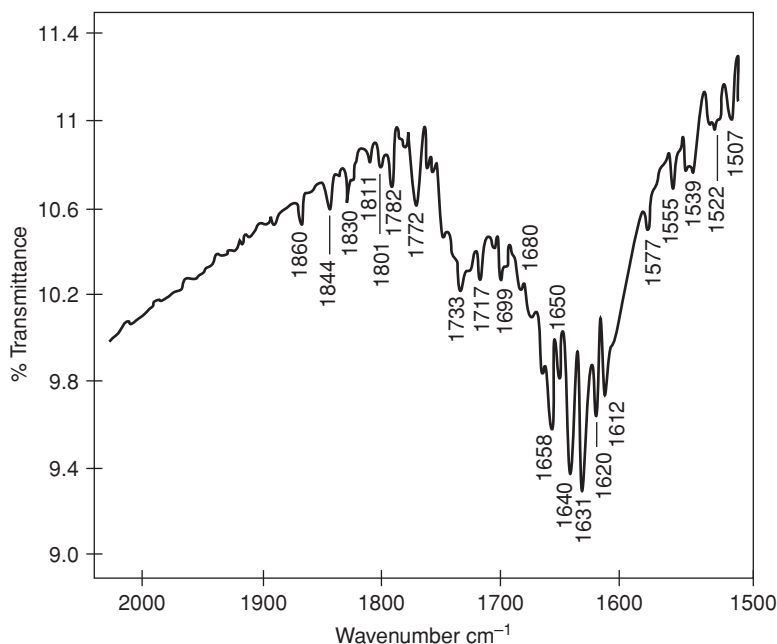
The sharp band at  $1194\text{ cm}^{-1}$  also shows the presence of C–CO–C stretching vibration. Aliphatic primary and secondary amines with primary carbons exhibit medium intensity bands at  $1138\text{ cm}^{-1}$  due to asymmetric C–C–N and C–N–C stretching vibration, respectively. The medium intensity band at  $1401\text{ cm}^{-1}$  is caused by the C–N stretching vibration of the amide III band. The absorption band at  $1467\text{ cm}^{-1}$  obtained by the methylene scissoring and methyl asymmetric bending modes. This shows the molecules contain both methyl and methylene groups.

Figure 9.6 shows the spectrum of the 2000–1600  $\text{cm}^{-1}$  region. A medium intensity band at  $1620\text{ cm}^{-1}$  caused by  $\text{NH}_2$  bending vibration appears in secondary amine salts. The symmetric and asymmetric NH bending vibration in primary amine salts occur at  $1620\text{ cm}^{-1}$  and  $1555$  and  $1507\text{ cm}^{-1}$  assigned to bands I, II, and III of the amide function of proteins (Silverstein *et al.*, 1991). Secondary acyclic amides display a weak amide II band at  $1577\text{ cm}^{-1}$  as a result of the presence of N–H bending vibration. The bands at  $1631$ ,  $1577$ , and  $1522\text{ cm}^{-1}$  are consistent with the skeletal vibration of the aromatic system. The medium intensity of the band at  $1640\text{ cm}^{-1}$  and  $1053\text{ cm}^{-1}$  represents the C=O stretching of uronic acids and the vibration

of the C–O–C bridge of glucosides (Yu *et al.*, 2003). The sulfated polysaccharides of seaweeds differ chemically and physicochemically from land plants. Primary amines exhibit medium-to-strong N–H in plane bending at  $1650\text{ cm}^{-1}$ , which evolved to a slightly greater frequency in hydrogen-bonded molecules. The band at  $1658\text{ cm}^{-1}$  was caused by C=C stretching with respect to the vinylidine group (Silverstein *et al.*, 1991).

The absorption bands at  $1680$  and  $1640\text{ cm}^{-1}$  indicate C=O stretching (amide I band) and N–H in-plane bending (amide I band) vibrations, respectively, of the primary amide group. The absorption band at  $1699\text{ cm}^{-1}$  was caused by the C=O absorption frequency. The effect of conjugation is at a maximum when the chromophores are coplanar. The steric effects, which disturb the coplanarity of the conjugated system, reduce the effect of conjugation. *cis*-non-planar conjugation shifts the C=O absorption to a higher frequency; thus the coplanarity is lost and, consequently, the effect of conjugation is reduced.

The normal ketone will absorb at a frequency of  $1720\text{ cm}^{-1}$ . The bands at  $1717$  and  $1733\text{ cm}^{-1}$  are caused by carbonyl absorption. The normal ketone absorption is shifted as the result of  $\alpha$ -halo ketones. Because  $\alpha$ -halo ketones are greater carbonyl absorption compounds than the parent ketones the shift increases with increasing dipole moment of C–X bond. This is due to the electrostatic



**Figure 9.6** FTIR spectra of seaweed extract (2000–1600  $\text{cm}^{-1}$ ).

interaction between two similarly oriented dipoles and is operative only when C–X and C=O bonds are approximately coplanar.

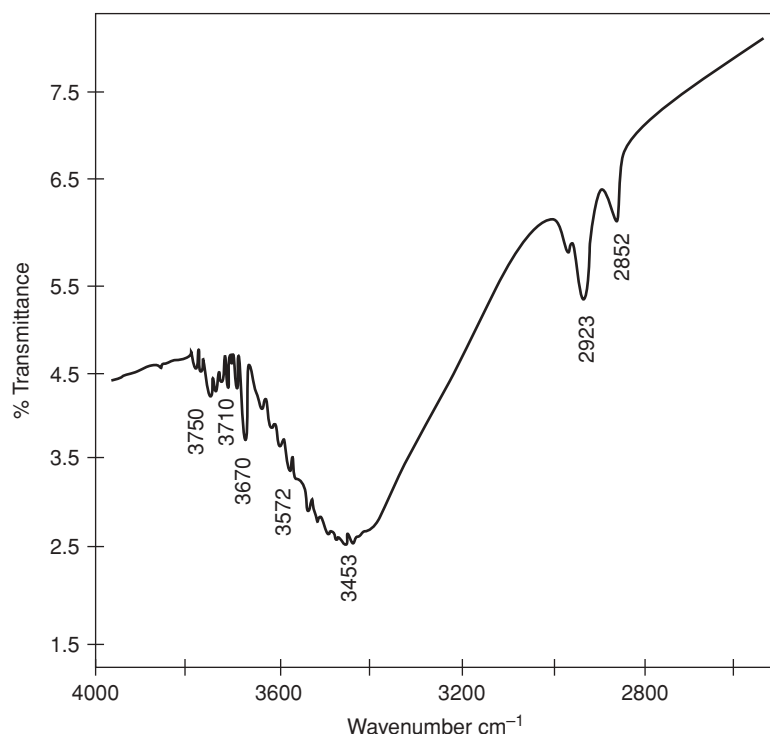
The halogen atom in equatorial orientation is near the carbonyl group, and the “field effect” causes an increase in the carbonyl stretching frequency. It shows the rigid molecule structure of  $\alpha$ -halo keto steroids.  $\alpha$ -halogen substitution increases in the C=O stretching frequency near 1770  $\text{cm}^{-1}$ . The absorption bands near 1782  $\text{cm}^{-1}$  show the presence of conjugated acyclic anhydride. The prominent absorptions at 1830  $\text{cm}^{-1}$  represent asymmetric C=O stretching vibration of an acyclic anhydride group since the higher frequency C=O band is more intense.

The strong band at 1860 and 1772  $\text{cm}^{-1}$  is the characteristic spectral feature of the C=O stretching absorption of an acid halide. Acid halides exhibit strong C=O stretching absorption in this region. FTIR spectral data and spectrum show new signals, one at 1733  $\text{cm}^{-1}$  (assigned to the C=O stretching vibration of a carboxyl acid group) and another, a small signal at 817.8  $\text{cm}^{-1}$ , which was at a lower wavenumber than the expected value (820  $\text{cm}^{-1}$ ) for the S–O stretching vibration of the primary sulfate group (Nakamoto, 1986). The absence of peaks in the region of 800–1000  $\text{cm}^{-1}$  indicates that the extract does not contain any 3,6-anhydrogalactosyl residues, which should appear at 930  $\text{cm}^{-1}$  (Matsuhiro *et al.*, 2005).

Figure 9.7 shows the spectrum of the 4000–2600  $\text{cm}^{-1}$  region. The bands at 2923 and 2852  $\text{cm}^{-1}$  represent asymmetric and symmetric C–H stretching vibrations of alkyl groups in seaweed. The corresponding bending vibrations occur at 1733  $\text{cm}^{-1}$ , respectively. The weak band at 3453  $\text{cm}^{-1}$  occurs because of the stretching vibrations of free N–H. It indicates the *trans*-configuration of a secondary amide. The prominent O–H stretching bond absorption was obtained in the range at 3750  $\text{cm}^{-1}$  and 3710 and 3670  $\text{cm}^{-1}$  (Nakamoto, 1986; Stancioff and Stanley, 1969). The additional bands at 3572 and 3453  $\text{cm}^{-1}$  appear at a lower frequency because of the intermolecular hydrogen bonding of the free hydroxyl group.

### Transmission electron microscope (TEM) studies

Nanotechnology involves the investigation and design of materials or devices at the atomic and molecular levels in the length scale of approximately 1–100 nm range. Nanostructures are capable of enhancing the physical properties of conventional textiles, in areas such as antimicrobial properties, water repellence, soil-resistance, antistatic, antiinfrared and flame-retardant properties, dyeability, color fastness, and strength of the textile materials. For the application of seaweed nanoparticles onto cotton fabric the extracted



**Figure 9.7** FTIR spectra of seaweed extract (4000–2900  $\text{cm}^{-1}$ ).

seaweed powder was characterized by TEM at greater magnifications and is shown in Figure 9.8.

It is clearly seen that the extracted granules of seaweed are in the nanometer scale range and possess different shapes. The variation of both shape and size among the granules

may be due to the nature and the shape of various functional groups present in the extract.

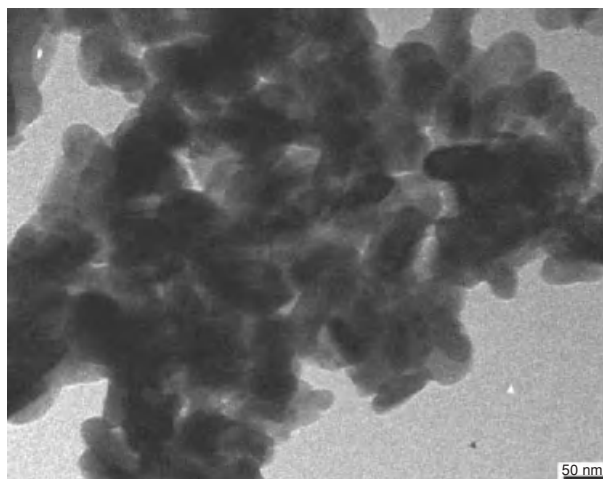
## 9.6 Antibacterial finishing

### 9.6.1 Padding of extract

The finishing solution was prepared by mixing the solvent extract (20 ml/l) and starch solution (30 g/l) with liquor ratio 1:20. The bioscourd and dyed cotton fabric was placed in the finishing solution and kept there for 20 min. Then the fabric was taken out and padded in the padding mangle (Figure 9.9) with 80% wet pick up to get an even distribution of finishing nanoparticles and was then air dried at room temperature (Mercy Sheeba and Thambidurai, 2009). The wet pick up or percent expression is calculated as follows:

$$\text{Percentage expression} = \frac{W_1 - W_0}{W_0} \times 100$$

Where  $W_1$  = weight of the fabric after padding, and  $W_0$  = weight of the fabric before padding



**Figure 9.8** TEM of seaweed extract at 50 nm.

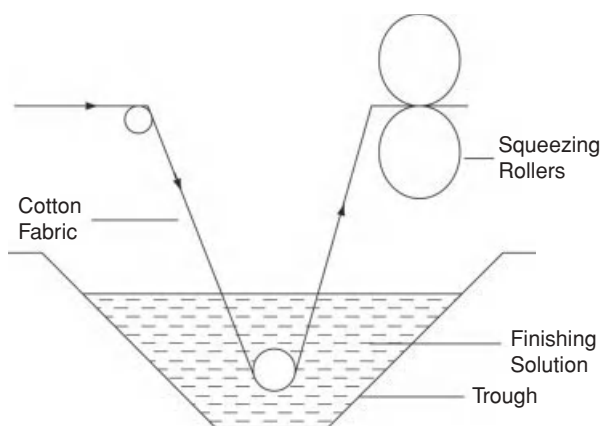


Figure 9.9 Two bowl padding mangle.

### 9.6.2 Antibacterial test

The antibacterial tests were performed as per the standard test procedure ATCC 6538 according to SN 195920. Treated cotton fabric was tested against the Gram-negative bacterium *Escherichia coli*. Fresh inoculants for antibacterial assessment were prepared on nutrient agar at 37 °C for 48 h. All tests on specimens in liquid culture were conducted in nutrient broth. To prepare an agar plate, solid culture was prepared by mixing 2 g of agar-agar, 0.5 g of peptone, and 0.3 g of beef extract in 100 ml of distilled water. One hundred microliters of microbial culture was uniformly distributed on the plate. Treated cotton fabric of 5 mm width was placed on the plate. The plate was placed in an incubator for 24 h at 37 °C. The zone of inhibition was then measured and recorded (Mercy Sheeba and Thambidurai, 2009).

### 9.6.3 Antibacterial property

From the antibacterial activity test, the inhibition zone height of 3 mm is noted. This inhibition zone confirms the antibacterial activity of the acetone-extracted particles. The presence of groups such as amine, sulfate, bromine, and phenols (Murphy *et al.*, 2007) may be the reason for the antibacterial activity of the seaweed extract. While the amine absorbs the bacteria, it consequently stops the production of new bacteria and retards the cellular metabolism, thereby killing the bacteria. In conclusion, the acetone extracts show good antibacterial activity as do other solvents (Selvasubha *et al.*, 2007; Zheng *et al.*, 2001; Kumar and Rengasamy, 2000; Febles *et al.*, 1995). When the finished fabric is washed with water, the applied extract is lost and hence the amine group content diminished, thereby weakening the antibacterial strength (Mercy Sheeba and Thambidurai, 2009).

## 9.7 Permanent finish

To improve the durability of the antibacterial finished product, research is going on to find out a suitable method of application. Basically, cotton fabrics have certain limitations such as poor crease resistance, durability, and they are easy to wrinkle (Abidi *et al.*, 2005). These drawbacks have been removed by chemical modifications and grafting process. Chemical modifications such as acetylation using acetic anhydride, benzooylation using benzoyl chloride, and cyanoethylation using acrylonitrile were carried out after swelling with 18–24% sodium hydroxide. Grafting was carried out using various monomers such as acrylonitrile, acrylamide and methyl methacrylate after swelling with 1–3% sodium hydroxide. For durable finishing, various crosslinking agents such as formaldehyde, dimethyl dihydroxy ethylene urea, citric acid, carboxylic acid, and 1,2,3,4-butane tetracarboxylic acid were padded with fabric and cured at above 150 °C.

However, after these processes the reactivity of the cellulose is reduced because of high degree of hydrophobicity or blocking of hydroxyl groups. To increase the reactivity even after chemical modification, the subsistent should contain a functional group such as amine or halogens. Micheal *et al.* (2002) effectively modified the cotton cellulose and increased the dye uptake by introducing amino groups as a new active center.

Recently, Selvasubha and Thambidurai (2006) reported work on the introduction of an amide group in the cellulose structure with addition of solvents either acetone or ethanol and acrylonitrile. During this process, the nitrile groups ( $C\equiv N$ ) were substituted in the  $C_2$  or  $C_3$  positions hydroxyl groups and were converted into amine groups by solvent-induced hydroxylation process. These amine groups were proved to have more reactivity (Selvasubha and Thambidurai, 2008) than the hydroxyl groups when the dyeing was carried out with reactive dyes in the absence of either an exhausting agent (sodium chloride) or a fixing agent (sodium hydroxide) or both. Hence, during application of seaweed a suitable medium or agent is also needed to create a bond between the hydroxyl group of cellulose with any one of the functional groups present in the seaweed extract, such as sulfur dioxide, amide, halides, or phenol groups.

## Acknowledgments

The author would like to thank the UGC, New Delhi, for providing financial assistance and Dr. P. Manisankar, Professor and Head, Department of Industrial Chemistry for his encouragement. Also, he would like to thank the Scholars J. Mercy Sheeba and S. Anandhavelu for their help.

## References

- Abdou, E.S., Elkholy, S.S., Elsabee, M.Z. and Mohamed, E. (2008) Improved antimicrobial activity of polypropylene and cotton nonwoven fabrics by surface treatment and modification with chitosan. *J. Appl. Polym. Sci.*, **108**, 2290–2296.
- Abidi, N., Hequet, E., Turner, C. and Sari-Sarraf, H. (2005) FTIR analysis of crosslinked cotton using a ZnSe-universal attenuated total reflectance. *J. Appl. Polym. Sci.*, **96**, 392–399.
- Anandhavelu, S. and Thambidurai, S. (2011) Preparation of chitosan–zinc oxide complex during chitin deacetylation. *Carbohydr. Polym.*, **83**, 1565–1569.
- Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turk, M. (1966) Antimicrobial susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, **45**, 493–496.
- Baumgartner, J.N. and Yang, C.Z. (1997) Physical property analysis and bacterial adhesion on a series of phosphonated polyurethanes. *Biomaterials*, **18**, 831–837.
- Bikales, N.M. and Segal, L. (eds) (1971) *High Polymers*, Vol. V, Part V, *Cellulose and Cellulose Derivatives*. John Wiley & Sons, Inc., New York.
- Buschle-Diller, G., Radhakrishnaiah, R. and Freeman, H. (2001) Environmentally benign preparatory processes-introducing a closed-loop system. National Textile Center Annual Report, C99–A07.
- Chen C. and Chang, W-Y. (2007) Antimicrobial activity of cotton fabric pretreated by microwave plasma and dyed with onion skin and onion pulp extractions. *Ind. J. Fibre Textile Res.*, **3b2**, 122–125.
- Cho, S-H., Kang, S-E., Cho, J-Y., *et al.* (2007) The antioxidant properties of brown seaweed (*Sargassum siliculosum*) extracts. *J. Med. Food*, **10**, 479–485.
- Choudhury, S., Sree, A., Mukherjee, S.C., *et al.* (2005) Antibacterial activity of organic extracts of selected marine algae and mangroves against fish pathogens. *Asian Fish. Sci.*, **18**, 285–294.
- Cohen, J.I., Castro, S., Han, J.-a., *et al.* (2000) Polycations. IX. polyammonium derivatives of cyclodextrins: syntheses and binding to organic oxyanions. *Heteroatom Chem.*, **11**, 546–555.
- Cox, S., Abu-Ghannam, N. and Gupta, S. (2010) An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *Int. Food Res. J.*, **17**, 205–220.
- Demirel, Z., Yilmaz-Koz, F.F., Karabay-Yavasoglu, U.N., *et al.* (2009) Antimicrobial and antioxidant capacity of brown algae collected from Aegean Sea. *J. Serbian Chem. Soc.*, **74**, 619–628.
- Duan, X-J., Zhang, W-W., Li, X-M. and Wang, B-G. (2006) Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chem.*, **95**, 37–43.
- Fang, N., Chan, V., Mao, H.Q. and Leong, K.W. (2001) Interactions of phospholipid bilayer with chitosan: effect of molecular weight and pH. *Biomacromolecules*, **2**, 1161–1168.
- Febles, C.I., Arias, A., Gil-Rodriguez, M.C., *et al.* (1995) *In vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). *Anuario del Instituto de Estudios Canarios*, **34**, 181–192.
- Gao, Y. and Cranston, R. (2008) Recent advances in antimicrobial treatments of textiles. *Textile Res. J.*, **78**, 60–72.
- Hebeish, A. (1994) *Development in Textile Chemistry and Chemical Technology* (Special Contribution), 2nd edn. Academy of Scientific Research and Technology, Cairo, Egypt, p. 149.
- Hebeish, A. and El-Hilw, Z.H. (2001) Chemical finishing of cotton using reactive cyclodextrin. *Coloration Technol.*, **117**, 104–110.
- Hermans, M.H. (2006) Silver-containing dressings and the need for evidence. *Am. J. Nursing*, **106**, 60–68.
- Huang, L.K. and Sun, G. (2003a) Durable and regenerable antimicrobial cellulose with oxygen bleach: concept proofing. *AATCC Rev.*, **3**, 17–21.
- Huang, L.K. and Sun, G. (2003b) Durable and oxygen bleach rechargeable antimicrobial cellulose: sodium perborate as an activating and recharging agent. *Ind. Engg Chem. Res.*, **42**, 5417–5422.
- Ibrahim, N.A., Gouda, M., El-Shafel, A.M. and Abdel, D.M. (2007) Antimicrobial activity of cotton fabrics containing immobilized enzymes. *J. Appl. Polym. Sci.*, **104**, 1754–1761.
- Joseph S., and Lipton, A.P. (2004) Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the peninsular coast of India. *J. Mar. Sci. Technol.*, **12**, 1–6.
- Joshi, M., Wazed Ali, S. and Rajendran, S. (2007) Antibacterial finishing of polyester /cotton blend fabrics using neem (*Azadirachta Indica*): A natural bioactive agent. *J. Appl. Polym. Sci.*, **106**, 793–800.
- Kubaneck, J., Jensen, P.R., Keifer, P.A., *et al.* (2003) Seaweed resistance to microbial attack: A targeted chemical defense against marine fungi. *Proc. Natl Acad. Sci. USA*, **100**, 6916–6921.
- Kumar, K.A. and Rengasamy, R. (2000) Evaluation of antibacterial potential of seaweeds occurring along the coast of Tamil Nadu, India against the plant pathogenic bacterium *Xanthomonas oryzae* pv. *Oryzae* (Ishiyama) dye. *Bot. Mar.*, **43**, 409–415.
- Lee, Y-H, Hwang, E-K. and Kim, H-D. (2009) Colorimetric assay and antibacterial activity of cotton, silk, and

- wool fabrics dyed with peony, pomegranate, clove, coptis chinensis and gallnut extracts. *Materials*, **2**, 10–21.
- Li, L.H., Deng, J.C., Deng, H.R., *et al.* (2010) Synthesis and characterization of chitosan/ZnO nanoparticles composite membranes. *Carbohydr. Res.*, **345**, 994–998.
- Li, Q., Chen, S.L. and Jiang, W.C. (2007) Durability of nano ZnO antibacterial cotton fabric to sweat. *J. Appl. Polym. Sci.*, **103**, 412–416.
- Lima-Filho, J.V.M., Carvalho, A.F.F.U., Freitas, S.M. and Melo, V.M.M. (2002) Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian coast. *Braz. J. Microbiol.*, **33**, 311–313.
- Liu, S. and Sun G. (2006) Durable and regenerable biocidal polymers: acyclic N-alamine cotton cellulose. *Ind. Engg Chem. Res.*, **45**, 6477–6482.
- Manilal, A., Sujith, S., Selvin, J., *et al.* (2009) Antibacterial activity of *Falkenbergia hillebrandii* (Born) from the Indian coast against human pathogens. *Phyton-Int. J. Exp. Bot.*, **78**, 161–166.
- Mao, J. and Murphy, L. (2001) Durable freshness for textiles. *AATCC Rev.*, **1**, 28–31.
- Mathlouthi, M. and Koenig, J.L. (1986) Vibrational spectra of carbohydrates. *Adv. Carbohydr. Chem.*, **44**, 7–66.
- Matsuhiro, B., Conte, A.F., Damonte, E.B., *et al.* (2005) Structural analysis and antiviral activity of a sulfated galactan from the red seaweed *Schizymenia binderi* (Gigartinales Rhodophyta). *Carbohydr. Res.*, **340**, 2392–2402.
- McDonnell, G. and Russell, A.D. (1999) Antiseptics and disinfectants: activity, action, and resistance. *Clin. Microbiol. Rev.*, **12**, 147–179.
- Mercy Sheeba, J. and Thambidurai, S. (2009) Extraction, characterization, and application of seaweed nanoparticles on cotton fabrics. *J. Appl. Polym. Sci.*, **113**, 2287–2292.
- Micheal, M.N., Tera, F.M. and Ibrahim, S.F. (2002) Effect of chemical modification of cotton fabrics on dyeing properties. *J. Appl. Polym. Sci.*, **85**, 1897–1903.
- Mtolera, M.S.P. and Semesi, A.K. (1996) Antimicrobial activity of extracts from six green algae from Tanzania. In: *Current Trends in Marine Botanical Research in the East African Region* (eds M. Björk, A.K. Semesi, M. Pedersen and B. Bergman). Gotab AB, Uppsala, Sweden, pp. 211–217.
- Murphy, V., Hughes, H. and McLoughlin, P. (2007) Cu (II) binding by dried biomass of red, green and brown macroalgae. *Water Res.*, **41**, 731–740.
- Nakamoto, K. (1986) *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 4th edn. John Wiley & Sons, Inc. New York, pp. 248–250.
- Obendorf, S.K. and Sun, G. (2007) Hybrid microporous membranes intended for protective clothing. National Textile Center Annual Report, C05–CR01.
- Parekh, K.S., Parekh, H.H. and Rao, P.S. (1984) Antibacterial activity of Indian seaweeds. *Phykos*, **23**, 216–221.
- Parthiban, M. and Gunasekaran, S. (2007) Effect of nano-silver application on antimicrobial finishing. *Melliand Int.*, **13**, 362–363.
- Patra, J.K., Patra, A.P., Mahapatra, N.K., *et al.* (2009) Antimicrobial activity of organic solvent extracts of three marine macroalgae from Chilika Lake, Orissa, India. *Malaysian J. Microbiol.*, **5**, 128–131.
- Purwar, R. and Joshi, M. (2004) Recent developments in antimicrobial finishing of textiles. *AATCC Rev.*, **4**, 22–26.
- Ray, B. and Lahaye, M. (1995) Cell-wall polysaccharides from the marine green alga *Ulva "rigida"* (Ulvales, Chlorophyta) Chemical structure of ulvan. *Carbohydr. Res.*, **274**, 313–318.
- Rosell, K.G. and Srivastava, L.M. (1987) Fatty acids as antimicrobial substances in brown algae. *Hydrobiologia*, **151/152**, 471–475.
- Sastry, V.M.V.S. and Rao, G.R.K. (1994) Antibacterial substance from marine algae: Successive extraction using benzene, chloroform and methanol. *Bot. Mar.*, **37**, 357–360.
- Selvasubha, A. and Thambidurai, S. (2006) Solvent-induced partial cyanoethylation and hydroxylation of cyanoethyl group. *J. Appl. Polym. Sci.*, **102**, 183–191.
- Selvasubha, A. and Thambidurai, S. (2008) Effect of solvent induced hydroxylation of cyanoethyl group on dye uptake of cotton fabrics. *J. Appl. Polym. Sci.*, **108**, 1373–1377.
- Selvasubha, A., Vijay Anand, A., Anita Hebsiba, G., Thambidurai, S. (2007) Finishing of cotton fabric with seaweed extract for anti-bacterial activity. *Colourage*, **54**, 80–90.
- Silverstein, R.M., Bassler, G.C., Morill, T.C. (1991) *Spectrometric Identification of Organic Compounds*. John Wiley & Sons, Inc., New York.
- Son, B., Yeom, B.Y., Song, S.H., *et al.* (2009) Antibacterial electro spun chitosan/poly(vinyl alcohol) nano fibres containing silver nitrate and titanium dioxide. *J. Appl. Polym. Sci.*, **111**, 2892–2899.
- Son, Y.A., Kim, B.S., Ravikumar, K. and Lee, S.G. (2006) A versatile strategy to fabricate hydrogel-silver nanocomposites and investigation of their antimicrobial activity. *Eur. Polym. J.*, **42**, 3059–3067.
- Stancioff, D.J. and Stanley, N.F. (1969) Infrared and chemical studies on algal polysaccharides. *Proceedings of International Seaweed Symposium*, **6**, 595–609.
- Sun, Y. and Sun, G. (2002) Durable and regenerable antimicrobial textile materials prepared by A continuous grafting process. *J. Appl. Poly. Sci.*, **84**, 1592–1599.

- Taskin, E., Ozturk, M., Taskin, E. and Kurt, O. (2007) Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *Afr. J. Biotechnol.*, **6**, 2746–2751.
- Trotman, E.R. (1984) *Dyeing and Chemical Technology of Textile Fibre*, 6th edn. John Wiley & Sons, Ltd, Chichester, Chapter, 2, pp. 31–32.
- Vigneshwaran, N., Kumar, S., Kathe, A.A., *et al.* (2006) Functional finishing of cotton fabrics using zinc oxide-soluble starch nanocomposites. *Nanotechnology*, **17**, 5087–5095.
- Vyas, S.K., Ingale, S.V., Mukhopadhyay, S. and Saraf, N. (2008) *Aloe vera* and neem as antimicrobial agents. [www.fibre2fashion.com](http://www.fibre2fashion.com), 22 October, 1–7. Available <http://www.fibre2fashion.com/industry-article/15/1469/aloe-vera-and-neem-as-antimicrobial-agents1.asp> (accessed 5 May 2011).
- Yadav, A., Prasad, V., Kathe, A.A., *et al.* (2006) Functional finishing in cotton fabrics using zinc oxide nanoparticles. *Bull. Mater. Sci.*, **29**, 641–645.
- Ye, W., Xin, J.H., Li, P., *et al.* (2006) Durable antibacterial finish on cotton fabric by using chitosan-based polymeric core-shell particles. *J. Appl. Polym. Sci.*, **102**, 1787–1793.
- Yu, P., Zhang, Q., Li, N., *et al.* (2003) Polysaccharides from *Ulva pertusa* (Chlorophyta) and preliminary studies on their antihyperlipidemia activity. *J. Appl. Phycol.*, **15**, 21–27.
- Zheng, Y., Chen, Y-S. and Lu, H-S. (2001) Screening for antibacterial and antifungal activities in some marine algae from the Fujian coast of China with three different solvents. *Chinese J. Oceanol. Limnol.*, **19**, 327–331.

# 10

## Enzyme-assisted Extraction and Recovery of Bioactive Components from Seaweeds

**You-Jin Jeon<sup>1</sup>, W.A.J.P Wijesinghe<sup>1</sup> and Se-Kwon Kim<sup>2</sup>**

<sup>1</sup>*School of Marine Biomedical Sciences, Jeju National University, Jeju, Republic of Korea*

<sup>2</sup>*Marine Bioprocess Research Center, Pukyong National University, Busan, Republic of Korea*

### 10.1 Introduction

Seaweeds are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. Macro algae can be classified as red algae (Rhodophyta), brown algae (Phaeophyta) or green algae (Chlorophyta), depending on their nutrient and chemical composition (Dawczynski *et al.*, 2007). Naturally growing seaweeds are an important source of food, especially in Asian countries such as China, Japan, and Korea (Marshall *et al.*, 2007; Rioux *et al.*, 2009). It well known that algae are at the bottom of the food chain in all aquatic ecosystems (Cardozo *et al.*, 2007).

The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity (Manilal *et al.*, 2009). In addition, seaweeds can be a very interesting natural source of new compounds with numerous biological activities that could be used as functional ingredients in many industrial applications (Plaza *et al.*, 2008). In contrast, seaweeds are a critical source of the chemical extracts used extensively in the food, pharmaceutical, and cosmetic industries. Thus, the investigation of new algal chemical compounds, a different source of natural products, has proved to be a promising area of pharmaceutical study. Many

reports have been published about isolated compounds from algae with biological activities, demonstrating their ability to produce metabolites unlike those found in terrestrial species. Moreover, algal products have already been used in the food, cosmetic, and pharmaceutical industries. An expanding market for these products is a fact and is facing a new challenge of growing algae on a large scale without harming any further the marine environment (Cardozo *et al.*, 2007).

In recent years, new kinds of extraction techniques appear to include enzymolysis and microwave-assisted extraction. The former has impressive effects with characteristics of high catalytic efficiency, high specificity, mild reactive conditions and preserving the original efficacy of active compounds to the maximum (Li *et al.*, 2006). The latter method has many advantages, such as shorter time, less solvent, higher extraction rate and better products with lower cost (Bayramoglu *et al.*, 2008; Proestos and Komaitis, 2008). In addition, various extractants were used to release soluble compounds from the algal matrix. The basic procedure for large-scale samples is to extract the algal powder with water or organic solvents. Under these conditions, the extraction yield varies from 8% to 30% of the algal dry yield (Robic *et al.*, 2009). In addition to the studies of the

soluble compounds, there are compounds attached to the cell wall (cell-wall-bound compounds), which cannot be easily extracted using aqueous solvents. This point of view this chapter is a discussion about the use of enzyme-assisted extraction as an alternative method to improve the recovery of bioactive compounds from seaweeds.

## 10.2 Extraction of bioactive compounds from seaweeds

The search for bioactive compounds from marine organisms has been a very attractive research area and number of recent research communications dealt with bioactive compounds isolated from seaweeds. Seaweeds have been identified as a rich source of bioactive compounds (Arunkumar, 2010). However, extraction is the most important step in isolating different types of bioactive compounds from plant materials. The ideal extraction method should be quantitative, non-destructive and time-saving. There are different extraction methods which can be employed in the extraction of active compounds (Yang *et al.*, 2010). The extraction efficiency of active compounds from any plant material can be affected by number of factors such as extraction solvent, particle size, temperature, time and pH, etc.

## 10.3 Role of cell wall degrading enzymes

Plant cell walls mainly consist of interconnecting polysaccharides, the most abundant source of organic carbon in the biosphere. Generally cell walls are made out of highly complex large biopolymers such as cellulose, hemicellulose, lignin and pectin. Cellulose, the most abundant carbohydrate polymer in nature, is the main structural component of plant cell walls and it is extremely difficult to degrade, as it is insoluble and is present as hydrogen-bonded crystalline fibres (Doi and Kosugi, 2004). However, seaweed cell walls and cuticles are chemically and structurally more complex and heterogeneous than those of land plants. They are composed of mixtures of sulfated and branched polysaccharides that are associated with proteins and various bound ions including calcium and potassium (Fuller and Gibor, 1987). Some of the red algae cell walls are made of cellulose, agars and carrageenans (Ali and Gamal, 2010). The presence of complicated cell wall polysaccharides limits the efficiency of general extraction procedures of active compounds from seaweeds. Enzymatic degradation of cell wall polymers has received attention for many years and is becoming a more and more attractive alternative to chemical

and mechanical processes. Therefore, the degradation of cell wall polysaccharide structures is a fundamental step in the release of active components. Hence, degradation of plant cell wall polysaccharides is of major importance in the many applications. In contrast, the degradation of cell wall polysaccharides by enzymes is a useful procedure which can be employed for extraction of bioactive compounds from seaweeds.

## 10.4 Importance of enzyme treatment prior to extraction of bioactive compounds

The enzyme-assisted extraction technique has been widely used to improve the extraction efficiency of bioactive components from land plants. In contrast, the successful use of this technique to enhance the recovery of polyphenols from citrus peel (Li *et al.*, 2006), grape skin (Pinelo *et al.*, 2006), apple skin (Pinelo *et al.*, 2008), unripe apples (Zheng *et al.*, 2009), blackcurrant (Landbo and Meyer, 2001) and *Ginkgo biloba* leaves (Chen *et al.*, 2011) have reported. However, the application of the enzyme-assisted extraction method to seaweed materials has been rarely reported. Seaweeds are a sustainable natural resource with industrial potential that is not fully utilized and there is considerable interest in the use of seaweed in commercial applications. In particular there is added value to extracted components for a wide range of uses such as cosmetic products, functional foods, and nutraceuticals. In order to identify conditions for improving algal extraction efficiency, their cell wall materials can be sequentially extracted by different solvents. The yields of extracts, the chemical composition, the chemical structure and the macromolecular properties of soluble extracts and insoluble residues depend on the nature of interactions of cell wall components. Therefore, alternative extraction conditions and techniques such as enzyme-assisted extraction can be employed on the basis of recovery of biologically active compounds from seaweeds. A summary of enzyme assisted extraction of components from seaweeds is given in Table 10.1.

## 10.5 Selection of the enzyme/s and the extraction conditions

Enzymes are biological catalysts. Selection of the appropriate hydrolytic enzyme or optimal mixture of enzymes is vital. Addressing the potency issue first one has to select the suitable enzyme to digest specific polymer bonds present in the intact seaweed material. After selection of the suitable

**Table 10.1** Summary of enzyme assisted extraction of components from seaweeds

Seaweed	Enzyme treated	Target active components	Reference
<i>Porphyra yezoensis</i> (red)	digestive enzymes obtained from the gut of abalone	Proteins	Amano and Noda, 1990
<i>Ulva pertusa</i> (green)	cellulase and Macerozyme mixture	Proteins	Amano and Noda, 1992
<i>Laminaria japonica</i> (brown)	an extract of gut from abalone in addition to the same enzymatic mixture used for the green seaweeds	Proteins	Amano and Noda, 1992
<i>Callymenia perforate</i> (red)	a mixture of digestive enzymes from the gut of abalone plus Macerozyme	Proteins	Amano and Noda, 1992
<i>Chondrus crispus</i> (red)	Carrageenase and cellulase	Proteins	Fleurence <i>et al.</i> , 1995
<i>Garcilaria verrucosa</i> (red)	Agarase and cellulase	Proteins	Fleurence <i>et al.</i> , 1995
<i>Sargassum horneri</i> (brown)	Commercial carbohydrases and proteases	Polysaccharides and proteins	Park <i>et al.</i> , 2004
<i>Scytosiphon lomentaria</i> (brown)	Commercial carbohydrases and proteases	Polysaccharides and proteins	Ahn <i>et al.</i> , 2004
<i>Ecklonia cava</i> (brown)	Food industrial carbohydrates	Polysaccharides	Heo <i>et al.</i> , 2005b
Seven species (brown)	Commercial carbohydrases and proteases	Polysaccharides and proteins	Heo <i>et al.</i> , 2005a
<i>Ecklonia cava</i> (brown)	Proteases	Proteins	Kim <i>et al.</i> , 2006a
<i>Hizikia fusiformis</i> (brown)	Alcalase and ultraflo	Polyphenols	Siriwardhana <i>et al.</i> , 2008
<i>Palmaria palmate</i> (red)	Umamizyme	Polyphenols	Wang <i>et al.</i> , 2010

enzymes various process conditions can be employed in order to obtain the maximum recovery of active components. There are several factors conditioning directly the effect of enzymes on the degradation of the cell wall polymers, and the release of target active compounds can be modified by manipulation and finding the optimal conditions. Simply there are optimal conditions which their activity is greatest.

Incubation time-temperature combination of the enzymatic treatment prior to extraction is possibly one of the most important factors to be considered. In the case of enzymatic reactions, many enzymes are adversely affected by high temperatures. In addition, when extracting polyphenols, the temperature cannot be increased indefinitely because of their instability. Further, enzymes are affected by changes in pH. The most favorable pH value, the point where the enzyme is most active is known as the optimum pH. Therefore, to perform the enzyme activity at maximum level, temperature and pH should be adjusted to their optimal conditions. The optimum reaction conditions (pH and temperature) of commonly used enzymes are shown in Table 10.2.

The enzyme concentration or the enzyme/substrate ratio is one of the most considerable variables. Type of extrac-

tion solvent used and the solvent to material ratio are also other important factors to be considered. In fact, the type of solvent is one of the most influencing variables in the extraction process. Aqueous organic solvents such as ethanol and methanol are the commonly employed solvent types for extraction of polyphenols. In addition, particle size and as well as agitation also play a critical role during the extraction process.

## 10.6 Bioactive peptides from seaweeds

The role of proteins as physiologically active components in the diet is being increasingly acknowledged. The primary structure of natural proteins consists of certain amino acid sequences that have the ability to exert physiological benefits in human beings. Those kinds of peptides are inactive within the sequence of the parent protein and can be released in different ways such as enzyme hydrolysis by digestive enzymes and hydrolysis by proteolytic microorganisms. Recently marine peptides have opened a new perspective for pharmaceutical developments (Aneiros and Garateix,

**Table 10.2** Optimum conditions for some commonly used enzymes

Enzyme	Optimum conditions		Reference
	pH	Temperature (°C)	
Viscozyme	4.5	50	Heo <i>et al.</i> , 2005a, Kim <i>et al.</i> , 2006a
Celluclast	4.5	50	Heo <i>et al.</i> , 2005, Kim <i>et al.</i> , 2006
AMG	4.5	60	Heo <i>et al.</i> , 2005, Kim <i>et al.</i> , 2006
Termamyl	6.0	60	Heo <i>et al.</i> , 2005, Kim <i>et al.</i> , 2006
Ultraflo	7.0	60	Heo <i>et al.</i> , 2005, Kim <i>et al.</i> , 2006
Carrageenase	6.8	45	Fleurence <i>et al.</i> , 1995
Agarase	6.0	55	Fleurence <i>et al.</i> , 1995
Xylanase	5.0	55	Fleurence <i>et al.</i> , 1995
Cellulose	3.8	50	Fleurence <i>et al.</i> , 1995
Protamex	6.0	40	Heo <i>et al.</i> , 2005, Kim <i>et al.</i> , 2006
Kojizyme	6.0	40	Heo <i>et al.</i> , 2005, Kim <i>et al.</i> , 2006
Neutrase	6.0	50	Heo <i>et al.</i> , 2005, Kim <i>et al.</i> , 2006
Flavourzyme	7.0	50	Heo <i>et al.</i> , 2005, Kim <i>et al.</i> , 2006
Alcalase	8.0	50	Heo <i>et al.</i> , 2005, Kim <i>et al.</i> , 2006
Umamizyme	7.0	50	Wang <i>et al.</i> , 2010

2004). Biologically active peptides and proteins have been isolated not only from marine animals, but also from seaweeds. With respect to nutraceuticals and pharmaceutical potentialities of seaweeds, different proteins and peptides with various bioactivities have been discovered. Some red or green seaweed species contain considerably high amount of proteins. However, extraction of protein from most seaweed is difficult due to the presence of large amounts of cell wall polysaccharides, such as alginates of the Phaeophyta or the carrageenans of some Rhodophyta (Fleurence, 1999). Amano and Noda (1990) reported that the use of algal cell wall degradation enzymes to facilitate the extraction of proteins from red alga *Porphyra yezoensis*. Further, they demonstrated the use of an enzymatic mixture, including digestive enzymes to improve the protein accessibility. In addition, the technique was tested on several algae including *Ulva pertusa*, *Laminaria japonica*, and *Callymenia perforate* and significant differences were obtained in protein composition for all the treated seaweeds (Amano and Noda,

1992). Fleurence *et al.* (1995) reported that the simultaneous application of carrageenase and cellulase activities on red alga *Chondrus crispus* led to a 10-fold increase in extraction efficiency of the proteins. Further they demonstrated that coupled use of agarase and cellulase on another red alga *Gracilaria verrucosa*, led to a threefold increase in protein yield. Therefore, incorporation of polysaccharidases is an attractive and efficient alternative method which facilitating access to the algal protein fraction. Moreover, in the case of enzymatic hydrolysis, the initial non-specific breakdown or the depolymerization of structural and storage polysaccharides could enhance the easy access of proteases to their respective substrates, which are located inside the cells.

The antioxidant activity of water-soluble natural extracts from edible brown seaweed, *Sargassum horneri*, was evaluated by examining the radical scavenging activities of the enzyme extracts of hydrolyzates from *S. horneri* (Park *et al.*, 2004). The brown seaweed was enzymatically hydrolyzed to obtain water soluble extracts using commercially available proteases and carbohydrases. Further they reported that Alcalase and Viscozyme extracts were more effective than the other extracts tested. However, enzyme-assisted extraction could be employed in order to degrade seaweed tissues, which helps the release of a variety of bioactive compounds, and in addition the technique allowed successful production of water-soluble materials from seaweeds with a considerably high yield.

### 10.6.1 Polyphenols and brown algal phlorotannins

Polyphenolic compounds are large and diverse group of naturally occurring compounds containing phenolic functionality and secondary metabolites which exists both in terrestrial and aquatic environments (Shibata *et al.*, 2002; Susanto *et al.*, 2009). Polyphenolic compounds are usually referred to as compounds containing multiple phenolic functionalities (Handique and Baruah, 2002). Since these naturally occurring polyphenols are known to have numerous biological activities such as antioxidant (Ahn *et al.*, 2007; Heo *et al.*, 2009; Kang *et al.*, 2005; Li *et al.*, 2009), anti-inflammatory (Ryu *et al.*, 2008), antiallergic (Le *et al.*, 2009), antibacterial (Al-Mola, 2009; Suffredini *et al.*, 2004), antiplasmin inhibitory (Fukuyama *et al.*, 1990), matrix metalloproteinase inhibitory (Kim *et al.*, 2006b) and anticancer (Kong *et al.*, 2009), they are found to be potential candidates for number of industrial applications such as functional foods, pharmaceuticals, nutraceuticals and functional cosmetics. Phlorotannins are polyphenolic compounds found exclusively in brown algae. Phlorotannins, a subgroup of tannins, are produced entirely by

polymerization of phloroglucinol units (Kang *et al.*, 2007; Koivikko *et al.*, 2005). During last two decades, the roles and functions of phlorotannins have been the subject of many studies.

Some plant polyphenols appear to be twisted together with cell wall polysaccharides via tight hydrophilic and hydrophobic bonds. Therefore the release of these polyphenolic compounds can be enhanced by enzyme-enhanced degradation of cell wall polysaccharides (Pinelo *et al.*, 2006). However, polyphenolic compounds existing in plants are not always associated with the plant cell walls (Pinelo *et al.*, 2006). The ability of tannins to form strong complexes with proteins, either reversibly by hydrogen bonding through peptide or amide linkages, or irreversibly by covalent condensations, is well known (Stern *et al.*, 1996). In addition, protein-binding activity depends mainly on the structure of the protein as well as the length and the structure of the tannin polymer (Haslam, 1996; Waterman and Mole, 1994). Moreover, phlorotannins are found to form covalent bonds with proteins; further, the protein precipitation of phlorotannins varies in a pH-dependent as well as concentration-dependent fashion (Stern *et al.*, 1996). This interaction between phlorotannins and proteins can be one of the important factors in the extraction of either proteins or phlorotannins. The procedures used for extraction of tannins from plant materials are widely variable. However the solvents most commonly used to extract phlorotannins have been aqueous organic solvents such as ethanol. It may be that the organic solvent increases the total extraction yield by inhibiting interactions or breaking hydrogen bonds between tannins and proteins during extraction.

Heo *et al.* (2005a) reported the potential antioxidant activities of enzymatic extracts from seven species of brown seaweeds using four different reactive oxygen species (ROS) scavenging assays namely DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical, superoxide anion, hydroxyl radical and hydrogen peroxide scavenging assays. The brown seaweeds were enzymatically hydrolyzed to prepare water-soluble extracts by using five different commercially available carbohydrate-degrading enzymes and five proteases. Further they reported that among the enzyme extracts, two extracts showed remarkable scavenging effects in DPPH free radical scavenging assay and the activity indicated a marked correlation with phenolic contents.

In one of the recent studies the effect of various protease and carbohydrase treatments on the extraction of polyphenols and other antioxidant ingredients from the red algae *Palmaria palmata* (dulse) was investigated (Wang *et al.*, 2010). The results of the study showed that enzyme-assisted extraction was effective in enhancing the recovery of polyphenols and other hydrophilic antioxidant compounds from a red algae *P. palmata*. In addition, Siriwardhana *et al.*

(2008) reported that effective extraction of algal bioactive compounds can be achieved by treatments such as pH control, heat and enzymatic hydrolysis. Further they demonstrated that integration of those optimized treatments in the extraction sequence of heat, enzymatic hydrolysis and pH control was the most effective sequence to extract antioxidants from the brown algae, *Hizikia fusiformis*.

Taken together, enzyme treatment prior to extraction has resulted in improved yields in the case of bioactive components from seaweeds. In all instances mentioned above, hydrolytic enzymes have been used in different combinations as agents that interact on cell walls, breaking down the structural integrity and rendering the intracellular materials more exposed for solvent extraction. Following this approach, enzymes have been explored as a mean to enhance the extraction of active compounds in seaweeds.

### 10.6.2 Carotenoids

Carotenoids are tetraterpenoid organic pigments that occur naturally in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus, some bacteria, and at least one species of aphid. People consuming diets rich in carotenoids from natural foods, such as fruits and vegetables, are healthier and have lower mortality from a number of chronic illnesses (Diplock *et al.*, 1998). Carotenoids with molecules containing oxygen, such as lutein and zeaxanthin, are known as xanthophylls. Fucoxanthin is a xanthophyll, with formula  $C_{42}H_{58}O_6$ . It is found as an accessory pigment in the chloroplasts of brown algae and most other heterokonts, giving them a brown or olive-green color. Currently, the most common way for extraction is by liquid solvent extraction using toluene, hexane, or petroleum ether (Roh *et al.*, 2008).

Barzana *et al.* (2002) reported that successful use of enzyme-mediated solvent extraction of carotenoids from marigold flower (*Tagetes erecta*). In contrast, 97% recovery yield of carotenoids was obtained under the optimal conditions. Generally, enzyme extraction prior to mechanical or solvent extraction has improved yields of target components. However, to date there has been no literature report found on extraction of carotenoids from seaweeds using enzyme-assisted extraction process. The process could be employed to enhance the extraction efficiency of carotenoids from brown seaweeds.

### 10.6.3 Polysaccharides

Marine algae appear to be good sources of fibres presenting great chemical, physicochemical and rheological diversities (Lahaye, 1991). Dietary fibers from edible seaweeds are little

or non-digested by man (Ruperez *et al.*, 2002). In addition, sulfated polysaccharides of soluble fibers from marine algae are not toxic for human. Sulfated polysaccharides are found in cell wall of the seaweeds. Several polysaccharides isolated from seaweeds exhibited promising antioxidant, antiproliferative, anticoagulant potentials (Costa *et al.*, 2010). Especially, fucans and alginic acid derivatives are known to exhibit different biological properties such as anticoagulant, anti-inflammatory, antiviral and antitumoral activities (Boisson-Vidal *et al.*, 1995).

*Ecklonia cava*, a kind of brown alga that is found abundantly in the subtidal regions of Jeju Island, Korea and Japan (Kim *et al.*, 2006a; Kim *et al.*, 2008) and it has long been utilized as a traditional food and also as a traditional folk herb (Li *et al.*, 2009; Shim *et al.*, 2009). In addition, this brown seaweed has a variety of compounds including peptides, carotenoids, fucoidans, and phlorotannins showing different biological activities (Heo *et al.*, 2009). Therefore, it is thought that *E. cava* would be a very useful material in production of functional foods. Thus, food grade extraction of *E. cava* is required. The brown seaweed *E. cava* was enzymatically hydrolyzed to prepare water-soluble extracts, using five carbohydrases and five proteases, and their potential antioxidant activities were reported (Kim *et al.*, 2006a). Their results indicated the >30 kDa fraction of the Cellulase enzymatic extract possesses good antioxidant activity against H<sub>2</sub>O<sub>2</sub> mediated cell damage in vitro.

Hydrolytic enzymes can convert water-insoluble seaweed materials into water-soluble materials. Hence, different bioactive properties including antioxidant activities will be expected with the resulting smaller materials of enzymatic extracts from seaweeds. Similarly Ahn *et al.* (2004) reported the radical scavenging activities of the enzymatic extracts hydrolyzed from a brown seaweed *Scytosiphon lomentaria*. According to the results obtained they have reported the potent antioxidant properties of the enzymatic extracts of *S. lomentaria*.

Heo *et al.* (2005b) suggested that the enzymatic extraction of seaweeds for the purpose of obtaining natural antioxidants would provide advantages of simple and large-scale production-process of perfect water-soluble antioxidant extracts. In addition, they have reported the potential antioxidant activity of enzymatic extracts from brown seaweed *E. cava* evaluated by DPPH, hydroxyl and alkyl radical scavenging using electron spin resonance (ESR). Further their results confirmed the carbohydrase extracts of *E. cava* as potent antioxidants.

The production of different bioactive polysaccharides with lyases is required for the development of more functional ingredients for industrial use. Commonly these polysaccharides have been extracted using water or aqueous organic solvents. Since the cell wall consist of complex polymers, it is not easy to extract active polysaccharides us-

ing solvent extraction process. Therefore, enzyme-assisted extraction can be employed as an alternative method to improve the extraction efficiency of bioactive polysaccharides from seaweeds.

## 10.7 Conclusions

Bioactive compounds discussed here are obtained from different seaweeds exhibiting different chemical structures and displaying a large variety of biological effects on specific targets. On the other hand, these components seem to be very useful and promising for biological research to clarify mechanism of action in the human body as well as in the design of very specific and potent new functional ingredients for a wide variety of industrial applications such as functional foods, pharmaceuticals, and functional cosmetics. Enzyme-assisted extraction of bioactive compounds from seaweeds could be a useful technique and the process may provide a valuable alternative to overcome physical and chemical extraction barriers. Therefore, from this point it is suggested that the potential use of enzyme-assisted extraction to improve value-added utilization of seaweed extracts as biologically active ingredients in appropriate industrial applications. Thus, enzyme treatment has been proposed as an alternate stage to solvent extraction processes to improve the yield and quality of several products.

## References

- Ahn, C.B., Jeon, Y.J., Kang, D.S., Shin, T.S. and Jung, B.M. (2004) Free radical scavenging activity of enzymatic extracts from a brown seaweed *Scytosiphon lomentaria* by electron spin resonance spectrometry. *Food Res. Int.*, **37**, 253–258.
- Ahn, G. N., Kim, K. N., Cha, S. H., *et al.* (2007) Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H<sub>2</sub>O<sub>2</sub>-mediated DNA damage. *Eur. Food Res. Technol.*, **226**, 71–79.
- Ali, A. and Gamal, E. (2010) Biological importance of marine algae. *Saudi Pharm. J.*, **18**, 1–25.
- Al-Mola, H. F. (2009) Antibacterial activity of crude extracts and phlorotannin isolated from the diatom *Cymbella* spp. *J. Pharmacy Res.*, **2**, 304–308.
- Amano, H. and Noda, H. (1990). Proteins of protoplasts from red alga *Porphyra yezoensis*. *Nippon Suisan Gakkaishi*, **56**, 1859–1864.
- Amano, H. and Noda, H. (1992) Proteins of protoplasts from several seaweeds. *Nippon Suisan Gakkaishi*, **58**, 291–299.
- Aneiros, A. and Garateix, A. (2004) Bioactive peptides from marine sources: pharmacological properties and isolation procedures. *J. Chromatogr. B*, **803**, 41–53.

- Arunkumar, K., Sivakumar, S.R. and Rengasamy, R. (2010) Review on biopotential in seaweeds (marine macroalgae): A special emphasis on bioactivity of seaweeds against plant pathogens. *Asian J. Plant Sci.*, **9**, 227–240.
- Bayramoglu, B., Sahin, S. and Sumnu, G. (2008) Solvent free microwave extraction of essential oil from oregano. *J. Food Engng*, **88**, 535–540.
- Barzana, E., Rubio, D., Santamariya, R.I., *et al.* (2002) Enzyme-mediated solvent extraction of carotenoids from Marigold flower (*Tagetes erecta*). *J. Agric. Food Chem.*, **50**, 4491–4496.
- Boisson-Vidal, C., Haroun, F., Ellouali, M., *et al.* (1995) Biological activities of polysaccharides from marine algae. *Drugs Future*, **20**, 1237–1249.
- Cardozo, K.H.M., Garatini, T., Barros, M.P., *et al.* (2007) Metabolites from algae with economical impact. *Comp. Biochem. Physiol. C*, **146**, 60–78.
- Chen, S., Xing, X.H., Huang, J.J. and Xu, M.S. (2011) Enzyme-assisted extraction of flavonoids from *Ginkgo biloba* leaves: Improvement effect of flavonol transglycosylation catalyzed by *Penicillium decumbens* cellulose. *Enzyme Microb. Technol.*, **48**, 100–105.
- Costa, L.S., Fidelis, G.P., Cordeiro, S.L., *et al.* (2010) Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomed. Pharmacother.*, **64**, 21–28.
- Dawczynski, C., Schubert, R. and Jahreis, G. (2007) Amino acids, fatty acids and dietary fibre in edible seaweed products. *Food Chem.*, **103**, 891–899.
- Diplock1, A.T., Charleux, J.L., Crozier-Willi, G., *et al.* (1998) Functional food science and defence against reactive oxidative species. *Br. J. Nutr.*, **80**, 77–112.
- Doi, R.H., Kosugi, A.O. (2004). Cellulosomes: plant-cell-wall-degrading enzyme complexes. *Nat. Rev. Microbiol.*, **2**, 541–551.
- Fleurence, J. (1999) The enzymatic degradation of algal cell walls: a useful approach for improving protein accessibility. *J. Appl. Phycol.*, **11**, 313–314.
- Fleurence, J., Massiani, L., Guyader, O. and Mabeau, S. (1995) Use of enzymatic cell wall degradation for improvement of protein extraction from *Chondrus crispus*, *Gracilaria verucosa* and *Palmaria palmata*. *J. Appl. Phycol.*, **7**, 393–397.
- Fukuyama, Y., Kodama, M., Miura, I., *et al.* (1990) Anti-plasmin inhibitor VI. Structure of phlorofucofuroeckol A, a novel phlorotannin with both dibenzo-1, 4-dioxin and dibenzofuran elements, from *Ecklonia kurome* OKA-MURA. *Chem. Pharm. Bull.*, **38**, 133–135.
- Fuller, M.P. and Gibor, A. (1987) Microorganisms as digesters of seaweed cell walls. *Hydrobiologia*, **151/152**, 405–409.
- Haslam, E. (1996). Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *J. Nat. Prod.*, **59**, 205–215.
- Handique, J.G. and Baruah, J.B. (2002) Polyphenolic compounds: an overview. *Reactive and Functional Polymers*, **52**, 163–188.
- Heo, S.J., Park, E.J., Lee, K.W. and Jeon, Y.J. (2005a) Antioxidant activities of enzymatic extracts from brown seaweeds. *Biores. Technol.*, **96**, 1613–1623.
- Heo, S.J., Park, P.J., Park, E.J., Kim, S.K. and Jeon, Y.J. (2005b) Antioxidant activity of enzymatic extracts from brown seaweed *Ecklonia cava* by electron spin resonance spectrometry and comet assay. *Eur. Food Res. Technol.*, **221**, 41–47.
- Heo, S.J., Ko, S.C., Cha, S.H., *et al.* (2009) Effect of phlorotannins isolated from *Ecklonia cava* on melanogenesis and their protective effect against photo-oxidative stress induced by UV-B radiation. *Toxicology in Vitro*, **23**, 1123–1130.
- Kang, K.A., Lee, K.H., Chae, S., *et al.* (2005) Eckol isolated from *Ecklonia cava* attenuates oxidative stress induced cell damage in lung fibroblast cells. *FEBS Lett.*, **579**, 6295–6304.
- Kang, K.A., Lee, K.H., Park, J.W., *et al.* (2007) Triphlorethol-A induces heme oxygenase-1 via activation of ERK and NF-E2nrelated factor 2 transcription factor. *FEBS Lett.*, **581**, 2000–2008.
- Kim, K.N., Heo, S.J., Song, C.B., *et al.* (2006a) Protective effect of *Ecklonia cava* enzymatic extracts on hydrogen peroxide-induced cell damage. *Proc. Biochem.*, **41**, 2393–2401.
- Kim, M.M., Ta, Q.V., Mendis, E., *et al.* (2006b) Phlorotannins in *Ecklonia cava* extract inhibit matrix metalloproteinase activity. *Life Sci.*, **79**, 1436–1443.
- Kim, S.K., Lee, Y., Jung, W.K., *et al.* (2008) Effect of *Ecklonia cava* ethanolic extracts on airway hyperresponsiveness and inflammation in a murine asthma model: Role of suppressor of cytokine signaling. *Biomed. Pharmacother.*, **62**, 289–296.
- Koivikko, R., Lopenen, J., Honkanen, T. and Jormalainen, V. (2005) Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological aspects. *J. Chem. Ecol.*, **31**, 195–212.
- Kong, C.S., Kim, J.A., Yoon, N.Y. and Kin, S.K. (2009) Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cancer cell. *Food Chem. Toxicol.*, **47**, 1653–1658.
- Lahaye, M. (1991) Marine algae as sources of fibres: determination of soluble and insoluble dietary fibre contents in some “sea vegetables”. *J. Sci. Food Agric.*, **54**, 587–594.
- Landbo, A.K. and Meyer, A. (2001) Enzyme-assisted extraction of antioxidative phenols from blackcurrant juice press residues (*Ribes nigrum*). *J. Agric. Food Chem.*, **49**, 3169–3177.

- Le, Q.T., Li, Y., Qian, Z., Kim, M.M. and Kim, S.K. (2009) Inhibitory effects of polyphenols isolated from marine alga *Ecklonia cava* on histamine release. *Process Biochemistry*, **44**, 168–176.
- Lee, S.H., Han, J.S., Heo, S.J. Hwang, J.Y. and Jeon, Y.J. (2010) Protective effects of dieckol isolated from *Ecklonia cava* against high glucose-induced oxidative stress in human umbilical vein endothelial cells. *Toxicology in Vitro*, **24**, 75–381.
- Li, B. B., Smith, B., and Hossain, M., (2006) Extraction of phenolics from citrus peels 11, Enzyme-assisted extraction method. *Separation and Purification Technology*, **48**, 189–196.
- Li, Y., Quian, Z.J., Ryu, B., Lee, S.H., Kim, M.M. and Kim, S.K. (2009) Chemical components and its antioxidant properties in vitro: an edible marine brown alga, *Ecklonia cava*. *Bioorg. Med. Chem.*, **17**, 1963–1973.
- Manilal, A., Sujith, S., Kiran, G.S., et al. (2009) Biopotentials of seaweeds collected from southwest coast of India. *J. Mar. Sci. Technol.*, **17**, 67–73.
- Marshall, S., Scott, G.W. and Tobin, M.L. (2007) Comparison of nutritive chemistry of a range of temperate seaweeds. *Food Chem.*, **100**, 1331–1336.
- Park, P.J., Shahidi, F. and Jeon, Y.J. (2004) Antioxidant activities of enzymatic extracts from an edible seaweed *Sargassum horneri* using ESR spectrometry. *Journal of Food Lipids*, **11**, 15–27.
- Pinelo, M., Arnous, A. and Meyer, A.S. (2006). Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends Food Sci. Technol.*, **17**, 579–590.
- Pinelo, M., Zornoza, B. and Meyer, A.S. (2008). Selective release of phenols from apple skin. *Separation and Purification Technology*, **63**, 620–627.
- Plaza, M., Cifuentes, A. and Ibanez, E. (2008) In the search of new functional food ingredients from algae. *Trends Food Sci. Technol.*, **19**, 31–39.
- Proestos, C. and Komaitis, M. (2008) Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. *LWT-Food Science and Technology*, **41**, 652–659.
- Rioux, L.E., Turgeon, S.L. and Beaulieu, M. (2009) Effect of season on the composition of bioactive polysaccharides from the brown seaweed *Saccharina longicruris*. *Phytochemistry*, **70**, 1069–1075.
- Robic, A., Mouro, C.R., Sassi, J.F., Lerat, Y. and Lahaye, M. (2009) Structure and interactions of ulvan in the cell wall of the marine green algae *Ulva rotundata* (Ulvales, Chlorophyceae). *Carbohydr. Polym.*, **77**, 206–216.
- Roh., M.K., Uddin, S. and Chun, B.S. (2008) Extraction of fucoxanthin and polyphenol from *Undaria pinnatifida* using supercritical carbon dioxide with co-solvent. *Biotechnol. Bioproc. Engng*, **13**, 724–729.
- Ruperez, P., Ahrazem, O. and Leal, J.A. (2002) Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *J. Agric. Food Chem.*, **50**, 840–845.
- Ryu, B. Li, Y., Qian, Z.J., Kim, M.M. and Kim, S.K. (2008) Exhibitory effects of compounds from brown alga *Ecklonia cava* on the human osteoblasts. *J. Biotechnol.*, **136S**, S588.
- Shibata, T., Yamaguchi, K., Nagayama, K., Kawagushi, S. and Nakamura, T. (2002) Inhibitory activity of brown algal phlorotannins against glycosidases from the viscera of the turban shell *Turbo cornutus*. *Eur. J. Phycol.*, **37**(4), 493–500.
- Shim, S.Y., To, L.Q., Lee, S.H. and Kim, S.K. (2009) *Ecklonia cava* extract suppresses the high-affinity IgE receptor, FcRIε expression. *Food Chem. Toxicol.*, **47**, 555–560.
- Siriwardhana, N., Kim, K.N., Lee, K.W., et al. (2008) Optimization of hydrophilic antioxidant extraction from *Hiziki fusiformis* by integrating treatments of enzymes, heat and pH control. *International J. Food Sci. Technol.*, **42**, 587–596.
- Stern, J.L., Hagerman, A.E., Steinberg, P.D. and Mason, P.K. (1996) Phlorotannin-protein interactions. *J. Chem. Ecol.*, **22**, 1877–1899.
- Suffredini, I.B., Sarder, H.S. and Goncalves, A.G. (2004) Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic Forest. *Brazilian J. Med. Biol. Res.*, **37**, 379–384.
- Susanto, S., Feng, Y. and Ulbricht, M. (2009) Fouling behavior of aqueous solutions of polyphenolic compounds during ultrafiltration. *J. Food Engng*, **91**, 333–340.
- Wang, T., Jonsdottir, R., Kristinsson, H.G., et al. (2010) Enzyme-enhanced extraction of antioxidant ingredients from red algae *Palmaria palmate*. *LWT- Food Science and Technology*, **43**, 1387–1393.
- Waterman, P.G. and Mole, S. (1994) *Analysis of Phenolic Plant Metabolites*. Blackwell Scientific Publications, Oxford.
- Yang, B., Jiang, Y., Shi, J., Chen, F. and Ashraf, M. (2010) Extraction and pharmacological properties of bioactive compounds from longan (*Dimocarpus longan* Lour.) fruit – A review. *Food Res. Int.*, doi:10.1016/j.foodres.2010.10.019
- Zheng, H.Z., Hwang, I.W. and Chung, S.K. (2009). Enhancing polyphenol extraction from unripe apples by carbohydrate-hydrolyzing enzymes. *Journal of Zhejiang University Science B*, **10**, 912–919.

# 11

## Structure and Use of Algal Sulfated Fucans and Galactans

**Vitor H. Pomin**

*Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA, and Federal University of Rio de Janeiro, Medical Biochemistry Institute, Rio de Janeiro, RJ, Brazil*

### 11.1 Introduction

The most studied sulfated polysaccharides (SPs) from the marine environment are the algal sulfated fucans (SFs) and sulfated galactans (SGs) (Pomin and Mourao, 2008; Pomin, 2009, 2010). These molecules are also the most abundant SPs found in nature. Another source of these compounds is marine invertebrates. In terms of total biomass, SFs and SGs are more abundant than the mammalian sulfated glycosaminoglycans (Pomin and Mourao, 2008). Interest in marine SPs is dominated by SFs and SGs, as demonstrated by the quantity of research publications and industrial/pharmacological applications. The SFs and SGs are composed mostly of sulfated fucose or sulfated galactose residues, respectively. However the definition of these nomenclatures has changed over time. In the first official report of isolation of an SF (almost one century ago), the original name for the high fucose containing glycan was fucoidin (Killing, 1913). Approximately 45 years later, McDowell renamed it fucoidan (Percival and McDowell, 1967). This chapter uses the nomenclature SF instead of fucoidan in agreement with IUPAC recommendations, which defines SFs as polysaccharides consisting of at least 90% sulfated L-fucose by monosaccharide composition. However one should note that the name fucoidan is still in common use (Mourao, 2004). Several other sulfated fucose-rich heteropolysaccharides composed of many other sugars

have been isolated and characterized from marine algae (see Albuquerque *et al.*, 2004; Silva *et al.*, 2005; Barroso *et al.*, 2008; Medeiros *et al.*, 2008). They also have different nomenclatures (Albuquerque *et al.*, 2004; Silva *et al.*, 2005; Barroso *et al.*, 2008; Medeiros *et al.*, 2008), such as ascophyllan or xylofucoglycuronan (Larsen *et al.*, (1966); Kloareg *et al.*, 1986) and sargassan or glycuronofucoglycan (Percival, 1968; Kloareg *et al.*, 1986; Larsen *et al.*, (1966); Medcalf *et al.*, 1978). Another example is the sulfated galactofucan (SGF) isolated from *Laminaria abyssalis*, which proved to be a glycan composed of 5–12% sulfated galactosyl units with the balance consisting of sulfated fucosyl units. Sometimes it is difficult to determine whether or not other non-fucosyl units are a consequence of incomplete purification procedures or whether they are components of the SFs from seaweeds (Mourao, 2004; Pomin and Mourao, 2008). We will not discuss the more heterogeneous SPs in this chapter.

Like fucoidan for SFs, other nomenclatures such as agaran and carrageenan co-exist in SGs in order to describe sub-types of galactose composed polysaccharides (Lahaye, 2001). The origin of the name carrageenan comes from a small village, Carragheen, on the Irish coast, where the carrageenan-bearing seaweed *Chondrus crispus* or “Irish moss” grows (Bixler, 1994). The name agaran (proposed by Knutsen *et al.*, 1994; see also Lahaye, 2001) was originally derived from the word “agar”, which means jelly in the Malay language (*agar-agar*) (Pomin and Mourao, 2008).

Algal SFs and SGs are located in the cell wall of seaweeds from where they can be extracted using hot water (Percival and Ross, 1950), acidic solutions (Black, 1954), or proteolytic digestions (Leite *et al.*, 1998; Martinez-Rumayor and Januzzi, 2006). These polymers may account for more than 40% of the total dry weight of isolated algal cell walls (Kloareg, 1984). The composition of these glycans may be very complex. This hinders both complete structure determination (Pomin and Mourao, 2008) as well as accurate molar composition percentages. In addition, some structures have been reported to change according to species (Percival and Ross, 1950; Mian and Percival, 1973), extraction procedures (Mabeau *et al.*, 1990), seasons of harvest (Imbs *et al.*, 2009) as well as local climatic conditions (environmental abiotic effects) (Black, 1954; Honya *et al.* (1999)) and growing places (regions of isolation). Usually other sugars in minor amounts, such as mannose, xylose, arabinose, hexuronic acids and sometimes even short polypeptides, can be detected in even the most homogeneous preparations of SFs and SGs (Mourao, 2004), which may complicate purification and/or structural determination procedures.

## 11.2 Phylogenetic distribution

Among all algae, SFs are reported mostly in brown algae (Phaeophyta) (Patankar *et al.*, 1993; Nishino *et al.*, 1995; Chevolut *et al.*, 1999, 2001; Chizhov *et al.*, 1999; Pereira *et al.*, 1999; Bilan *et al.*, 2002; Yoon *et al.*, 2007). SFs are absent or occur in almost insignificant amounts in green algae (Chlorophyta), red algae (Rhodophyta), and golden algae (Xanthophyta) (Pomin and Mourao, 2008). SFs are unquestionably the most abundant marine SPs since the brown algae dominate the near-shore environment in number of species (1500–2000 species) as well as in terms of biomass (Pomin and Mourao, 2008). As will be discussed later, the structures of SFs are directly related to the species of extraction (Mourao, 2004), in which one single species may show one or more structures (Mourao, 2004), although similar structures can also be found in different species as well (Pomin and Mourao, 2008).

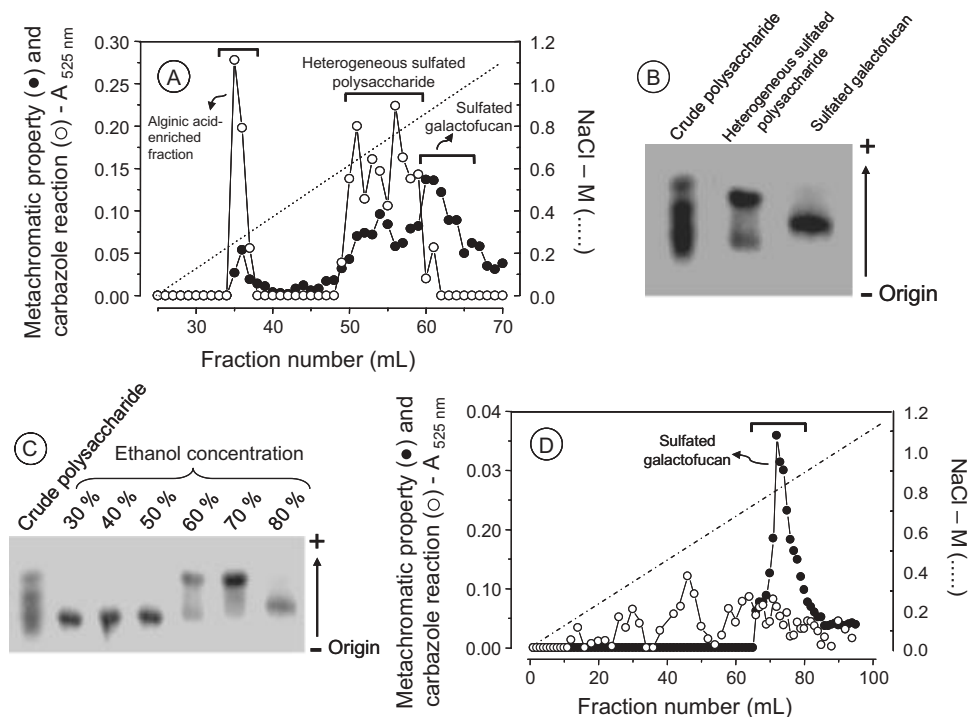
On the other hand, algal SGs are reported to be widely abundant in red algae, whereas in green algae they occur less abundantly (Pomin and Mourao, 2008). Like brown algal SFs, the structures of SGs from red and green algae are also reported to be strictly dependent upon the species from which it has been extracted (Pomin and Mourao, 2008) or on other environmental/laboratorial conditions (Pereira *et al.*, 2005). And as discussed below, the major structural features of SGs are also related to macroalgal class (green or red).

## 11.3 Common methods for extraction and structural analyses

### 11.3.1 Methods for isolation

As these marine SPs are located at the cell wall of seaweeds, lysed preparations containing this outer part of the alga is essential for isolation of their components. This lysis procedure is usually efficiently carried out with non-specific proteolytic enzymes such as trypsin, papain, max-atase and others (Pereira *et al.*, 1999, 2005; Farias *et al.*, 2008). Sometimes components from the algal cell wall, including SPs, are extracted using hot water (Lee *et al.*, 2008) or acidic solutions (Nishino *et al.*, 1994). This first step of isolation of SPs from the cell wall is usually followed by a second procedure based on specific precipitation of polyanionic compounds, in which SFs and SGs are included (Farias *et al.*, 2008). Although the resulting preparation is mainly SPs and polycationic compounds are removed, it may be contaminated with small amounts of nucleic acids, resistant small peptides and anionic amino acids and pigments. Precipitation can be achieved by using either organic solvents such as: ethanol (Figure 11.1 or Pereira *et al.*, 2005), isopropanol (Freile-Pelegrin *et al.*, 2006; Sen *et al.*, 1994), acetone (Farias *et al.*, 2008), or others; or specific zwitterionic/cationic/nonionic detergents such as cetyl-pyridinium-chloride (CPC) (Freile-Pelegrin *et al.*, 2006), Triton X-100 (Bourgougnon *et al.*, 1993), sodium deoxycholate (Bae *et al.*, 2005), CHAPS (Norman *et al.*, 1990), among others. A serial precipitation with increasing volumes of the detergent/organic solvent can also be used (as exemplified in Figure 11.1C). This will lead to SPs in the fractions containing different amounts, structures/compositions and/or degrees of sulfation (Farias *et al.*, 2008). This is typically suggested for sophisticated isolation procedures such as those required for brown algal SFs which are likely to contain the most difficult molecules for isolation, purification and structural determination (Pereira *et al.*, 2005).

The initial crude isolation steps are usually followed by a more refined purification using anion exchange chromatography using either columns filled with cationic resins such as DEAE cellulose (Figure 11.1A) (Pereira *et al.*, 2005), DOWEX<sup>®</sup> (Farias *et al.*, 2000), Lewatit<sup>®</sup> (Figure 11.2) (Farias *et al.*, 2008) or commercial pre-packed cationic columns, made of porous silica or silica gel, destined for high pressure liquid chromatography (HPLC) or fast protein liquid chromatography (FPLC) systems. One or more runs on anionic columns are usually required for complete purification of the algal SFs and SGs. This final procedure is sufficient to separate the SP from other negatively charged

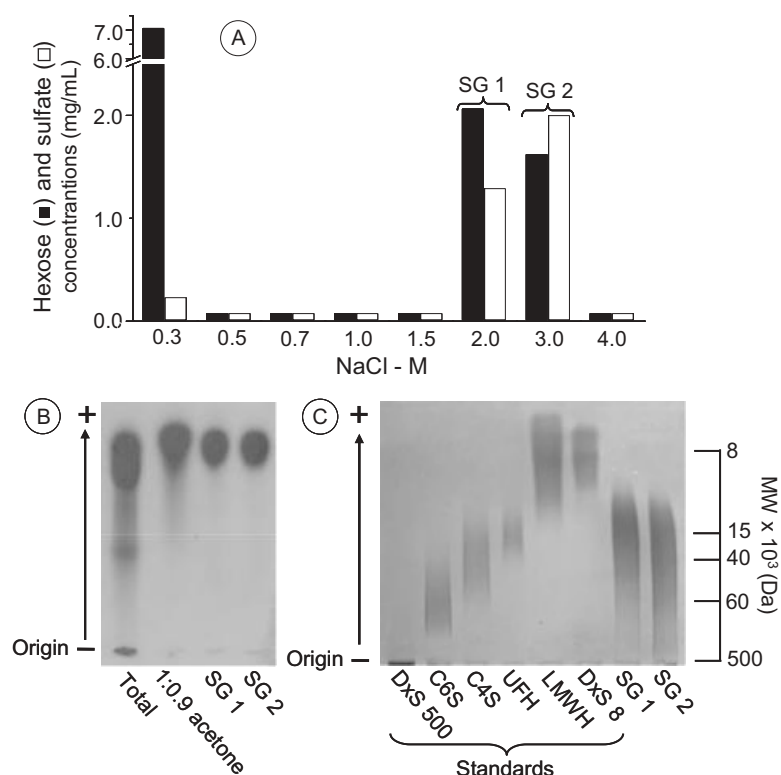


**Figure 11.1** Comparison of two procedures to purify the sulfated galactofucan from the brown alga *Laminaria abyssalis*: one based on anionic-exchange chromatography (A) and the other based on serial precipitation with increasing concentrations of ethanol (C). The respective fractions obtained from each case were analyzed by agarose gel electrophoresis (B, C). (A) crude polysaccharides were applied to a DEAE-cellulose column and eluted with a linear gradient of NaCl, up to 1.2 m, as indicated by the dashed line. The fractions eluted (1 ml each) were checked by their metachromatic properties (closed circles) with DMB (Farias *et al.*, 2008) and hexuronic acid content (open circles) using the carbazole reaction (ref). Absorbance measurements were taken for both at 525 nm as described in Section 11.3.2 methods for detection and quantitation. The three fractionated peaks, as indicated on the panel, were pooled, desalted by dialysis, and lyophilized. (B) The crude polysaccharide (unfractionated extraction), the heterogeneous fraction and the sulfated galactofucan were analyzed by agarose gel electrophoresis. The alginic acid enriched fraction was not applied due to its low metachromatic property. (C) The crude polysaccharides of the brown alga were also purified by a serial-precipitation with increasing concentrations of ethanol and the different precipitates were analyzed by agarose electrophoresis. Their hexuronic acid contents were estimated by the carbazole reaction, as described in Section 11.3.2 methods for detection and quantitation. (D) The 80% ethanol-precipitated fraction was applied to the DEAE-cellulose column. The eluted fractions (1 ml each) were checked by metachromasy (closed circles) and carbazole reaction (open circles).

materials (Figures 11.1A and 11.2A). Homogeneous fractions in the chromatogram may signify homogeneous populations of structures (Figures 11.1B and 11.2B) as illustrated for the SGF isolated from the brown alga *L. abyssalis* (Figure 11.1) and for the SG from the green alga *Codium isthmocladum* (Farias *et al.*, 2008). Fractions are usually eluted from the column using NaCl or volatile quaternary ammonium salt gradients (Figures 11.1A and 11.1D) (Farias *et al.*, 2008; Vogl *et al.*, 2000) or in stepwise salt washes (Figure 11.2A). Anion exchange chromatography can also be used analytically to verify the homogeneity of fractions (Figure 11.1D).

### 11.3.2 Methods for detection, quantization, and purity control

Usually, SPs, including SFs and SGs, are detected by their electrostatic interactions with specific dyes such as 1,9-dimethyl-methylene blue chloride (DMB) (Farias *et al.*, 2000; Pereira *et al.*, 2005), and stains such as toluidine blue (Farias *et al.*, 2000; Pereira *et al.*, 2005). These coloring reagents are widely used by microbiologists and other scientists to stain cells based on their charged contents (mainly in coloration of mast cells). The reaction has a property called metachromasia, which



**Figure 11.2** Purification of the fractions SG 1 and SG 2 isolated from the green alga *Codium isthmocladum* by ion-exchange chromatography (A), and electrophoretic analysis in agarose gel (B) and polyacrylamide gel (C). (A) The fraction precipitated with 0.9 volume of acetone (~12 mg) was applied to a Lewatit<sup>®</sup> column and the elution was carried out in a stepwise system, initiating with 0.3 M NaCl, followed by 0.5 M, 0.7 M, 1.0 M, 1.5 M, 2.0 M, 3.0 M, and 4.0 M NaCl. The eluted fractions were analyzed for their hexose content (black bars) and for sulfate content (white bars). (B) The crude polysaccharides before acetone precipitation (Total), the fraction precipitated with 1:0.9 acetone, and SG 1 and SG 2 were applied to a 0.5% agarose gel in 1,3-diaminopropane:acetate (pH 9.0) and run for 1 h at 110 V as indicated by the arrow. The sulfated polysaccharides in the gel were fixed, dried, and stained with toluidine blue. (C) SG 1 and SG 2 (~10 µg of each) were analyzed by PAGE. The molecular weights (MW-Da) of standard compounds are indicated at the right. These standards were: high-molecular-weight dextran sulfate (~500 kDa), chondroitin 6-sulfate from shark cartilage (~60 kDa), chondroitin 4-sulfate from whale cartilage (~40 kDa), unfractionated heparin from porcine intestinal mucosa (~15 kDa), low-molecular-weight heparin (~7.5 kDa), and low-molecular-weight dextran sulfate (~8 kDa). Data from (Farias *et al.*, 2008).

is the capacity to change colors (in this case, from dark blue to light pink, including several intermediate colors) in direct proportion to the degree of interactions between the tested sample and the stain. The cationic nature of the colorants allows them to interact with SFs and SGs through electrostatic forces (Farias *et al.*, 2000, Pereira *et al.*, 2005). Assays of samples with DMB can confirm the presence of SPs and when used with standards enables quantization of the concentration of unknown SP samples. The DMB assay can also be used for monitoring chromatographic column eluate (Figure 11.1A and D) at an optimum wavelength of 525 nm (Farias *et al.*, 2000, Pereira *et al.*, 2005). Staining with toluidine blue may offer the possibility to qualitatively

or quantitatively detect SPs using mostly electrophoretic methods. See Figures 11.1B, C and 11.2B, C.

Another method widely used in carbohydrate analysis is the phenol-H<sub>2</sub>SO<sub>4</sub> (phenol-sulfuric) reaction (Dubois *et al.*, 1956). This permits the measurement of hexose content ( $\lambda_{\max} = 490$  nm). Brown algal cell walls possess high amounts of alginic acid containing polymers. These uronic acid enriched materials can be detected by the carbazole reaction ( $\lambda_{\max} = 530$  nm; Dische, 1947). This technique is commonly used for tracking contaminants or other negatively charged molecules in ionic exchange chromatography during purification or qualitative analyses of SFs (Figure 11.1A, D).

Agarose gel horizontal electrophoresis is a common and cheap method for checking homogeneity of SF and SG containing samples after purification by liquid chromatography (see item 11.5.2). As observed in Figures 11.1B, C and 11.2B, these electrophoretic gels, after proper staining with toluidine blue, reveal the level of purity of the fractionated samples. The gels in Figure 11.1B and C show the level of homogeneity of brown algal SGF fractions isolated using either ion chromatography or serial precipitation with increasing volumes of ethanol. The 80% ethanol precipitated fraction proved to be the most homogeneous fraction and proved additionally by ion exchange chromatography (Figure 11.1D). A high level of purity was observed for the SG1 and SG2 fractions from the precipitate, with 0.9 volume of acetone, from the *C. isthmocladum* SG (Figure 11.2B) (Farias *et al.*, 2008).

### 11.3.3 Methods for molecular weight determination

The molecular weight (MW) of SFs and SGs are most often estimated by polyacrylamide gel vertical electrophoresis (Figure 11.2C) and less often by high precision size-exclusion chromatography in HPLC or FPLC systems. Although these methods are not as accurate as those from mass spectrometry (MS) they are used due to the high MWs for these molecules. Marine sulfated glycans exhibit high MW, maybe over 100 thousand Da. The MWs of SG1 and SG2 around 14 and 20 kDa, respectively, are one of the rare examples showing very low MWs (Farias *et al.*, 2008). Thus the high MW of the SFs and SGs usually makes MW estimation difficult using MS methods. The limitations are due to instrumental detection limits (MS regularly analyzes glycans no larger than a couple of kDa) and/or due to the high order of complexity in the spectra from these sugars. Spectra may contain several peaks of very complex unpredictable structures along with different levels of ionization or sodiation (depending on the respective counter-ion of the sample). The determination of MWs of these marine compounds via MS is rare and when they are successfully accomplished publication is quite straightforward (Daniel *et al.*, 2007).

### 11.3.4 Methods for structural characterization

So far, desulfation and methylation are the most used and informative methods among all chemical reactions employed for structural characterization of SFs and SGs. Desulfated derivatives analyzed by nuclear magnetic resonance (NMR) spectroscopy are compared to spectra of the na-

tive compound. In addition, MS of methylated derivatives, both native and desulfated samples, offer structural information regarding the position of sulfation as well as other substituents such as pyruvates, acetyls, and glycosylation sites (position of the glycosidic bonds). Both 1D and 2D NMR spectroscopy experiments,  $^1\text{H}$  and  $^{13}\text{C}$  homonuclear and heteronuclear, are quite useful for structural characterization of these marine glycans. Basically all structures deposited in the international literature are characterized via NMR spectroscopy (Mulloy *et al.*, 1994). This chapter does not intend to describe either the types or objectives of NMR experiments since these subjects are widely discussed in many other papers and textbooks. However we will pick, as a single illustrative example, the preponderantly 4-sulfated, 3-linked SG from the green alga *C. isthmocladum*, which was fully characterized by all these methods (Farias *et al.*, 2008). This example describes how these experiments can be successfully applied in order to accomplish structural determination of SPs isolated from seaweeds.

#### Proving 4- and 6-sulfation in the SG fractions from the green alga *C. isthmocladum*

The attempt to determine the structure of the *C. isthmocladum* SG was accomplished using the methylated version of the native polysaccharide as well as its chemically desulfated derivative. In spite of certain limitations concerning these chemical reactions (desulfation and methylation), such as a partial reaction or preferential sites of reaction toward specific positions of the sugar ring, these types of analyses provide generally valuable information regarding the analyzed structures. The methylated derivatives of SG fractions (SG1 and SG2) from *C. isthmocladum* were identified by MS and are listed in Table 11.1.

Comparing the methyl galactitols produced from the native SG and desulfated derivative (Table 11.1), we can observe the disappearance of 2-*O*-methyl galactitol and significant increase in 2,4-di-*O*-methyl galactitol (indicative of 4-sulfation) and 2,4,6-tri-*O*-methyl galactitol (indicative of 4- and 6-sulfation). The production of significant amounts of 2,4,6-tri-*O*-methyl galactitol from methylation of the desulfated galactans suggests the predominance of 3-linked galactose units. In addition, the production of significant amounts of 2,4- and 2,6-di-*O*-methyl galactitols may indicate partial desulfation of the molecules, the possibility of branching residues or the presence of other types of substituents (such as pyruvate) as will be discussed below. Therefore, the data about methylated derivatives of the native and desulfated fractions of the *C. isthmocladum* SG support the presence of 4- and 6-sulfation in these molecules.

**Table 11.1** Methylated galactose derivatives and desulfated galactans obtained from native SG1 and SG2 from the green alga *C. isthmocladum*

Methylated sugars <sup>a</sup> (as alditol acetates)	<i>t<sub>R</sub></i> <sup>c</sup> (min)	% of total peak area <sup>b</sup>			
		SG1		SG2	
		Native	Desulfated	Native	Desulfated
2,4,6-Met <sub>3</sub> -Gal	34.4	10	34	7	36
2,6-Met <sub>2</sub> -Gal	36.9	40	41	32	38
2,4-Met <sub>2</sub> -Gal	40.8	<1	25	<1	22
2-Met-Gal	42.3	50	<1	61	4
	4-substituted		49%		51%
	6-substituted		24%		29%

<sup>a</sup>After the methylation reaction the methylated galactans were hydrolyzed and the products analyzed as their alditol acetate derivatives by GLC-MS using a DB-1 capillary column (25 m × 0.3 mm).

<sup>b</sup>The proportions of the methylated acetates are based on the integral of each peak compared with the sum of integrals.

<sup>c</sup>Retention time of the alditol acetate derivatives in the GLC-MS on the DB-1 capillary column (25 m × 0.3 mm).

### Proving the dominance of 4-sulfated, 3-linked $\beta$ -D-galactopyranosyl units

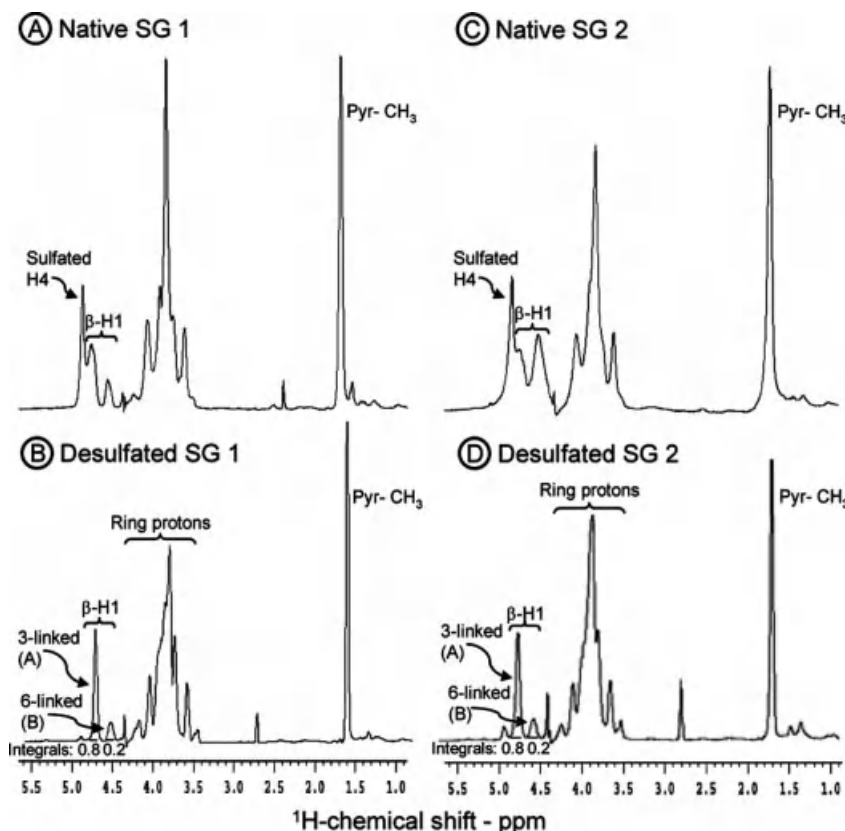
For a more detailed structural analysis of the SGs from the green alga *C. isthmocladum*, one-dimensional and two-dimensional NMR spectroscopy, were employed. The fractions of native SGs and their desulfated derivatives produced similar signals in the <sup>1</sup>H 1D NMR spectra (Figure 11.3), which indicates similar structures for SG1 and SG2. Therefore the 2D NMR spectra were recorded exclusively with fraction SG2 and its desulfated derivative since this fraction looked primarily more sulfated (Figure 11.2A).

The <sup>1</sup>H-signals between 4.4 and 4.9 ppm in the spectra of the SGs contain a mixture of signals from the  $\beta$ -anomers of galactopyranoses and of H-4 from the sugar rings; these exhibited a downfield shift (~0.6 ppm) due to sulfation (Pomin *et al.*, 2005a, b; Bilan *et al.*, 2007). This conclusion is reinforced by the analysis of the <sup>1</sup>H/<sup>13</sup>C Distortionless Enhancement by Polarization Transfer-Heteronuclear Single Quantum Coherence (DEPT-HSQC) spectrum of the native polysaccharide (Figure 11.4A) and the disappearance of the signal at  $\delta_{\text{H}}/\delta_{\text{C}}$  4.94/77.8 ppm, after desulfation (Figure 11.4B). The <sup>1</sup>H 1D (Figure 11.3) and <sup>1</sup>H/<sup>13</sup>C DEPT-HSQC (Figure 11.4) spectra show two preponderantly anomeric signals, designated as A and B, for the native SG2 as well as for its desulfated derivative.

Through the 2D Correlation Spectroscopy (COSY) spectra (Figure 11.5A and C) and Total Correlation Spectroscopy (TOCSY) spectra (Figure 11.5B and D), it was possible to trace the spin systems of these two signals (A and B), especially for the desulfated SG2 (Figure 11.5A

and B). Thus we obtained the <sup>1</sup>H chemical shifts indicated in Table 11.2. Only the chemical shifts of H-6 could not be determined. However, these values were easily deduced due to the negative-phase signals relative to CH<sub>2</sub> (blue-contour peaks in Figure 11.4B) in the <sup>1</sup>H/<sup>13</sup>C DEPT-HSQC spectrum. Two signals of H-6/C-6 associated with spin systems A and B (preponderant and minor blue signals, respectively) were identified. Analysis of the <sup>13</sup>C chemical shift values indicate unequivocally that the units designated A and B (structures 1 and 2 in Table 11.2, respectively) were associated with 3- and 6-linked  $\beta$ -D-galactopyranose residues, respectively, as indicated by the typical low-field shift of carbons (~10 ppm) in sites of glycosylation (Table 11.2). This <sup>13</sup>C shift was also seen in reference compounds of 3- and 6-linked  $\beta$ -D-galactosyl units (structures 5 and 6 in Table 11.2) and in galactose residues located at non-reducing terminals (structure 7 in Table 11.2). The spin system traced for the native SG2 was more complex due to the greater heterogeneity of the polymer. However, two spin systems, denoted A and B (Figure 11.4C and D) (Table 11.2), were identified. Again it was difficult to identify the signals that correspond to H-6, but the DEPT-HSQC spectrum (Figure 11.4A) was especially useful for this assignment (blue-contour peaks).

Signals from glycosylated 6-position ( $\delta_{\text{H,H'}}/\delta_{\text{C}}$  4.36, 4.01/69.9 ppm of units (denoted as B) and unsubstituted 6-position (units A) were identified by comparison with the spectrum of the desulfated SG2 (Figure 11.3B). Moreover, we identified another signal ( $\delta_{\text{H,H'}}/\delta_{\text{C}}$  4.42, 4.32/66.8 ppm) with a typical <sup>1</sup>H low-field shift (~0.6 ppm) that indicates 6-sulfation (Figure 11.3A).



**Figure 11.3**  $^1\text{H}$ -NMR spectra at 400 MHz of the native SG1 (A), native SG2 (C) from *C. isthmocladum*, and the desulfated derivatives of SG1 (B) and SG2 (D). About 5 mg of each were dissolved in 0.5 ml  $\text{D}_2\text{O}$  and the 1D NMR spectra were recorded at 50 °C. The residual water signal was suppressed by presaturation. Chemical shifts are relative to external trimethylsilylpropionic acid at 0 ppm. The H4 signals correspond to 4-sulfated galactose units. The  $\beta$ -H1 signals correspond to the  $\beta$ -anomeric protons. The signals denoted by A and B correspond to 3-linked and 6-linked galactose units, respectively. The integrals of each anomeric peak of the desulfated galactan are indicated under the peak. Pyr- $\text{CH}_3$  indicates methyl signals from pyruvate groups.

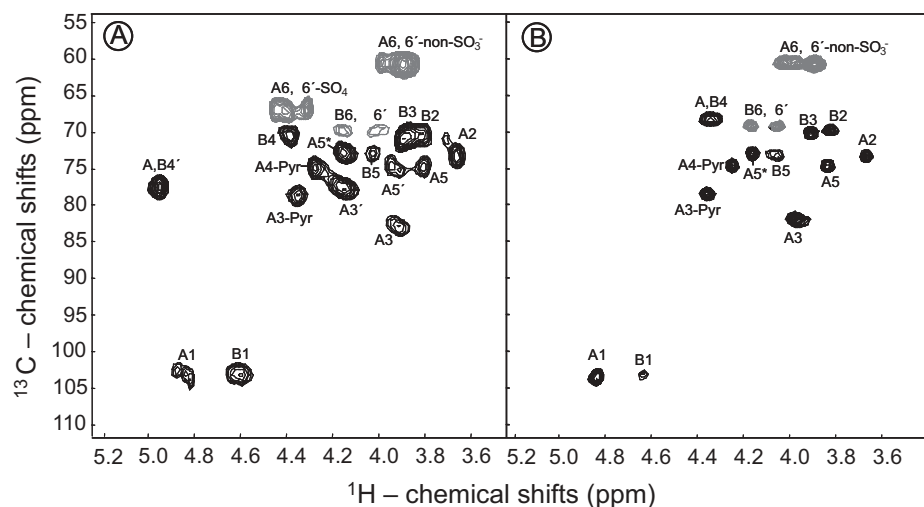
The 6-sulfation is necessarily associated with system A, while system B is glycosylated at this site. System A is mainly sulfated at C-4 as indicated by the typical low-field shift ( $\sim 0.65$  ppm) of the H-4 (structure 3 in Table 11.2). But there are also minor amounts of non-sulfated units as indicated by methylation analysis. System B is mainly 4-sulfated (as indicated by the down-field shift of H-4 and structure 4 in Table 11.2), but non-sulfated units also co-exist.

In synthesis, these results indicate, for the SGs from the green alga *C. isthmocladum*, a preponderant constitution of 3- $\beta$ -D-Galp-1, about 80% of the total residues, as indicated by the integrals of the NMR signals (Figure 11.3B and D). Most of these units are 4-sulfated, but, in minor amounts, there are also units sulfated at the 6-position, as well as non-sulfated units. The sulfated galactans also contain 6-linked units of  $\beta$ -D-Galp-4( $\text{SO}_3^-$ ), and, in minor amounts, 6-linked non-sulfated galactopyranosyl units.

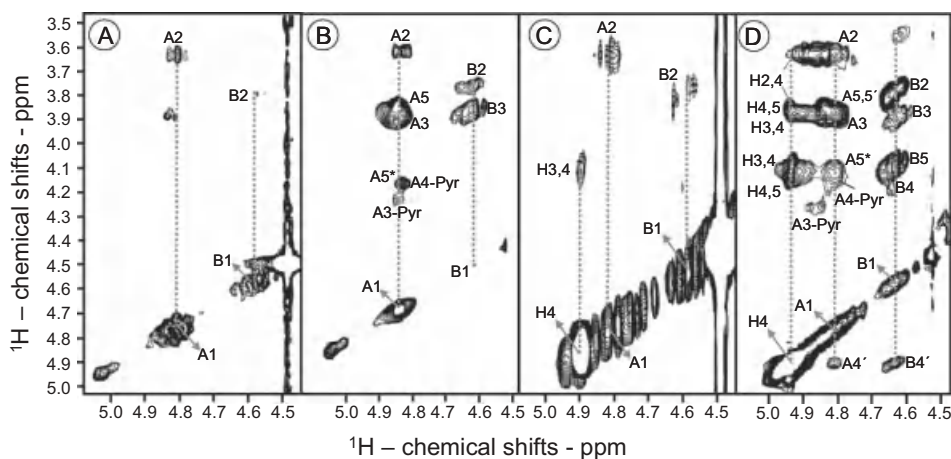
Nuclear Overhauser enhancement spectroscopy (NOESY) spectra (data not shown) did not reveal a clear distribution pattern for these minor residues in the structure of the polymer.

#### Occurrence of 3,4-O-(1' carboxy)-ethylidene units in the SGs from *C. isthmocladum*

Curiously, in addition to the  $^1\text{H}$ - and  $^{13}\text{C}$ -signals from the galactose ring and its anomeric protons and carbons, the native SG1 and SG2 and their desulfated derivatives show an intense high-field  $^1\text{H}$ -signal at  $\sim 1.7$  ppm (Figure 11.3), which strongly indicates methyl groups. Furthermore, in methylation analysis the desulfated galactan was still highly substituted at C-3 and C-4 as observed from the production of 2,6-di-O-methyl galactitols (Table 11.1). These data together suggest the presence of an extra structure with methyl group, bound to C-3 and/or C-4 of the galactose ring.



**Figure 11.4**  $^1\text{H}/^{13}\text{C}$  DEPT-HSQC spectra of the native SG2 (A) and its desulfated derivative (B). The assignments were based on 2D NMR experiments (COSY and TOCSY), and HSQC of the depyruvylated and desulfated derivative (data not shown). The gray-contour peaks are due to the negative phase from  $\text{CH}_2$  groups, and the black-contour peaks are due to the positive phase from CH and  $\text{CH}_3$  groups. The values of chemical shifts are relative to external trimethylsilylpropionic acid at 0 ppm for  $^1\text{H}$  and methanol for  $^{13}\text{C}$ . The signals are denoted by A for 3-linked and by B for 6-linked  $\beta$ -D-galactopyranosyl units. The peaks denoted by A3-Pyr, A4-Pyr and A5\* indicate  $^1\text{H}$ -chemical shifts of H3, H4, and H5 of the 3,4-(1'-carboxy)-ethylidene- $\beta$ -D-galactopyranose residues, respectively. The peaks denoted by A3', A4-B4', and A5' correspond respectively to signals from H3 and H4 of the 4-sulfated, 3-linked  $\beta$ -D-galactopyranosyl units; H4 of the 4-sulfated, 6-linked  $\beta$ -D-galactopyranosyl units; and H5 of the 4-sulfated, 3-linked  $\beta$ -D-galactopyranosyl units.



**Figure 11.5** Strips of the anomeric regions (expansions from 5.1 to 4.5 ppm) from the COSY (A and C) and TOCSY (B and D) spectra of the desulfated galactan 2 (A and B) and the native SG 2 (C and D) from *C. isthmocladum*. About 5 mg of each were dissolved in 0.5 ml  $\text{D}_2\text{O}$  and the 2D NMR spectra were recorded at 50  $^\circ\text{C}$  at 400 MHz. The spin systems are denoted by A for 3-linked and by B for 6-linked  $\beta$ -galactose units. The peaks denoted by A3-Pyr, 4-Pyr and A5\* indicate  $^1\text{H}$ -chemical shifts of H3, H4, and H5 of the 3,4-(1'-carboxy)-ethylidene- $\beta$ -D-galactopyranose residues, respectively. The peaks denoted by A4' and B4' correspond to signals H4 of the 4-sulfated, 3-linked and 6-linked galactosyl units, respectively.

**Table 11.2**  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts (ppm) for native SG 2 from the green alga *C. isthmocladum* and its desulfated derivative from the green alga *C. isthmocladum*

Polysaccharide	Structure	$^1\text{H}$ and $^{13}\text{C}$ chemical shift <sup>a</sup> (ppm)					
		H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6,6' C-6
Desulfated galactan from <i>C. isthmocladum</i>	1) Unit A <sup>c</sup> : 3- $\beta$ -D-Galp-1	4.81	3.64	3.92	4.39	3.86	3.94,3.85
		102.6	73.8	82.9	67.1	75.2	60.3
	2) Unit B <sup>d</sup> : 6- $\beta$ -D-Galp-1	4.62	3.82	3.89	4.38	4.09	4.36,4.01
		103.1	70.1	70.2	67.0	73.9	69.9
Native galactan from <i>C. Isthmocladum</i>	3.1) Unit A: 3- $\beta$ -D-Galp-4(SO <sub>4</sub> )-1 (preponderant)	4.84	3.71	4.16	<b>4.94</b>	3.95	3.96,3.86
		103.1	71.9	77.6	<b>77.8</b>	74.8	60.4
	3.2) Unit A: 3- $\beta$ -D-Galp-4,6-di(SO <sub>4</sub> )-1 (minor)	4.84	3.71	4.16	<b>4.94</b>	3.95	<b>4.42,4.32</b>
		103.1	71.9	77.6	<b>77.8</b>	74.8	<b>66.8</b>
Desulfated galactan from <i>C. yezoense</i> <sup>b</sup>	4) Unit B: 6- $\beta$ -D-Galp-4(SO <sub>4</sub> )-1 (minor)	4.63	3.83	3.89	<b>4.94</b>	4.13	4.36,4.01
		103.2	70.2	70.2	<b>77.8</b>	73.7	69.9
	5) 3- $\beta$ -D-Galp-1	4.69	3.79	3.86	4.21	3.73	3.79
		105.4	71.5	83.2	69.7	76.0	62.3
Native galactan from <i>C. yezoense</i> <sup>b</sup>	6) 6- $\beta$ -D-Galp-1	4.47	3.57	3.69	3.97	3.92	4.04,3.91
		104.8	72.2	73.9	69.9	74.8	70.5
	7) $\beta$ -D-Galp-1	4.62	3.54	3.65	3.92	3.70	3.78
		105.7	72.2	73.9	69.9	76.4	62.2
Native galactan from <i>C. yezoense</i> <sup>b</sup>	8) 3- $\beta$ -D-Galp-4(SO <sub>4</sub> )-1	4.70	3.82	4.08	<b>4.82</b>	3.81	3.74
		105.4	72.2	79.0	<b>78.8</b>	76.0	62.3

<sup>a</sup>Chemical shifts are relative to external trimethylsilylpropionic acid at 0 ppm for  $^1\text{H}$  and methanol for  $^{13}\text{C}$ . Values in boldface indicate sulfate position and in italic indicate glycosylated positions.

<sup>b</sup>Reference values from (Bilan *et al.*, 2007; Shashkov *et al.*, 2000).

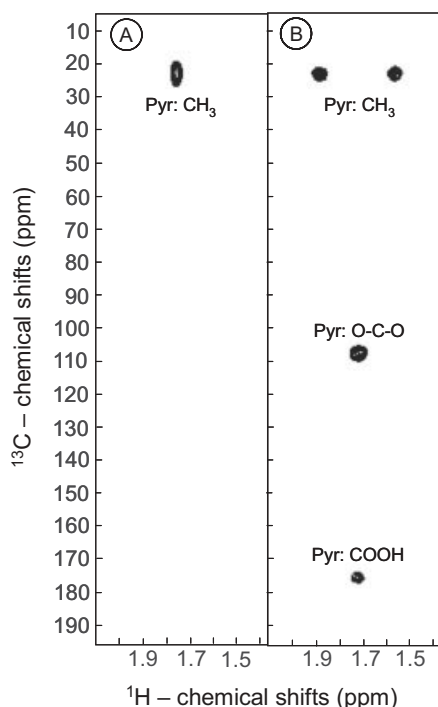
<sup>c</sup>3-linked - $\beta$ -D-galactopyranose.

<sup>d</sup>6-linked - $\beta$ -D-galactopyranose.

The use of  $^1\text{H}/^{13}\text{C}$  heteronuclear NMR experiments proves that this methyl group comes from pyruvate groups. The HSQC spectrum (Figure 11.6A) only reveals a singlet at  $\delta_{\text{H}}/\delta_{\text{C}}$  1.77/23.4 ppm that may correspond to methyl signals from pyruvic acid. The HMBC spectrum (Figure 11.6B) shows a doublet signal at  $\delta_{\text{H}}/\delta_{\text{C}}$  1.87/23.4 ppm and  $\delta_{\text{H}}/\delta_{\text{C}}$  1.67/23.4 ppm due to the occurrence of  $^1\text{H}$ - $^{13}\text{C}$  coupling during the acquisition time. Moreover, due to identified signals of proton nuclei bound to carbon nuclei that are separated by more than one bond, we assigned two other signals coupled to the  $\delta_{\text{H}}$  1.77 ppm peak. These peaks resonate at  $\delta_{\text{C}}$  109.1 ppm and  $\delta_{\text{C}}$  176.6 ppm and correspond respectively to the groups O-C-O and COOH of pyruvate (Figure 11.6B and Table 11.3). The low-field proton chemical shift of this system ( $\sim$ 1.77 ppm) clearly reveals a typical pyruvate involved in a five-membered cyclic ke-

tal including O-3 and O-4 of the non-reducing terminal galactoses instead of a six-membered cyclic ketal including O-4 and O-6 positions (Shashkov *et al.*, 2000; Bilan *et al.*, 2007). These data together indicate the presence of galactose residues with 3,4-O-(1'-carboxy)-ethylidene cyclic ketals.

The occurrence of 3,4-O-(1'-carboxy)-ethylidene-galactose residues in the SGs from the green alga forced us to reinterpret the methylation analysis of these polysaccharides. The observation that significant amounts of 2,6-di-O-methyl derivative obtained from the desulfated galactans (Table 11.1) may be explained by the presence of pyruvylated groups substituted at 3- and 4-positions of the galactoses that are located at non-reducing ends of the polysaccharide. In addition, a re-analysis of the NMR spectra (TOCSY and DEPT-HSQC) of spin-system



**Figure 11.6**  $^1\text{H}/^{13}\text{C}$  HSQC (A) and HMBC (B) spectra of the methyl region of the pyruvate group from native SG 2. Pyr:CH<sub>3</sub>, Pyr: O-C-O and Pyr: COOH indicate signals from the CH<sub>3</sub>, O-C-O and COOH groups of the 3,4-*O*-(1'carboxy)ethylidene  $\beta$ -D-galactopyranosyl units, respectively. The singlet and doublet signals of the Pyr:CH<sub>3</sub> in HSQC and HMBC spectra, respectively, are due to the decoupling and coupling during the acquisition time in the pulse sequences.

A reveals an additional heterogeneity compatible with 3,4-*O*-(1'carboxy)-ethylidene- $\beta$ -D-galactopyranosyl units from non-reducing terminals, especially coincident signals

of H-3 and H-4 of pyruvated units and the low-field shift of the adjacent H-5 (denoted by A5\*) (Figures 11.4 and 11.5).

### Major conclusions about the structure of the SGs from the green alga *C. isthmocladum*

The SG from the green alga *C. isthmocladum* is a complex polysaccharide with different structural components. The main variations come from different positions of glycosidic linkages (3- and 6-linked units), different sulfation sites (positions 4 and/or 6), and from the presence of pyruvate groups involved in cyclic ketals with the positions O-3 and O-4 of the  $\beta$ -D-galactoses located at non-reducing ends. Methylation studies and NMR spectra indicate that 3- $\beta$ -Galp-4(SO<sub>3</sub><sup>-</sup>)-1 is the preponderant unit of these polysaccharides (Figure 11.7A). However galactopyranosyl units linked by  $\beta$ 1 $\rightarrow$ 3 linkages and sulfated at 4- and 6-positions are also found (Figure 11.7B), as well as 4-sulfated and 6-linked residues (Figure 11.7C). Finally, units at non-reducing terminals contain 3,4-*O*-(1'carboxy)-ethylidene (Figure 11.7D). Another possible source of heterogeneity in these molecules is the presence of minor amounts of non-sulfated units. The attempt to determine a repetitive pattern of distribution in the polymer using NMR (especially the NOESY spectra) proved to be unsuccessful.

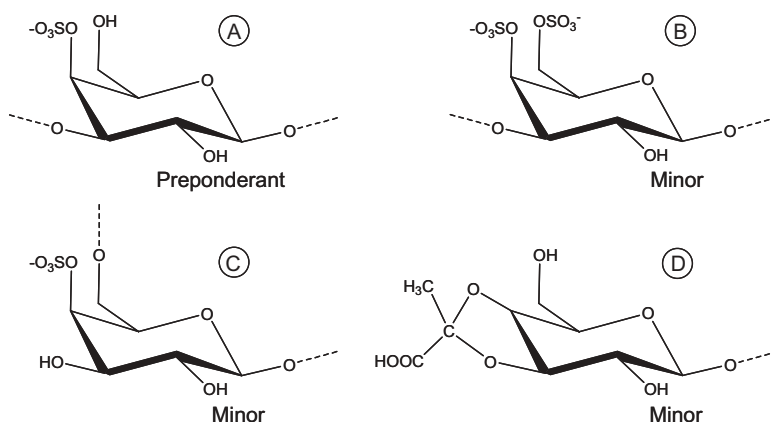
The SGs from *C. isthmocladum* appear to be similar polysaccharides that one from *C. yezoense* (Bilan *et al.*, 2007). But a more in depth analysis of the structure of these two polysaccharides reveals some differences. In particular, the SG from *C. isthmocladum* has a more simple structure than the polysaccharide from *C. yezoense*, as revealed by HMQC spectra (compare Figure 11.4 and the results described in Bilan *et al.*, 2007) and methylation analysis. Clearly the SG from *C. isthmocladum* has no 3,6-linked units and no six-membered cyclic ketals including O-4 and O-6 positions. In addition the sulfated galactan from *C. isthmocladum* is less branched than the polymer from *C. yezoense*.

**Table 11.3**  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts<sup>a</sup> of the correlation peaks –  $\delta_{\text{H}}/\delta_{\text{C}}$  – (ppm) of pyruvate involved in cyclic ketals with non-reducing-terminal galactopyranoses derived from the  $^1\text{H}/^{13}\text{C}$  HMBC spectra

Chemical group	Pyruvylated galactose found in <i>C. isthmocladum</i>	Reference values <sup>b</sup>	
		Five-membered ring (O-3 and O-4 substituted)	Six-membered ring (O-4 and O-6 substituted)
CH <sub>3</sub>	1.77/23.4	1.62/26.4	1.48/26.4
O-C-O	1.77/109.1	1.62/108.3	1.48/102.0
COOH	1.77/176.6	1.62/178.5	1.48/177.2

<sup>a</sup>Chemical shifts are relative to external trimethylsilylpropionic acid at 0 ppm for  $^1\text{H}$  and methanol for  $^{13}\text{C}$ .

<sup>b</sup>Data from (Bilan *et al.*, 2007).



**Figure 11.7** Proposed structures of the components found in the sulfated galactan from the green alga *C. isthmocladum*. (A) The preponderant component is 3-β-D-Galp-4(SO<sub>4</sub>)-1. Other components also found but in minor amounts are (B) 3-β-D-Galp-4,6di(SO<sub>4</sub>)-1, (C) 6-β-D-Galp-4(SO<sub>4</sub>)-1 and (D) 3,4-O-(1' carboxy)ethylidene-β-D-Galp-1 of the non-reducing terminals.

## 11.4 General structural features related to phylogenetic occurrence

### 11.4.1 Phylogenetic implications: how has the 3-linked, β-galactopyranose unit occurred in the marine environment throughout the course of evolution?

A comparison among SGs from different marine organisms indicates that polysaccharides with the glycosidic linkage β(1→3) are strongly conserved in some taxonomic groups of eukaryotes (rhodophytes, chlorophytes, angiosperms, echinoderms, and mollusks). The SGs found among these phyla differ mainly in sulfation sites, with a strong tendency toward 4-sulfation in plants (algae and marine angiosperms), and 2-sulfation in invertebrate animals. The 6-sulfation is dispersed in minor amounts throughout phylogeny. Similar distribution of the sulfation pattern is not observed for the SFs. These observations provide grounds for speculation about the evolutionary history of SPs.

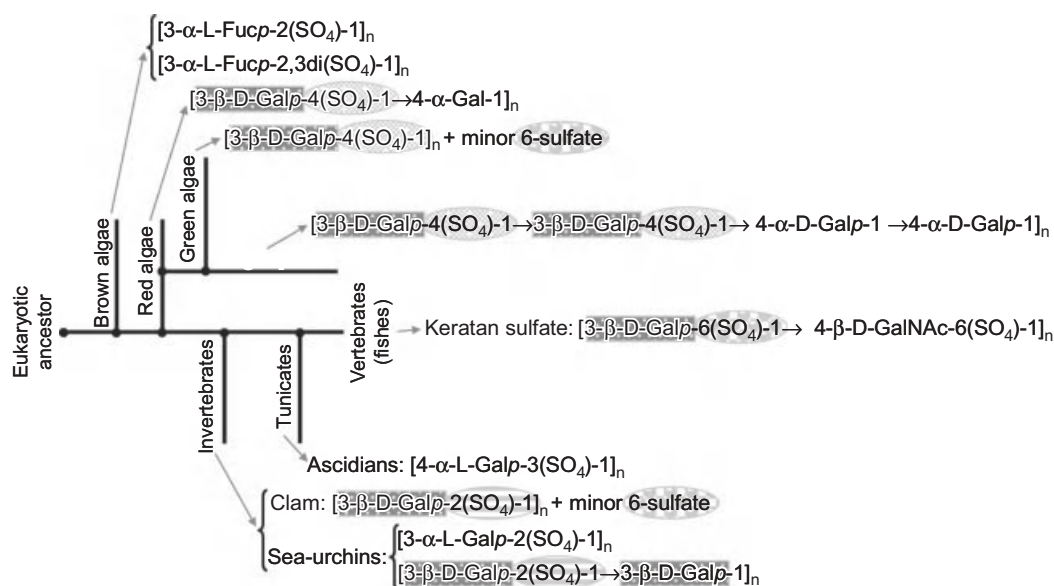
Occurrence of the 3-β-D-Galp-1 unit in the SGs from the sea urchin *G. crenularis* and its presence in SGs from green algae (Bilan *et al.*, 2007; Farias *et al.*, 2008) (Figure 11.7), and from sea grass (Aquino *et al.*, 2005) stimulated us to review the distribution of this structure in marine organisms from the animal and plant kingdoms (Whittaker, 1969) in order to propose a phylogenetic relationship of this unit (Figure 11.8). Although this comparison is based only on structural components of the SGs, which are products of action of several genes and biosynthetic enzymes, this

taxonomic comparison might allow us to ask whether there is a relationship among the marine organisms that express sulfated 3-β-D-Galp-1.

Thus the hypothetical cladogram (Figure 11.8) shows that the sulfated 3-β-D-Galp-1 units are preserved among species of specific phyla that inhabit the marine environment, including green algae, red seaweeds, marine sea grass (Angiospermae, Spermatophyta), invertebrates (sea urchins, clams, and tunicates) and vertebrates such as fishes that express keratan sulfate (Scudder *et al.*, 1986).

Although the 3-β-D-Galp-1 unit has been preserved in the major phyla during evolution (with the only exception being the most ancient brown algae), the preferential sulfation site on this structure varies in a tendency toward 2-sulfation for animals, 4-sulfation for plants (algae and marine angiosperms), and a dispersive distribution of 6-sulfation.

These observations raise the hypothesis that the galactosyltransferases responsible for incorporation of 3-β-D-Galp-1 units in the biosynthesis of SG backbones have been maintained during evolution in specific phyla of marine organisms. But a variation in the distribution of sulfotransferases types seems to occur. In favor of this hypothesis is the evidence that the basic backbones are the same but there is a great variation in the position of sulfation from species to species. To some extent these results are analogous to the biosynthesis of the glycosaminoglycans from vertebrates, where the glycosidic chains vary relatively little among polymers constructed in different tissues, organs, or species. Modifications of the glycosidic core occur mostly after chain elongation when the principal modification is the sulfation at different sites. Unfortunately the



**Figure 11.8** Phylogenetic tree showing the proposed relationship among sulfated polysaccharides from marine organisms of different phyla. The structure 3- $\beta$ -D-Galp-1 is identified by dark gray boxes and sulfate positions are indicated differently with light gray ellipses. The brown algae (Phaeophyta) exhibit polymers of  $\alpha$ -L-fucose bound by (1 $\rightarrow$ 3) and (1 $\rightarrow$ 4) glycosidic linkages, and with different patterns of sulfation (Pereira *et al.*, 1999; Berteau and Mulloy, 2003). The red algae (Rhodophyta) exhibit sulfated galactans composed mainly by the sequence  $[3-\beta\text{-D-Galp-1}\rightarrow 4-\alpha\text{-D-Galp-1}]_n$  (Pereira *et al.*, 2005). Most of them are composed of 4- $\alpha$ -D-3,6-AnGalp-1 (3,6-anidrogallactose residues) and 3- $\beta$ -D-Galp-4(SO<sub>4</sub>)-1, as found in carrageenans, the most common sulfated polysaccharides from red algae (Murano *et al.*, 1997). The preponderant residue of the sulfated galactans from green algae (Clorophyta) is 3- $\beta$ -D-Galp-4(SO<sub>4</sub>)-1 (Bilan *et al.*, 2007). The marine angiosperms exhibit the repeating sequence:  $[3-\beta\text{-D-Galp-4}(\text{SO}_4)\text{-1}\rightarrow 3-\beta\text{-D-Galp-4}(\text{SO}_4)\text{-1}\rightarrow 4-\alpha\text{-D-Galp-1}\rightarrow 4-\alpha\text{-D-Galp-1}]_n$ , comprising structural features of algal and invertebrate sulfated polysaccharides. In invertebrates, the sulfated polysaccharides from two species of sea urchins (Echinodermata) *E. lucunter* (Alves *et al.*, 1997) and *Glyptocidaris crenularis* (Castro *et al.*, 2009) exhibit repeating sequences:  $[3-\alpha\text{-L-Galp-2}(\text{SO}_4)\text{-1}]_n$  and  $[3-\beta\text{-D-Galp-2}(\text{SO}_4)\text{-1}\rightarrow ]_n$  and  $[3-\beta\text{-D-Galp-2}(\text{SO}_4)\text{-1}\rightarrow 3-\beta\text{-D-Galp-1}\rightarrow ]_n$ , respectively. The clam *Mere-trix petechialis* (Mollusca) has a polysaccharide composed of the backbone 3- $\beta$ -D-Galp-1, mainly 2-sulfated and to some extent 6-sulfated (Amornrut *et al.*, 1999). Some species of ascidians (Urochordata) *Ascidia nigra*, *Clavelina oblonga*, *Styela plicata* and *Herdmania monus* (Pavao *et al.*, 1989, 1990; Albano *et al.*, 1990; Santos *et al.*, 1992) exhibit  $[4-\alpha\text{-L-Galp-3}(\text{SO}_4)\text{-1}]_n$ . The glycosaminoglycan keratan sulfate can be found in minor amounts as  $[3-\beta\text{-D-Galp-6}(\text{SO}_4)\text{-1}\rightarrow 4-\beta\text{-D-GalNAc-6}(\text{SO}_4)\text{-1}]_n$  (Scudder *et al.*, 1986).

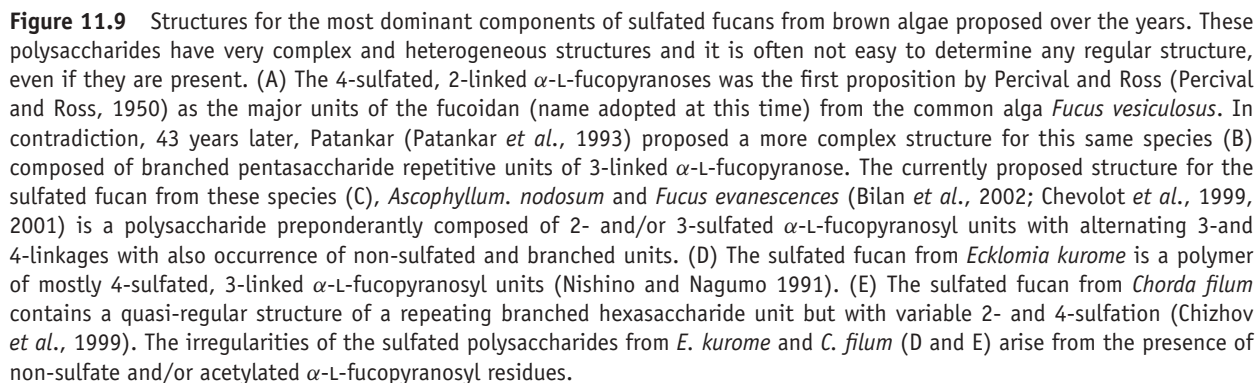
biosynthesis of SGs from marine organisms is virtually unknown and therefore it is not yet possible to compare the expression of these molecules. The alternative, and just as likely hypothesis, is that the presence of these SGs in such distantly related organisms is an example of independent convergent evolution of biosynthetic pathways. This hypothesis is not based on gene sequences, not even on the sequence of proteins, and requires future work to propose a firm theory.

#### 11.4.2 Restricted occurrence of SFs in brown algae

During our studies of occurrence and distribution of structures of SPs in marine organisms we found out that brown

algal SFs are in general more complex and heterogeneous than green or red algal SGs. SFs were reported exclusively in brown algae and so far not in any other algal classes (Pomin and Mourao, 2008). The structures of these brown algal SFs are so complex and hard to determine in terms of structure since controversial published data by different authors occurs. The complexity of the system is increased by the number of types and positions of glycosidic bonds, heterogeneous sulfation patterns and the presence of branching residues.

The initial structural study of an SF was obtained from the widely studied brown alga *Fucus vesiculosus*. Early on Percival and Ross (1950) suggested a polysaccharide composed of  $\alpha$ -L-fucopyranose, mainly bound by 1 $\rightarrow$ 2 glycosidic linkages and 4-sulfated (Figure 11.9A). Patankar



branches of non-sulfated fucose residues (Chevolot *et al.*, 1999; Pereira *et al.*, 1999). More recently NMR analysis of SFs from other species of brown algae revealed unique structures (Figure 11.9D and E). In particular the occurrence of *O*-acetylation is also commonly present (Chizhov *et al.*, 1999). Contradictions related to the structures of brown algal SFs arise from the complexity of the molecules as well as difficulties in purification. The complexity is due to the presence of branching, random distribution of sulfation, different types of glycosidic linkages and the presence of other heterogeneities such as acetylation, methylation,

and pyruvylation (Figure 11.9) (Bilan *et al.*, 2007; Chizhov *et al.*, 1999).

### 11.4.3 SGs in green algae

Recently a couple of SGs from green algae have been structurally reported, particularly those from the genus *Codium*. The *Codium* species have shown large amounts of galactose-composed polysaccharides in their cell wall together with other heteropolysaccharides. In the past 2 years structures of SGs isolated from *C. isthmocladum* (see Section 11.3.4) and *C. jezoense* (Bilan *et al.*, 2007) have been reported. Both molecules were studied by a combination of chemical reaction analysis (a combination of methylation and desulfation) plus NMR spectroscopy, as commonly employed for structural determination of many SGs. Coincidentally the green algal SG from these two species exhibit similar backbones composed preponderantly of 3- $\beta$ -D-Galp-1 units mainly 4-sulfated (as described for the polysaccharide of *C. isthmocladum*, Figure 11.7A), plus minor amounts of other structures (Figure 11.7B–D). These green algal SPs are also highly pyruvylated at the non-reducing terminal residues forming cyclic ketals such as 3,4-O-(1'-carboxi)-ethylidene- $\beta$ -D-Galp-1 units (Figure 11.7D). The green algal SGs seem to be more complex than those from red algae. Partial characterization of SGs from other green algal species is also available in the literature. SG from *C. fragile* and *C. cylindricum* reveal heterogeneous polymers. In addition to galactose residues, *C. fragile* is also composed of arabinose residues (sulfated arabinogalactan) (Love and Percival, 1964) and *C. cylindricum* of additional glucose residues (Matsubara *et al.*, 2001). However the 3-linked 4-sulfated  $\beta$ -D-galactopyranosyl units seem to be the most dominant component in the quite heterogeneous backbone of SGs from green algae.

### 11.4.4 Red algal SGs occur usually in disaccharide repeating units within heterogeneous sulfation patterns: carrageenans and agarans

Marine SGs are widely abundant in red algae. Carrageenans and agarans are the most common SGs from this type of macroalgae. These polymers are widely used in industry. Both of these red algal polysaccharides usually have a linear backbone made of alternating 3-linked  $\beta$ -D-galactopyranose and 4-linked  $\alpha$ -galactopyranose residues (Figure 11.10A), showing a 'masked repeat' unit of disaccharides similar to the animal glycosaminoglycans. The  $\beta$ -galactoses are always D-enantiomers, whereas the  $\alpha$ -galactose residues may be present in the D or L configu-

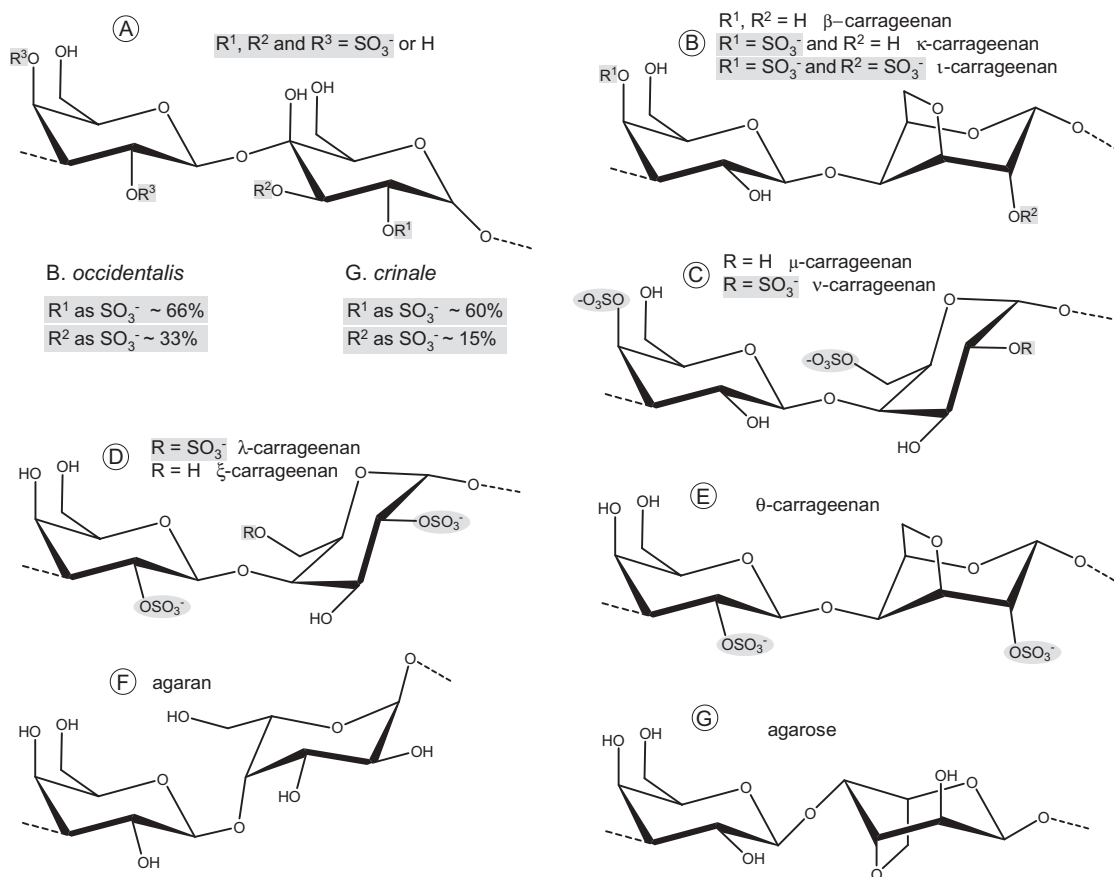
ration (Usov, 1998). A substantial portion may also exist in the form of 3,6-anhydro derivatives (Figure 11.10B, E and G). Considerable structural variation in red algal SGs occur among different species and in samples collected at different environments or in different seasons of the year (Pereira *et al.*, 2005). Furthermore, various hydroxyl groups may be substituted by a sulfate ester, a methyl group or pyruvic acid (Usov, 1998). The major structural variation in these polysaccharides is the sulfation pattern. The sulfate distribution along the galactose backbone is quite heterogeneous as in animal glycosaminoglycans and the sulfate contents are markedly different between different species as depicted in the comparison between the SGs from the red algal species *Botryocladia occidentalis* and *Gelidium crinale* (Figure 11.10A) (Pereira *et al.*, 2005).

Carrageenans are traditionally classified by a Greek prefix according to their sulfation pattern (Figures 11.10B–E) and the presence of a 3,6-anhydro bridge (carrageenose) on the 4-linked  $\alpha$ -D-galactose (van de Velde *et al.*, 2004) (Figures 11.10B and E). We will not discuss variations in the structures of this class of polysaccharide since this topic has been extensively covered in several other reviews (Lahaye, 2001; Usov, 1998; van de Velde *et al.*, 2004). The carrageenans and agarans are extensively exploited due to their industrial applications. The wide uses of these SPs are based on their unique properties to form strong aqueous gels. These molecules are the major hydrocolloids used as texturing agents for food. A small change from  $\alpha$ -D-galactopyranoses in carrageenans to  $\alpha$ -L-galactopyranoses in agarans is enough to promote great changes in the physical-properties of these molecules (Lahaye, 2001). Other modifications in the backbone of the SG can greatly change their physicochemical properties and, consequently, in industrial applications and biological activities. For example, high levels of 3,6-anhydro- $\alpha$ -L-galactopyranosyl units in agar group polysaccharides (also known as agarose, Figure 11.2G) and low sulfate contents are the major structural requirements for gelling (Lahaye, 2001). Several types of these gels are widely exploited by industries in their attempt to obtain the best and specific gel formation under different conditions (regulated by temperature and the combination of ingredients) (Lahaye, 2001).

## 11.5 Industrial applications

### 11.5.1 SFs/fucoidans as food supplements and cosmetic hydrators

The SFs or fucoidans are being submitted to substantial pharmaceutical research, focusing primarily on two distinct classes: F-fucoidan, which is composed of >95%



**Figure 11.10** Chemical structures of repeating disaccharide units of SGs from red algae. These units are composed mainly of alternating 3-linked  $\beta$ -D-galactopyranose and 4-linked  $\alpha$ -D-galactopyranose residues. The species *Botryocladia occidentalis* and *Gelidium crinale* (A) contain SGs which differ exclusively in sulfate contents (Fonseca *et al.*, 2008; Farias *et al.*, 2000; Pereira *et al.*, 2005). The most common SGs from red algae are carrageenans, which differ in sulfation patterns (B–E) and also in replacement of the  $\alpha$ -D-galactoses by 3,6-anhydro- $\alpha$ -D-galactose units (B and E). The structural variety of carrageenans is classified by Greek letters, such as (B)  $\beta$ - (beta),  $\iota$ - (iota) and  $\kappa$ - (kappa), (C)  $\mu$ - (mu) and  $\nu$ - (nu), (D)  $\lambda$ - (lambda) and  $\xi$ - (xi) and (E)  $\theta$ - (theta) carrageenan. For more details and carrageenan structures, see (Lahaye, 2001; Usov, 1998; van de Velde *et al.*, 2004). The polysaccharides composed of  $\alpha$ -L-galactoses (F) or 3,6-anhydro- $\alpha$ -L-galactose units (G) are named agaran and agarose, respectively (Lahaye, 2001). These polymers can also show different sulfation patterns. All sulfate groups are highlighted in light gray.

sulfated fucose-containing polymers, and U-fucoidan, which has approximately an additional 20% glucuronic acid. As a consequence of this research U-fucoidan and F-fucoidan are now being separately marketed as a nutraceutical and food supplement.

A study released in 2005 by Japanese researchers (Aisa *et al.*, 2005), indicated that F-fucoidan can induce apoptosis in human lymphoma cell lines. French researchers showed in 2002 that F-fucoidan can inhibit hyperplasia in rabbits (Deux *et al.*, 2002). A study at the Staten Serum Institute, in Copenhagen, showed that after pretreatment with fucoidan, deaths of rats infected with meningitis may increase. Twenty

one out of 45 rats that had been given fucoidan and then treated after infection with an antibiotic died; as compared with 5 deaths out of 29 for those had not been given fucoidan and had been treated with only the antibiotic. Another clinical study of ingested fucoidan from the genus *Undaria* revealed that this molecule showed effects on hematopoietic stem cells (Irhimeh *et al.*, 2007). These analyses were carried out at UTAS, Australia. Scientists found that there was a small increase in the total numbers of  $\text{CD34}^+$  cells and a profound increase, from 40% to 90%, in the proportion of  $\text{CD34}^+$  cells that expressed CXCR4 when 75% *Undaria*'s fucoidan was ingested. A smaller increase was

observed when *Undaria* containing 10% w/w fucoidan was ingested.

Even though some clinical tests are ongoing, SFs/fucoidans are already being exploited in industry due to evidence of their ability to enhance health: (1) supportive contribution to the immune system, (2) catalytic replacement of dead body cells due to acceleration of tissue/cell regeneration (cell proliferation), (3) maintenance of cholesterol levels, and (4) boosting apoptosis (the natural process of eliminating harmful cells). Thus fucoidans (SFs) are currently being explored as dietary supplements either inside of digestive capsules or by forming a swallowing gel which makes better and quicker absorption, enhances bioavailability of active ingredients, enhances portability and makes easier the uptake (no pills to swallow).

In addition to the SFs role in the assembly of the cell wall, these molecules also form mucus on the surface of brown algae. This outside mucus plays a role in keeping the long blades from sticking together which would reduce the surface area available for photosynthesis. This algal class forms big forests deep in the sea since they proliferate widely and also forms big blades (laminae), so the SF-based mucus is quite important to keep these blades separate. Due to this physical property of SFs, today there is an interest in exploring and processing these molecules as skin hydrators in the cosmetic industry for skin care purposes. Fucoidan/SFs are now known to be a potent nutrient-drenched hydrator that creates a protective barrier to help seal in and sustain moisture. Seaweed SFs aid in protecting the skin's collagen supply, maintaining its firmness and supporting the skin's levels of line-filling hyaluronic acid, a component glycosaminoglycan in skin's connective tissue that is essential for the balance of rigidity and softness. But above all, the oily properties of algal glycans reduces the loss of water from epidermal tissues which makes these molecules a target for exploring hydrating actions which help to moisturize the skin.

### 11.5.2 Carrageenans and agarans: the most industrially used SG molecules

#### *The utility and processing of carrageenans*

Carrageenans and agarans are the most explored SGs in industry. Carrageenans were introduced on an industrial scale in the 1930s. They were first used in China around 600 BCE (where *Gigartina* was used) and in Ireland around 400 CE. The largest current producer in contemporary times is the Philippines, where cultivated seaweed produces about 80% of the world supply. The most commonly used are *Cottonii* (*Kappaphycus alvarezii*, *K. striatum*) and *Spinousum* (*Eucheuma denticulatum*), which together provide about

three-quarters of the world production. These grow from the sea surface to a depth of about 2 m. The seaweed is normally grown on nylon lines strung between bamboo floats and harvested after 3 months or so when each plant weighs around 1 kg. The *Cottonii* variety has been reclassified as *Kappaphycus cottonii* by Maxwell Doty (1988), thereby introducing the genus *Kappaphycus*, on the basis of the phycocolloids produced (kappa carrageenan).

After harvest, the seaweed is dried, baled, and sent to the carrageenan manufacturer. There the seaweed is ground, sifted to remove impurities such as sand, and washed thoroughly. After treatment with a hot alkali solution (e.g., 5–8% potassium hydroxide), the cellulose is removed from the carrageenan by centrifugation and filtration. The resulting carrageenan solution is then concentrated by evaporation. It is dried and ground to specification. There are three types of processing:

- 1 Semirefined – this is only performed using *Eucheuma cottonii* or *Eucheuma spinosum*. The raw weed is first sorted and crude contaminants removed by hand. The weed is then washed to remove salt and sand, and then cooked in hot alkali to increase the gel strength. The cooked weed is washed, dried, and milled. *E. spinosum* undergoes a much milder cooking cycle as it dissolves quite readily. The product is called semi refined carrageenan, Philippines natural grade or in the United States it simply falls under the common carrageenan specification.
- 2 Refined – the essential difference in the refining process is that the carrageenan is dissolved and filtered to remove cell wall debris. The clear solution is then precipitated either by alcohol or by potassium chloride.
- 3 Mixed processing – a hybrid technology exists where weed is treated heterogeneously as in the semi refined process but alcohol or high salt levels are used to inhibit dissolution. This process is often used on South American weeds and gives some of the cost benefits of semirefined processing while allowing a wider range of weeds to be processed. Oddly the naturally low cellulose levels in some South American weeds allow them to be heterogeneously processed and still be sold under the EU refined specification.

The uses industrial for carrageenans are vast. They can be used to make:

- Personal lubricants
- Desserts, ice cream, cream, milk shakes, sweetened condensed milks, sauces – the gel formation allow increasing viscosity and consistency

- Beer – clarifier to remove haze-causing proteins
- Pâtés and processed meat – substitute fat to increase water retention and increase volume
- Toothpaste – stabilizer to prevent constituents separating
- Fruit Gushers – ingredient in the encapsulated gels
- Fire-fighting foam – thickener to cause foam to become sticky
- Shampoo and cosmetic creams – thickness;
- Air freshener gels
- Marbling – the ancient art of paper and fabric marbling uses a carrageenan mixture to float paints or inks upon; the paper or fabric is then laid on it, absorbing the colors
- Shoe polish – gel to increase viscosity
- Biotechnology – gel to immobilize cells/enzymes
- Pharmaceuticals – used as an inactive excipient in pills/tablets
- Carrageenan – used to thicken skim milk, in an attempt to emulate the consistency of whole milk. This usage did not become popular. It is used in some brands of soy milk
- Diet sodas
- Soy milk
- Pet food
- Alien saliva (movie effects)
- Laboratorial experiments – lambda-carrageenan is used in animal models of inflammation to test analgesics because dilute carrageenan solutions (1–2%) injected subcutaneously cause swelling and pain
- Shaving ham sold at restaurants and commercial delicatessens.

#### *Carrageenans as microbicide sexual lubricants*

Studies at the Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, Maryland, USA, suggest that carrageenans might function as a topical microbicide, especially against the following viruses:

- 1 Herpes simplex virus (HSV): There are indications that a carrageenan-based gel may offer some protection against HSV-2 transmission by binding to the receptors on the

herpes virus thus preventing the virus from binding to cells. Research has shown that a carrageenan-based gel effectively prevented HSV-2 infection at a rate of 85% in a mouse model. Some personal and condom lubricants are already made with carrageenan and several of these products (such as Divine) were found to be potent as human papillomavirus (HPV) inhibitors in the study (though others that listed carrageenan in their ingredients were not) (Buck *et al.*, 2006).

- 2 HPV – Laboratorial studies have shown that carrageenans are extremely potent inhibitors of HPV infection *in vitro* and in animal challenge models (Buck *et al.*, 2006). Clinical trial results announced at the 2010 International Papillomavirus Conference held in Montreal, Canada indicate that a carrageenan-based personal lubricant called Carraguard is effective for preventing HPV infection in women. The clinical results suggest that use of carrageenan-based personal lubricant products, such as Divine No 9, BIOglide (Buck *et al.*, 2006) or Oceanus Carrageenan may likewise be effective for preventing HPV infection.
- 3 Human immunodeficiency virus (HIV): A phase 3 clinical trial by Population Council examined whether the carrageenan-based product Carraguard was effective as a topical microbicide for blocking HIV infection in women. The trial ran from 2004 to 2007, with more than 4000 South African women completing the study, but found no statistical difference in infection between those who used the lubricant and those who did not. The trial did provide information about usage patterns, however, and showed that the gel is at least safe – not increasing infection any more than the baseline or causing significant side effects. As such, they expect to use it as a stable delivery vehicle for experimental anti-retrovirals in future studies. Concurrent studies in macaques found the same Carrageenan gels used in clinical trials to be effective against simian immunodeficiency virus (SIV) challenge. This was in direct contrast of *in vitro* findings, where the compound was found to enhance HIV and SIV infections in various assays. Although compliance was believed to be one issue in clinical versus animal trials, the high viscosity and controlled nature of animal–viral inoculations (atraumatic introduction of virus using a French Catheter) may be why the latter animal study observed a positive outcome (Turville *et al.*, 2008).

As carrageenans have potency to form gels in solution, these molecules can also be explored as lubricants, especially design for sexual purposes. The antiviral action described above coupled to the lubricant mechanism can provide a very useful and competitive product in the market.

### *Health concerns in using carrageenans*

The Joint FAO/WHO expert committee on food additives states, “based on the information available, it is inadvisable to use carrageenan or processed eucheuma seaweed in infant formulas”. There is evidence from studies performed on rats, guinea pigs and monkeys, indicating that degraded carrageenan (poligeenan) may cause ulcerations in the gastrointestinal tract and gastrointestinal cancer (Tobacman, 2001). Poligeenan is produced from carrageenan subjected to high temperatures and acidity. The average carrageenan molecule weighs over 100 kDa while poligeenans have a molecular weight of less than 50 000 Da. A scientific committee working on behalf of the European Commission has recommended that the amount of degraded carrageenan be limited to a maximum of 5% (the limit of detection) of total carrageenan mass. Upon testing samples of foods containing high molecular weight carrageens, researchers found no poligeenan.

A recent publication (Borthakur *et al.*, 2007) indicates that carrageenan induces inflammation in human intestinal epithelial cells in tissue culture through a BCL10-mediated pathway that leads to activation of NF $\kappa$ B (NF $\kappa$ B) and interleukin-8 (IL-8). Carrageenan is assumed to be immunogenic due to its unusual  $\alpha$ -1,3-galactosidic link that is part of its disaccharide unit structure. Consumption of carrageenan may have a role in intestinal inflammation and possibly inflammatory bowel disease, where mutations are associated with genetic proclivity to Crohn's disease. Carrageenan is reported to interfere with macrophage activity as well (Rumjanek *et al.*, 1977; Catanzar *et al.*, 1971).

### *The utility of agarans*

Agarans (agar or agar-agar) exhibit hysteresis, melting at 85 °C (358 K) and solidifying from 32–40 °C (305–313 K). In microbiology, nutrient agar is used throughout the world to provide a solid surface containing medium for the growth of bacteria and fungi. Agar is typically sold commercially as a powder that can be mixed with water or buffer and prepared similarly to gelatin before use as a growth medium. The basic agar formula can be used to grow most of the microbes whose needs are known. More specific nutrient agars are available, since some microbes prefer certain environmental conditions over others.

### *In motility assays*

As a gel, an agarose (Figure 11.10G) medium is porous and therefore can be used to measure microorganism motility and mobility. The gel's porosity is directly related to the concentration of agarose in the medium, so various levels of effective viscosity (from the cell's “point of view”) can

be selected, depending on the experimental objectives. A common identification assay involves culturing a sample of the organism deep within a block of nutrient agar. Cells will attempt to grow within the gel structure. Motile species will be able to migrate, albeit slowly, throughout the gel and infiltration rates can then be visualized; whereas non-motile species will only show growth along the now-empty path introduced by the invasive initial sample deposition. Another setup commonly used for measuring chemotaxis and chemokinesis utilizes the under-agarose cell migration assay whereby a layer of agarose gel is placed between a cell population and a chemoattractant. As a concentration gradient develops from the diffusion of the chemoattractant into the gel, various cell populations requiring different stimulation levels to migrate can then be visualized over time using microphotography as they tunnel upward through the gel against gravity along the gradient.

### *In molecular biology*

Agar can be used as a heterogeneous mixture of two classes of polysaccharide: agarpectin and agarose. Although both polysaccharide classes share the same galactose-based backbone, agarpectin is heavily modified with acidic functional groups, such as sulfate and pyruvate. The neutral charge and lower degree of chemical complexity of agarose (Figure 11.10G) make it less likely to interact with biomolecules. Agarose has therefore become the preferred matrix for work with proteins, nucleic acids, and other polysaccharides. Gels made from purified agarose have a relatively large pore size, making them useful for separation of large molecules such as proteins and protein complexes >200 kDa, as well as DNA fragments >100 base-pairs. Agarose has been widely used for immunodiffusion and immunoelectrophoresis as the agarose fibers function as an anchor for immunocomplexes. Agarose is generally used as the medium for analytical scale electrophoretic separations in agarose gel electrophoresis and for column-based preparative scale separations such as gel filtration chromatography and affinity chromatography.

### *In plant biology*

Research grade agar is used extensively in plant biology as it is supplemented with a nutrient and vitamin mixture that allows for seedling germination in petri dishes under sterile conditions (given that the seeds are sterilized as well). Nutrient and vitamin supplementation for *Arabidopsis thaliana* is standard across most experimental conditions. Murashige & Skoog (MSO) nutrient mix and Gamborg's B5 vitamin mix are generally used. A 1.0% agar/0.44% MSO + vitamin dH<sub>2</sub>O solution is suitable for growth media in normal growth temperatures. The solidification of the agar within

any growth media (GM) is pH-dependent, with an optimal range between 5.4–5.7. Usually the application of KOH is needed to increase the pH to this range. A general guideline is about 600  $\mu$ l 0.1 M KOH per 250 ml GM. This entire mixture can be sterilized using the liquid cycle of an autoclave. This medium nicely lends itself to the application of specific concentrations of phytohormones etc. to induce specific growth patterns in that one can easily prepare a solution containing the desired amount of hormone, add it to the known volume of GM, and autoclave to both sterilize and evaporate off any solvent that may have been used to dissolve the often polar hormones. This hormone/GM solution can be spread across the surface of petri dishes sown with germinated and/or etiolated seedlings. Experiments with the moss *Physcomitrella patens*, however, have shown that choice of the gelling agent – agar or Gelrite – does influence phytohormone sensitivity of the plant cell culture.

#### *In culinary/cuisine*

Agar-agar is a natural vegetable gelatin counterpart. White and semitranslucent, it is sold in packages as washed and dried strips or in powdered form. It can be used to make jellies, puddings, ice-creams, and custards. For making jelly, it is boiled in water until the solids dissolve. Sweetener, flavoring, coloring, fruit or vegetables are then added and the liquid is poured into molds to be served as desserts and vegetable aspics, or incorporated with other desserts, such as a jelly layer in a cake. Agar-agar is approximately 80% fiber, so it can serve as an intestinal regulator. Its bulk quality is behind one of the latest fad diets in Asia, the *kanten* (the Japanese word for agar-agar) diet. Once ingested, *kanten* triples in size and absorbs water. This results in the consumer feeling fuller. Recently this diet has received some press coverage in the United States as well. The diet has shown promise in obesity studies (Maeda *et al.*, 2005). One use of agar in Japanese cuisine is *anmitsu*, a dessert made of small cubes of agar jelly and served in a bowl with various fruits or other ingredients. It is also the main ingredient in Mizuyōkan, another popular Japanese food. In Indian cuisine, agar-agar is known as “China grass” and is used for making desserts. In Burmese cuisine, a sweet jelly known as *kyauk kyaw* is made with agar.

## 11.6 Pharmacological properties

The marine SFs and SGs exhibit potential pharmacological effects in mammalian systems such as thrombosis/coagulation, inflammation, angiogenesis, parasitosis, oncogenesis/tumorigenesis, and on cellular actions such as in-

ducing cell growth, migration and adhesion. These pharmacological properties are the main reason for research and studies on these molecules. Herein we will systematically describe these actions individually, trying to make, whenever possible, a correlation between the species-specific structural features and the levels of biological action.

### 11.6.1 Antiviral actions

Similar to the antiviral and lubricant actions of carrageenans described earlier, SFs and SGs may also be used for these purposes. Although antiviral effects are the most studied pharmacological actions of SFs and SGs, the clinical use of these molecules is still under research and/or clinical test. A long road seems to exist until the final target of commercialization of these compounds as oral or topical antiviral drugs. The following compiled information is a summary of the main papers published since 1999 concerning the pharmacological actions of SFs/fucoidans and SGs.

#### *SFs against HSV and influenza virus*

In the work of Feldman *et al.* 1999, three fractions of SFs (Ee, Ec and Ea) isolated from the brown seaweed *Leathesia difformis* were found to be selective antiviral agents against HSV types 1 and 2 and human cytomegalovirus (Feldman *et al.*, 1999). Fraction Ea showed the most activity, with  $IC_{50}$  values in the range 0.5–1.9  $\mu$ g/ml without affecting cell viability at concentrations up to 400  $\mu$ g/ml. The antiherpetic activity of Ea was assessed by three different methods: plaque reduction, inhibition of virus yield and prevention of HSV-2 induced shut-off of cell protein synthesis. This demonstrated that the inhibitory effect was independent of the antiviral assay and the multiplicity of infection. The mode of action of Ea could be ascribed to an inhibitory action on virus adsorption. These SF fractions did not inhibit the blood coagulation process even at concentrations exceeding more than 100 times the  $IC_{50}$  value. Another work from Lee *et al.* (2004) showed antiviral activity of SF against the same virus types, HSV-1, HSV-2, and cytomegalovirus (Lee *et al.*, 2004). The SF from this work was isolated from the sporophyll of *Undaria pinnatifida* (Mekabu) and exhibited a similar ratio of ~10% of galactose units to the galactofucan previously described from *L. abyssalis*, item 3.

Indeed, the antiviral activity of SFs against herpes virus is the most studied one. In addition to the two works described in the previous paragraph, the papers by Sinha *et al.* (2010), Adhikari *et al.* (2006), and Hayashi *et al.* (2008) also describe antiherpetic actions of SFs. In the paper of Adhikari *et al.* (2006) a SF containing fraction (SmWE)

was isolated from a water extraction of the brown seaweed *Stoechospermum marginatum* collected from the Arabian Sea. Anion exchange chromatography of the crude fraction results in the production of a SF fraction (F3) having a molecular mass of 40 kDa. NMR spectroscopic studies and methylation analysis suggest that the polymer consists of a backbone of (1–4) and (1–3) linked  $\alpha$ -L-fucopyranosyl residues that are substituted at C-2 and C-3. The studies also found that the fucosyl residues are sulfated mostly at C-2 and/or C-4. SmWE and F3 are selective inhibitors of HSV-1 and HSV-2 in Vero cells with antiviral effective concentration 50% (EC<sub>50</sub>) values in the range 0.63–10.0  $\mu$ g/ml. These compounds are highly selective due to the lack of cytotoxicity. Antiviral activity was dependent upon the presence of the SFs during the adsorption period. No direct inactivating effect on virions was observed in a virucidal assay. The absence of anticoagulant activity at concentrations near EC<sub>50</sub> confirmed that there was no correlation between the antiviral and anticoagulant properties.

Another work using crude water extraction (CiWE) of SF-containing fractions isolated from the brown seaweed *Cystoseira indica* was performed (Mandal *et al.*, 2007). Both CiWE and the main fraction (CiF3) obtained by anion exchange chromatography showed potent antiviral activity against HSV-1 and HSV-2 without cytotoxicity for Vero cell cultures. The mode of action of these compounds was mainly ascribed to an inhibitory effect on virus adsorption. Chemical, chromatographic and spectroscopic methods showed that the major polysaccharide had an apparent molecular mass of 35 kDa and contained a backbone of  $\alpha$ -(1–3)-linked fucopyranosyl residues substituted at C-2 with fucopyranosyl and xylopyranosyl residues. This SF, considered the principal active component of the *C. indica* water extract, also contained variously linked xylose, galactose and glucuronic acid residues. Sulfate groups are located mostly at C-4 of (1–3)-linked fucopyranosyl units and showed to be crucial for the antiherpetic activity of this polymer.

In the work of Hayashi *et al.* (2008) the researchers additionally proved that oral intake of the SF from an edible brown alga *Undaria pinnatifida* exhibits antiviral effects through direct inhibition of viral replication and stimulation of both innate and adaptive immune defense functions. In this study, the effects of the SF from *U. pinnatifida* were examined on *in vivo* viral replication and in the host's immune defense system. Oral administration of the SF was proven to protect mice from infection with HSV-1 as judged by survival rate and lesion scores. Phagocytic activity of macrophages and B cell blastogenesis *in vitro* were significantly stimulated by the SF while no significant change in the release of NO<sub>2</sub> by macrophages was observed. In *in vivo* studies, oral administration of the SF produced augmentation of natural killer (NK) activity in HSV-1-infected mice

immunosuppressed by 5-fluorouracil treatment. Cytotoxic T lymphocyte activity in HSV-1-infected mice was also enhanced by oral administration of the SF. The production of neutralizing antibodies in the mice inoculated with HSV-1 was significantly promoted during the oral administration of the SF for 3 weeks.

In the paper by Sinha *et al.* (Sinha *et al.*, 2010) HSV is described to display biological affinity for cell-surface heparan sulfate proteoglycans during the virus entrance into the host cells. Therefore an approach for inhibiting HSV infection by using the SF from *Sargassum tenerrimum* competing then with the entry receptors was studied. This SP showed apparent molecular masses of  $30 \pm 5$  kDa. The IC<sub>50</sub> values against HSV-1 were in the range 0.5–15  $\mu$ g/ml and they lacked cytotoxicity at concentrations up to 1000  $\mu$ g/ml. The anti-HSV activity increased with increasing sulfate ester content in studies using chemically sulfated derivatives. These results suggest the feasibility of inhibiting HSV infection by blocking viral entry with polysaccharide having specific sulfation degrees.

Influenza seems to be the second target in antiviral tests of SFs. Two major works have been published in the last two years. Taoda and coworkers proved that the monosaccharide L-fucose only is not enough to prevent parainfluenza virus type 2 infection into LLCMK2 cells, but the entire SF is required, although L-fucose partly inhibits cell fusion and hemadsorption (Taoda *et al.*, 2008).

In the important work of Makarenkova (Makarenkova *et al.* 2010), the antiviral activity of the SF from the brown sea algae *Laminaria japonica* against infection caused by the highly virulent avian influenza virus (Alduck/Novosibirsk/02/05, H5N1) was evaluated in pig embryo kidney cell cultures. This SF was determined to have no cytotoxic activity and to reveal virucidal activity against influenza A/H5N1 virus. When given at concentrations of 50–500  $\mu$ g/ml, SF protected cell cultures from the cytopathogenic activity of influenza virus in a dose of 0.01 TCID<sub>50</sub>/1.0 ml and was able to suppress influenza A/H5N1 virus production within 24 hours of infection when either prophylactic only or therapeutic and prophylactic treatment regimens were used.

## SGs

The structure-antiviral effect relationship of red algal SGs against viruses has been reported in several papers as well. Similar to SFs, the anti-herpetic activities of red algal SGs are the most studied antiviral activity of these marine galactose-composed polysaccharides (Chattopadhyay *et al.*, 2008; Caceres *et al.*, 2000; Duarte *et al.*, 2001, 2004; Matsuhira *et al.*, 2005; Talarico *et al.*, 2004; Mazumder *et al.*, 2002). These compounds were shown to interfere with the

initial adsorption of viruses into host cells. Matsuihro and coworkers performed structural analysis and studied antiherpetic activity for the SG from the red seaweed *Schizymenia binderi* (Gigartinales, Rhodophyta). They showed that this SG is composed of sulfated groups mainly at O-2 positions of 3-linked  $\beta$ -D-galactopyranosyl residues and at O-3 positions of 4-linked  $\alpha$ -galactopyranosyl units, where the latter residue is partially glycosylated at O-2. The SG from *S. binderi* exhibited highly selective antiviral activity against HSV types 1 and 2 with selectivity indices (ratio cytotoxicity/antiviral property) >1000 for all assayed virus strains. Similarly, Talarico and coworkers have demonstrated that the SGs from the red seaweeds *Gymnogongrus griffithsia* and *Cryptonemia crenulata* lacked cytotoxic effects in Vero cells and showed a broad spectrum of antiviral activity against HSV-1 and HSV-2 with inhibitory concentration 50% (IC<sub>50</sub>) values in a good range of 0.5–5.6  $\mu$ g/mL. Most important from this work is that a significant protection against a murine vaginal infection with HSV-2 was afforded by topical treatment with these algal SGs (Talarico *et al.*, 2004).

Chemical investigation with antiherpetic correlation was also carried out for the high MW SG from the red alga *Gracilaria corticata* (Gracilariaceae, Rhodophyta). Most of the sulfate groups of this SG are located at C-4 of 1,3-linked D-galactosyl units and C-6 of the 1,4-linked L-galactose residues. This alga also exhibited a cell wall agar polymer with methyl groups at C-6 of its 1,3-linked D-galactosyl units and at C-2 of the 1,4-linked L-galactose residues. Bioassays showed that the high MW-SG exhibited selective antiherpetic activity against virus types 1 and 2 (Mazumder *et al.*, 2002).

Duarte and coworkers show in one paper that SGs from the red alga *Bostrychia montagnei* have antiherpetic properties correlated to the MW and sulfate content of the polysaccharides (Duarte *et al.*, 2001). In another paper from the same author (Duarte *et al.*, 2004) shows that the antiherpetic activity of the agaran sulfate from *Acanthophora spicifera* (Rhodomelaceae, Ceramiales) has a restrict activity correlated to the types of units of the backbone. These are: 3-linked  $\beta$ -D-galactopyranoses highly substituted with sulfate groups on C-2 (28–30%), sulfates on C-2 and 4,6-O-(1'-carboxyethylidene) groups (9–15%), and only the C-2 sulfate groups (5–8%) with small amounts of C-6 sulfate, 6-O-methyl, and non-substituted residues, and the 4-linked  $\alpha$ -galactoses are formed mainly by 3,6-anhydro- $\alpha$ -l-galactose (15–16%) and its precursor,  $\alpha$ -l-galactose 6-sulfate (10–17%), together with lesser amounts of 3,6-anhydro- $\alpha$ -l-galactose 2-sulfate,  $\alpha$ -l-galactose 2,6-disulfate,  $\alpha$ -l-galactose 2,3,6-tri-sulfate,  $\alpha$ -l-galactose 2,6-disulfate 3-xylose, 2-O-methyl- $\alpha$ -l-galactose, and unsubstituted  $\alpha$ -l-galactose.

Chattopadhyay and coworkers have also studied the structure–function relationship of the SG from *Gracilaria corticata*. They reported that the sulfate groups located at C-4 of 1,3-linked galactopyranosyl residues appear to be very important for the anti-herpetic activity of the polysaccharide. Some carrageenans also have promising antiherpetic activities as reported in the work with the tetrasporic *Stenogramme interrupta* (Phyllophoraceae). This red alga synthesizes zeta- and lambda-carrageenans.

Antiviral actions against four serotypes of dengue virus (DENV) of the sulfated carrageenans ( $\kappa$ ,  $\iota$ ,  $\nu$ ) from the red seaweeds *Gymnogongrus griffithsia* and *Cryptonemia crenulata* were also described in another work (Talarico *et al.*, 2005). Both seaweed derivatives were selective inhibitors of DENV-2 multiplication in Vero cells with IC<sub>50</sub> values around 1  $\mu$ g/mL and selectivity indices > 1000. The compounds had a lower antiviral effect against DENV-3 (IC<sub>50</sub> values in the range 13.9–14.2  $\mu$ g/mL), an even lower effect against DENV-4 (IC<sub>50</sub> values in the range 29.3 to >50  $\mu$ g/mL) and were totally inactive against DENV-1 (Talarico *et al.*, 2005).

### 11.6.2 The use of SFs and SGs in therapy for preventing thrombosis and coagulation

Indeed, the anticoagulant and antithrombotic properties of SFs and SGs are the most desirable pharmacological uses for these marine sugars. The existence and pressing need for new drugs is a consequence of the increasing incidence of thromboembolic diseases – cardiovascular diseases are the leading cause of death (30% of total) in the world. In addition, heparin preparations have several limitations due to collateral effects and limited source of material (Mourao, 2004) since this glycosaminoglycan is widely used for the treatment and prevention of arterial and venous thrombosis (Fareed *et al.*, 2000). The situation was further complicated recently due to contamination of heparin preparations with oversulfated chondroitin sulfate (Guerrini *et al.*, 2008). This contaminant induces hypotension associated with kallikrein release when administered by intravenous injection (Kishimoto *et al.*, 2008).

Red algal SGs (Pereira *et al.*, 2005) and green algae (Matsubara *et al.*, 2001) have been known for some time to act as modulators of coagulation. Most of their activities are mediated by both antithrombin and heparin cofactor II (HCII), although there is a particular case of a SG from a specific green alga that exhibits a serpin-independent anticoagulant effect, possibly due to the inhibition of fibrin polymerization (Matsubara *et al.*, 2001). However, relatively few studies have interpreted the biological activity of SGs in terms of a molecular structure. This is much more

difficult when the complex structures of brown algal SFs are analyzed even though these molecules can show potent anticoagulant/antithrombotic activities (Pereira *et al.*, 2002). The unclear structures of brown algal SFs disable a confident structure–function relationship.

A test of red algal SGs from *B. occidentalis* and from *G. crinale* (Figure 11.10A) on animal models of venous thrombosis revealed that these polysaccharides have a serpin-dependent anticoagulant activity due to inactivation of thrombin and factor Xa (Pomin, 2010). These polysaccharides also have a procoagulant effect due to activation of factor XII. The algal SGs differ in their venous antithrombotic activities in a sulfation pattern-dependent way, as will be described below. It is noteworthy that the algal SGs have no hemorrhagic effect even when tested at high doses (Fonseca *et al.*, 2008). The attempts to identify structural features in the algal polysaccharide necessary for anticoagulant activity have been limited by the fact that algal SGs are heterogeneous mostly on their sulfation patterns. Only in the cases for the SGs of the two red algal species above (Figure 11.10A) has it been shown that the occurrence of 2,3-disulfated  $\alpha$ -galactose units is a critical structural motif in promoting the interaction of the polysaccharide with the plasma protease and the serpins (Pereira *et al.*, 2005). Obviously the identification of specific structural requirements in the algal polysaccharides necessary for interaction with coagulation (co)factors is an essential step for more rational development of anticoagulant drugs and sometimes this approach is compromised by the present of complex and/or heterogeneous structures in algal polysaccharides, especially brown algal SFs.

### 11.6.3 Inhibiting inflammation

The amount of information concerning anti-inflammatory actions of SFs is much larger than those regarding SGs as described below.

#### SFs

In 1999, Del Bigio *et al.* published a paper showing that inflammatory cells are postulated to mediate some of the brain damage following ischemic stroke (Del Bigio *et al.*, 1999). In the particular case of intracerebral hemorrhage, this event is associated with more inflammation than ischemic stroke. The authors tested an SF which had been reported previously to reduce inflammatory brain damage in a rat model of intracerebral hemorrhage induced by injection of bacterial collagenase into the caudate nucleus. Rats were treated with 7 days of intravenous infusion of SF (30  $\mu$ g/h) or vehicle. The hematoma was assessed

*in vivo* by magnetic resonance imaging. Motor behavior, passive avoidance and skilled forelimb function were tested repeatedly for six weeks. SF-treated rats exhibiting evidence of impaired blood clotting and hemodilution, had larger hematomas, and tended to have less inflammation in the vicinity of the hematoma after 3 days. They showed significantly more rapid improvement of motor function in the first week following hemorrhage and better memory retention in the passive avoidance test. Acute white matter edema and eventual neuronal loss in the striatum adjacent to the hematoma did not differ between the two groups. Investigation of more specific anti-inflammatory agents and hemodiluting agents are warranted in intracerebral hemorrhage.

Maruyama and coworkers proved in 2005 that mekabu SF obtained from *Undaria pinnatifida* (Up) sporophylls augments the type 1 T-helper (Th1) cell response in normal BALB/c mice (Maruyama *et al.*, 2005). In this study, the authors examined the effects of this SF of mekabu on also the type 2 T-helper (Th2) response in bronchoalveolar lavage fluid (BALF) after ovalbumin (OVA) aerosol challenge. Mekabu SF (50 mg/kg) was injected intraperitoneally into BALB/c mice for 4 days and then the mice were sensitized with 50  $\mu$ g/mouse of OVA plus alum (1 mg/mouse), 1 and 8 days later. The mice were challenged with OVA delivered using a nebulizer 7, 8 and 9 days after the second challenge with OVA plus alum. After 24 h the T-cell responses in BALF were assessed by measuring the amount of Th2 cytokines (IL-4, IL-5, IL-13) and gamma-interferon (IFN- $\gamma$ ) produced by Th1 cells. Production of Th2 cytokines was suppressed ( $p < 0.05$ ), and the amount of IFN-gamma was not increased in the mice treated with mekabu SF. Anti-OVA immunoglobulin E (IgE) and IgE levels in serum determined after challenge with aerosolized OVA at the end of the experiment were lower ( $p < 0.05$ ) in the treated mice than in the control mice. Pulmonary inflammation was relieved by mekabu SF which also down-regulated Th2-dominated responses. These results indicate that mekabu SF modulates Th2 responses and might be useful for treating allergic inflammation.

Recently, Choi and coworkers showed effects of algal SF on aspirin-induced ulcers in rats, measured by both biochemical and immunological parameters (Choi *et al.*, (2010)). The status of stomach tissue glycogen storage and histological changes were also examined. Examination of basic biochemical parameters showed significant ( $p < 0.01$ ) alterations in aspartate (AST) and alanine (ALT) transaminases in ulcer-induced rats. Also, moderate alterations ( $p < 0.05$ ) were observed in the levels of cholesterol and blood urea nitrogen (BUN). Histopathological examination showed neutrophil infiltration and inflammation in oxyntic cells with altered glycogen storage. Analysis of serum

cytokines of aspirin-induced rats showed a moderate decrease in IL-10 with considerable increase of IL-6 and INF- $\gamma$  when compared with control. Administration of SF showed considerable ( $p < 0.05$ ) protection against ulceration by inhibiting the acute alterations of AST, ALT, cytokines and stomach glycogen. However, aggravated serum INF- $\gamma$  was observed in the SF-pretreated group. These findings suggest that the anti-ulcer property of SF might contribute in protecting the inflammatory cytokine-mediated oxidative damage to gastric mucosa.

### SGs

The main work concerning anti-inflammatory action of algal galactose-composed polysaccharide is that described by Burgermeister (Burgermeister *et al.*, 2002). In this work, highly sulfated branched  $\beta(1 \rightarrow 3)$ linked galactans were prepared from the arabinogalactan from *Larix decidua* Miller by partial hydrolysis and subsequent chemical sulfonation. This compound named LaPSvS1 exhibited high *in vivo* antiangiogenic and anti-inflammatory effects in two different modifications of the known CAM-assay. LaPSvS1 interacts with the fibroblast growth factor 2 (FGF-2) system, and this interaction is reported to be correlated with the potent inhibitor effect of LaPSvS1 on FGF-2 induced angiogenesis and inflammation since inflammation and angiogenesis are codependent in this mechanism.

#### 11.6.4 Pro- and antiangiogenic actions of SFs/fucoidans

Similarly to higher proportion of works describing the anti-inflammatory actions for SFs compared to those involving SGs, the amounts of work reporting effects of SGs on angiogenesis is very limited and the single major work available is the same as described earlier (Burgermeister *et al.*, 2002). However, nine main works have been published since 1999 concerning pro- or antiangiogenic actions of brown algal SFs. The summary of these articles is described below.

The Japanese investigator Shimeno demonstrated in 2000 that chemically oversulfated fucan (OSF) but not native SF (NSF) effectively suppresses the tube structure formation by human umbilical vein endothelial cells (HUVEC) on the basement membrane preparation, Matrigel (Soeda *et al.*, 2000). In their study, using more defined systems where basic fibroblast growth factor (bFGF) induces the tube formation by HUVEC on collagen gel, they investigated the mechanism responsible for the inhibition of angiogenesis by OSF *in vitro*. Unlike NF and desulfated fucan (desF), OSF potentially inhibited the bFGF-induced HUVEC migration and tube formation.

This same Japanese researcher published, later in 2003, that SF may suppress tumor growth by inhibiting tumor-induced angiogenesis since both NSF and OSF significantly suppressed the mitogenic and chemotactic actions of vascular endothelial growth factor 165 (VEGF(165)) on HUVEC by preventing the binding of VEGF(165) to its cell surface receptor (Koyanagi *et al.*, 2003). The suppressive effect of OSF was more potent than that of NSF, suggesting an important role for the numbers of sulfate groups in the SF molecule. Consistent with its inhibitory actions on VEGF(165), OSF clearly suppressed the neovascularization induced by Sarcoma 180 cells that had been implanted in mice. The inhibitory action of SF was also observed in the growth of Lewis lung carcinoma and B16 melanoma in mice. These results indicate that the antitumor action of SF is due, at least in part to its antiangiogenic potency and that increasing the number of sulfate groups in the SF molecule contributes to the effectiveness of its antiangiogenic and antitumor activities.

Because of the heparin-like structure of some SFs, Matou and coworkers in 2002 postulated that some SF might modulate heparin-binding angiogenic growth factor activity similarly (Matou *et al.*, 2002). They studied the effect of the SF from *A. nodosum* (Figure 11.9C) at antithrombotic concentrations on fibroblast growth factor (FGF)-2-induced proliferation and differentiation of HUVEC. The SF effect on HUVEC differentiation was evaluated by studying the expression of surface proteins (i.e., integrin, adhesion molecules) known to be modulated by FGF-2 and involved in angiogenesis and by quantifying closed areas delimited by vascular tubes formed on reconstituted basement membrane. This SF had no modulatory effect on the mitogenic activity of FGF-2 but significantly increased tubular structure density induced by FGF-2. Alone this SF increased  $\alpha(6)$  integrin subunit expression with only partially organized tubular structure. In the presence of FGF-2, SF enhanced  $\alpha(6)$ ,  $\beta(1)$  and PECAM-1 and inhibited  $\alpha(v)\beta(3)$  integrin expression. Assays using heparin showed minimum effect of this glycosaminoglycan in these systems. The most striking effect of SF was observed on  $\alpha(6)$  expression and tube formation was abolished by monoclonal anti- $\alpha(6)$  antibodies. SF plus FGF-2 effect on  $\alpha(6)$  expression was markedly decreased by monoclonal anti-FGF-2 antibodies, indicating that SF acts mainly via FGF-2. These results show that, at antithrombotic concentrations, contrary to heparin, SF can enhance vascular tube formation induced by FGF-2 with a modulation of the expression of surface proteins (mainly  $\alpha(6)$ ) involved in angiogenesis.

In addition of the previous work with SF from *A. nodosum* (Figure 11.9C), three other groups of authors published from 2003 to 2006 three distinct papers about

the influence of MW of this particular algal SF in angiogenesis. The group under the direction of Michel, in Paris, France, showed *in vitro* and in a model of critical hind limb ischemia in rat, the therapeutic potential of low-molecular-weight SF (LMWSF) derivative in an antithrombin-independent effect. *In vitro* results showed that LMWSF enhanced (FGF)-2-induced [(3H)]thymidine incorporation in cultured rat smooth muscle cells (Luyt *et al.*, 2003). Intravenous injection in rats of LMWSF significantly increased the stromal-derived factor (SDF)-1 level from  $1.2 \pm 0.1$  to  $6.5 \pm 0.35$  ng/ml in plasma. The therapeutic effect of LMWSF (5 mg/kg/day), FGF-2 (1  $\mu$ g/kg/day), and LMWSF combined with FGF-2 was assessed 14 days after induction of ischemia by (1) clinical evaluation of claudication, (2) tissue blood flow analysis, (3) histochemistry of muscle metabolic activity, and (4) quantification of capillary density. Both LMWSF and FGF-2 similarly improved residual muscle blood flow ( $62.5 \pm 6.5$  and  $64.5 \pm 4.5\%$ , respectively) compared with the control group ( $42 \pm 3.5\%$ ,  $p < 0.0001$ ). The combination of FGF-2 and LMWSF showed further significant improvement in tissue blood flow ( $90.5 \pm 3\%$ ,  $p < 0.0001$ ). These results were confirmed by phosphorylase activity, showing muscle regeneration in rats treated with the combination of FGF-2 and LMWSF. Capillary density count increased from  $9.6 \pm 0.7$  capillaries/muscle section in untreated ischemic controls to  $14.3 \pm 0.9$  with LMWSF,  $14.5 \pm 0.9$  with FGF-2, and  $19.1 \pm 0.9$  in combination ( $p < 0.001$ ). Thus, LMWSF potentiates FGF-2 activity, mobilizes SDF-1, and facilitates angiogenesis in a rat model. This algal compound was proved to be an alternative active molecule for conventional treatment in critical ischemia.

In 2005, Hirata's group of in Japan, evaluated in more details the reasons why some native SFs express antiangiogenic effect, whereas some LMWSF fractions exhibit otherwise the pro-angiogenic effect (Matsubara *et al.*, 2005). These authors addressed this concern involving different molecular sizes of SF fragments with opposite mechanisms on angiogenesis. In their studies, the effects of middle molecular weight SF (MMWSF) (15–30 kDa) on human umbilical vein endothelial cell functions were examined. SF (30 kDa) had similar effects to the high MWSF on inhibition of HUVEC tube formation and angiogenesis in an *ex vivo* model, although their effects were weaker than native SF. On the other hand, 15–20 kDa SF enhanced HUVEC migration but did not inhibit HUVEC tube formation. Thus 15–20 kDa SF would have some pro-angiogenic effect on angiogenesis. These results elucidate that 20–30 kDa would be a critical point to characterize the role of SFs on angiogenesis.

Letourneur's group, also from Paris, France, published in *The Journal of Biological Chemistry*, a therapeutic in-

duction of angiogenesis using SF as a potential strategy for the cases of chronic ischemia (Lake *et al.*, 2006). As heparan sulfate proteoglycans are known to play an important role by their interactions with pro-angiogenic growth factors such as VEGF. As some SFs mimic some biological activities of heparin or heparin sulfates, these researchers used cultured human endothelial cells (ECs) to investigate the possible ability of LMWSF to enhance the actions of VEGF(165). Data showed that LMWSF greatly enhances EC tube formation in growth factor reduced Matrigel. LMWSF is a strong enhancer of VEGF(165)-induced EC chemotaxis but not proliferation. In addition, LMWSF had no effect on VEGF(121)-induced EC migration, a VEGF isoform that does not bind to heparan sulfate proteoglycans. Then with binding studies using (125)I-VEGF(165) they observed that LMWSF enhances the binding of VEGF(165) to recombinant VEGFR-2 and neuropilin-1 (NRP1) but not to VEGFR-1 (VEGF receptor). Surface plasmon resonance analysis showed that LMWSF binds with high affinity to VEGF(165) (1.2 nm) and its receptors (5–20 nm) but not to VEGF(121). Preinjection of LMWSF on immobilized receptors shows that VEGF(165) has the highest affinity for VEGFR-2 and NRP1 as compared with VEGFR-1. Overall the effects of LMWSF were much more pronounced than those of LMW heparin. These findings suggested an efficient mechanism of action of LMWSF by promoting VEGF(165) binding to VEGFR-2 and NRP1 on ECs that could help in stimulating therapeutic revascularization.

NRP1 and NRP2 are cell surface receptors shared by class 3 semaphorins and VEGF. Ligand interaction with NRPs selects the specific signal transducer, plexins for semaphorins or VEGF receptors for VEGF, and promotes NRP internalization, which effectively shuts down receptor-mediated signaling by a second ligand. In the work of Narazaki *et al.* 2008, the authors proved that some SPs, dextran sulfate, and marine SF, but not others, reduce endothelial cell-surface levels of NRP1, NRP2, and to a lesser extent VEGFR-1 and VEGFR-2, and block the binding and *in vitro* function of semaphorin3A and VEGF(165) (Narazaki *et al.*, 2008). Administration of SF to mice reduces VEGF(165)-induced angiogenesis and tumor neovascularization *in vivo*. They found that dextran sulfate and marine SF can bridge the extracellular domain of NRP1 to that of the scavenger receptor expressed by endothelial cells I (SREC-I) and induce NRP1 and SREC-I coordinate internalization and trafficking to the lysosomes. Overexpression of SREC-I in SREC-I-negative cells specifically reduces cell-surface levels of NRP1 indicating that SREC-I mediates NRP1 internalization. These results demonstrate that engineered receptor internalization is an effective strategy for reducing levels and function of cell-surface receptors and identify certain SPs as "internalization inducers."

### 11.6.5 Algal SPs helping the fight against tumor

The same ratio of SFs and SGs contribution found in the literature about anti-inflammatory (Section 11.6.3) and antiangiogenic (Section 11.6.4) actions repeat for the available information concerning actions against tumors. The maintenance of this ratio in three different biological actions reflects the higher production and research with SFs. The research on SGs may represent a potential area for future projects, especially taking into account their simpler structures in more cases. The data from SGs applied to treatments or prevention of tumors are still quite restricted when compared to SFs. The researcher Zhou from the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China is the main contributor of results concerning anti-tumor effects of SGs. This researcher has three out of four papers published in this field since 1999 (Lin *et al.*, 2004; Zhou *et al.*, 2004, 2005, 2006).

#### SFs

Due to the fact that SFs, especially enzymatically-treated SF extracts, reveal anti-tumor potential, researches from Fukuoka, Japan, recently evaluated the effects of enzyme-digested SF extracts prepared from seaweed Mozuku of *Cladophora novae-caledoniae* kylin on *in vitro* invasion and angiogenesis abilities of human tumor cells (Ye *et al.*, 2005). First, they evaluated the effect of the SF extracts on oxidative stress of tumor cells, and demonstrated that intracellular  $H_2O_2$  levels and released  $H_2O_2$  from tumor cells were both greatly repressed upon the treatment with the SF extracts, suggesting that SF extracts ameliorate oxidative stress of tumor cells. Next they tested the effects of SF extracts on invasion ability of human fibrosarcoma HT1080 cells, showing that SF extracts significantly inhibit their invasion, possibly via suppressing matrix metalloproteinases (MMPs) MMP-2/9 activities. Further, they investigated the effects of the SF extracts on angiogenesis of human uterine carcinoma HeLa cells and found that SF extracts suppressed expression and secretion of an angiogenesis factor VEGF, resulting in suppressed vascular tubules formation of tumor cells. Their results clarified that enzyme-digested SF extracts from *Cladophora novae-caledoniae* kylin possess inhibitory effects on invasion and angiogenesis of tumor cells. These effects might, at least partially, be elicited by the antioxidative potential of enzyme digested SF extracts.

Maruyama and coworkers (also described earlier) used the same SF from Mekabu (sporophyll of *Undaria pinnatifida*), in antitumoral tests where its activity is possibly through enhancement of the immune response (Maruyama *et al.*, 2006). This report describes the effects of a di-

etary Mekabu SF on the tumor growth of mouse A20 leukemia cells and on T cell-mediated immune responses in T cell receptor transgenic (DO-11-10-Tg) mice. The animals were fed with a diet containing 1% Mekabu SF ( $0.034 \pm 0.003$  g/mouse/day) for 10 days and subcutaneously (s.c.) inoculated with A20 leukemia cells. Thereafter the mice were fed with the diet containing SF for 40 days. Mekabu SF inhibited tumors by 65.4 %. The authors studied how the killer activities of T cell-mediated and NK cells are augmented in DO-11-10 mice fed with Mekabu SF. The cytolytic activities of OVA, which is specific against OVA-transfected A20 (OVA-A20) B lymphoma cells, and NK cells against YAC-1 were significantly enhanced in the mice fed with SF compared with a basic diet. Thus, these findings suggested that Mekabu SF indeed mediates tumor destruction through Th1 cell and NK cell responses.

Another group also from Fukuoka, Japan published in 2009 that SF may induce apoptosis through activation of caspase-8 on human breast cancer MCF-7 cells (Yamasaki-Miyamoto *et al.*, 2009). Their results demonstrated that SF reduced the viable cell number of MCF-7 cells in a dose- and time-dependent manner. In contrast SF did not affect the viable cell number of normal human mammary epithelial cells. Results from the apoptosis assay demonstrated that SF induced internucleosomal DNA fragmentation, chromatin condensation, activation of caspase-7, -8, and -9, and cleavage of poly(ADP ribose) polymerase. Furthermore, expression of Bid was decreased, whereas truncated Bid was increased by SF treatment. There was also a decline in cytosolic Bax and a striking increase of cytosolic cytochrome C. Caspase-8-specific inhibitor, z-ITED-fmk, canceled the cytotoxicity of SF, activation of caspase-7, -8, and -9, and a series of changes in Bax, Bid, and cytochrome c. However, caspase-9-specific inhibitor exerted a moderate inhibitory effect on the cytotoxicity of SF. These data indicated that SF could induce apoptotic cell death through a caspase-8-dependent pathway in MCF-7 cells.

Another group from Hiroshima, Japan showed interestingly that SF-vitamin C complex suppresses tumor invasion through the basement membrane, with scarce injuries to normal or tumor cells, via decreases in oxidative stress and matrix metalloproteinases (Saitoh *et al.*, 2009). SF and vitamin C (VC) were dissolved in water and lyophilized then rinsed with ethanol until no detection for supernatant VC to form the SF-VC (1:0.23 wt/wt) inclusion body (SF-VC-IB). SF-VC-IB increased not only VC-stabilizing at 37 degrees, but also hydroxyl-radical scavenging as shown by ESR/spin-trap method, more markedly than a mere mixture of SF:VC (1:0.23 wt/wt). Invasion of human fibrosarcoma cells HT-1080 through the basement membrane was repressed by SF-VC-IB of non-cytotoxic concentrations without significant inhibition to human skin dermal fibroblasts DUMS-16

cells. SF-VC-IB suppressed the invasiveness-related gelatinases MMP-2/9, and diminished reactive oxygen species inside the cytoplasm around the nucleus, in HT-1080 cells as shown by electrophoretic zymography and the redox indicator NBT assay, respectively. Thus SF-VC-IB markedly exhibits antioxidant and MMP-suppressing activities and preferentially inhibited tumor invasion without cytotoxicity to normal cells, and is suggested as a potent tumor-invasion suppressor.

Some researchers from Korea demonstrated that the mechanism of SF-induced apoptosis in leukemic cells is related to ERK1/2, JNK, glutathione, and nitric oxide (Jin *et al.*, 2010). Jin and coworkers investigated the effects of SF on the apoptosis of human promyeloid leukemic cells and SF-mediated signaling pathways. SF induced apoptosis of HL-60, NB4, and THP-1 cells, but not K562 cells. SF treatment of HL-60 cells induced activation of caspases-8, -9, and -3, the cleavage of Bid, and changed mitochondrial membrane permeability. SF-induced apoptosis, cleavage of procaspases, and changes in the mitochondrial membrane permeability were efficiently blocked by depletion of mitogen-activated protein kinase (MAPK) kinase kinase 1 (MEKK1) and inhibitors of MAPK kinase 1 (MEK1) and c Jun NH2-terminal kinase (JNK). The phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and JNK was increased in SF-treated HL-60, NB4, and THP-1 cells, but not K562 cells. ERK1/2 activation occurred at earlier times than JNK activation and JNK activation was blocked by MEK1 inhibitor. In addition, SF-induced apoptosis was inhibited by addition of glutathione and/or L-NAME, and SF decreased intracellular glutathione concentrations and stimulated nitric oxide (NO) production. Buthionine-[R,S]-sulfoximine rendered HL-60 cells more sensitive to SF. Depletion of MEKK1 and inhibition of MEK1 restored the intracellular glutathione content and abrogated NO production, whereas inhibition of JNK activation by SP600125 restored intracellular glutathione content but failed to inhibit NO production in SF-treated HL-60 cells. These results suggest that activation of MEKK1, MEK1, ERK1/2, and JNK, depletion of glutathione, and production of NO are important mediators in SF-induced apoptosis of human leukemic cells.

### SGs

Zhou and coworkers have demonstrated in some articles the *in vivo* antitumor and immuno-modulation activities of lambda-carrageenan (Figure 11.10D) from the red seaweed *Chondrus ocellatus*, an important economic alga in China (Zhou *et al.*, 2005). These authors showed that the native lambda-carrageenan, its low-MW derivatives and co-administration with 5-Fu (a pyrimidine analog, which is largely used as a drug in the treatment of cancers), has great

benefit effects against S180 and H-22 tumors. The anticancer effects of these compounds were determined by the weight of immune organ, proliferation ratio of lymphocyte, concentration of Tumor necrosis factor-alpha (TNF- $\alpha$ ) as well as histopathology of tumors from transplanted S180 or H-22 tumor mice. The results indicated that the degraded lambda-carrageenan could enhance the antitumor activities of 5-Fu and improve the immuno-competence damaged by 5-Fu.

### 11.6.6 Combating infection of parasites with algal SPs: a new avenue against parasitoses

Some SPs are antimalarial *in vitro*, inhibiting the invasion of free *Plasmodium falciparum* parasites into erythrocytes (Clark *et al.*, 1997). A thrombospondin-related adhesion protein is implicated in host cell invasion; this binds to SF and heparin and probably to cell surface heparin sulfates (McCormick *et al.*, 1999). SF and LMWSF, but not desulfated fucoidan, inhibit *Plasmodium berghei* development in Hep G2 cells and sporozoite invasion of Chinese hamster ovary cells (Ying *et al.*, 1997). Hepatocytes bear a particularly highly sulfated heparin sulfate that is thought to be instrumental in the clearance by the liver of circulating sporozoites. However, SPs can enhance adhesion of infected erythrocytes to cells bearing CD36 (McCormick *et al.*, 2000). Chondroitin sulfate A has been identified as a cell-surface receptor for *P. falciparum*-infected erythrocytes (Rogerson *et al.*, 1995), with which neither fucoidan nor other highly SPs can compete.

Another widespread parasite, *Toxoplasma gondii*, is also affected by SFs. In this case low concentrations of SFs can enhance infection of fibroblasts in culture, though higher concentrations are inhibitory (Ostergaard *et al.*, 2000).

Results describing antiparasitic actions of SGs are virtually unknown as of now.

### 11.6.7 Effects on cellular growth, migration and adhesion

Like heparin, SF has antiproliferative effects on vascular smooth muscle cells (SMCs). A SF fraction from *A. nodosum* (Figure 11.10C) was more active than heparin (Logeart *et al.*, 1997); SFs were internalized by cells and perhaps transported to the nucleus. Patel and co-workers (2002) were able to distinguish between the modes of activity of SF and heparin; SF was active even for heparin-resistant SMCs (Patel *et al.*, 2002). In this study, crude commercial SF was more active than the purified material, indicating that some highly active fraction was discarded in the purification,

another indication that specific structures within these complex mixtures can be linked to particular biological effects. SF can also modulate proliferation of fibroblasts and here again it has been shown that antiproliferative and anticoagulant SF structures are different (Haroun-Bouhedja *et al.*, 2000). The situation for endothelial cells is complex and depends on the agent used to stimulate proliferation; heparin and SF affect growth and migration differently (Giroux *et al.*, 1998).

## 11.7 Major conclusions

In this chapter the main methods for extraction, isolation/purification, and structural characterization of SP from seaweeds is described. We took the SG from the green alga *C. isthmocladum* as the major example for explaining the application of some of these methods. Next, we pointed out the phylogenetic distribution of SFs and SGs, in which the former is found only at brown algae and the latter is found in green as well as in red algae. The complexity of structures rises from red algal, followed by green algal, and brown algal molecules. In red algae, carrageenans and agarans are well known and widely explored SG types, composed markedly of disaccharide units like glycosaminoglycans. Their main structures and industrial uses are reviewed herein. Several pharmacological actions of both SFs and SGs are also documented. These included actions involved in virosis, thrombosis/coagulation, inflammation, angiogenesis, tumorigenesis, parasitosis, and cellular actions such as cell growth, adhesion, and migration. Among several papers concerning structure and function of SFs and SGs available in the literature, some reviews are strongly recommended for future reading: (1) (Berteau and Mulloy, 2003); (2) plus comments from (Tissot and Daniel, 2003); (3) (Cumashi *et al.*, 2007); (4) (Pomin and Mourao, 2008); (5) (Pomin, 2009); (6) (Pomin, 2010), and (7) (Li *et al.*, 2008). The additional comments of (Tissot and Daniel, 2003) and the review of Berteau and Mulloy (2003) describe the potent inhibiting activity of algal SFs against the human complement system. The complement system is a major component of the immunity and is mainly involved in the innate and humoral immune response. It also allows the link between the innate immunity and the adaptive defense. An uncontrolled activation is harmful for the host organism as observed in ischemic and anaphylactic shocks or xenograft rejection (Mollnes and Fosse, 1994). The algal SF from the fucale *A. nodosum* (Figure 11.10 C) has been first described as an anticomplementary molecule by (Blondin *et al.*, 1994). Since this first report, other SFs from order Fuciales (*F. evanescens*, Figure 11.10 C), and from other brown algae of order Lam-

inales have been also described as inhibitors of the complement (Zvyagintseva *et al.*, 2000).

## Acknowledgments

I acknowledge Prof. Se-Kwon Kim for the kind invitation to contribute to this book; and Prof. Paulo A. S. Mourao for all background, knowledge and support, during my masters and PhD degree courses involving marine SFs and SGs. I thank Prof. James H. Prestegard for the opportunity of a post-doctorate in his laboratory at CCRC, UGA, USA, where this chapter was written. And finally, I am immensely grateful to Laura Morris for her careful editing on this material.

## References

- Adhikari, U., Mateii, C.G., Chattopadhyay, K., *et al.* (2006) Structure and antiviral activity of sulfated fucans from *Stoechospermum marginatum*. *Phytochemistry*, **67**, 2474–2482.
- Aisa, Y., Miyakawa, Y., Nakazato, T., *et al.* (2005) Fucoidan induces apoptosis of human hs-sultan cells accompanied by activation of caspase-3 and down-regulation of erk pathways. *Am. J. Hematol.*, **78**, 7–14.
- Albano, R.M., Pavao, M.S.G., Mourao, P.A.S. and Mulloy, B. (1990) Structural studies of a sulfated l-galactan from *Styela plicata* (tunicate) – analysis of the smith-degraded polysaccharide. *Carbohydr. Res.*, **208**, 163–174.
- Albuquerque, I.R.L., Queiroz, K.C.S., Alves, L.G., Santos, E.A., Leite, E.L. and Rocha, H.A.O. (2004) Heterofucans from *Dictyota menstrualis* have anticoagulant activity. *Brazilian J. Med. Biol. Res.*, **37**, 167–171.
- Alves, A.P., Mulloy, B., Diniz, J.A. and Mourao, P.A. S. (1997) Sulfated polysaccharides from the egg jelly layer are species-specific inducers of acrosomal reaction in sperms of sea urchins. *J. Biol. Chem.*, **272**, 6965–6971.
- Amornrut, C., Toida, T., Imanari, T., *et al.* (1999) A new sulfated beta-galactan from clams with anti-HIV activity. *Carbohydr. Res.*, **321**, 121–127.
- Aquino, R.S., Landeira-Fernandez, A.M., Valente, A.P., Andrade, L.R. And Mourao, P.A.S. (2005) Occurrence of sulfated galactans in marine angiosperms: evolutionary implications. *Glycobiology*, **15**, 11–20.
- Bae, S., Yim, J., Lee, H. And Pyo, S. (2005) Activation of macrophages by sulfated exopolysaccharide from marine microalga *Gyrodinium impudicum* (strain KG03): involvement of the NF-kB and maps pathway. *FASEB J.*, **19**, a551–a551.

- Barroso, E.M.A., Costa, L.S., Medeiros, V.P., *et al.* (2008) A non-anticoagulant heterofucan has antithrombotic activity in vivo. *Planta Med.*, **74**, 712–718.
- Berteau, O. and Mulloy, B. (2003) Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology*, **13**, 29r–40r.
- Bilan, M.I., Grachev, A.A., Ustuzhanina, N.E., Shashkov, A.S., Nifantiev, N.E. And Usov, A.I. 2002. Structure of a fucoidan from the brown seaweed *Fucus evanescens* c.ag. *Carbohydr. Res.*, **337**, 719–730.
- Bilan, M.I., Vinogradova, E.V., Shashkov, A.S. And Usov, A.I. (2007) Structure of a highly pyruvylated galactan sulfate from the pacific green alga *Codium yezoense* (Bryopsidales, Chlorophyta). *Carbohydr. Res.*, **342**, 586–596.
- Bixler, H.J. (1994) The carrageenan connection IV. *Br. Food J.*, **96**, 12–17.
- Black, W.A.P. (1954) The seasonal variation in the combined l-fucose content of the common British Laminariaceae and Fucaceae. *J. Sci. Food Agric.*, **5**, 445–448.
- Blondin, C., Fischer, E., Boissonvidal, C., Kazatchkine, M.D. And Jozefonvicz, J. (1994) Inhibition of complement activation by natural sulfated polysaccharides (fucans) from brown seaweed. *Molec. Immunol.*, **31**, 247–253.
- Borthakur, A., Bhattacharyya, S., Dudeja, P.K. And Tobacman, J.K. (2007) Carrageenan induces interleukin-8 production through distinct bcl10 pathway in normal human colonic epithelial cells. *American J. Physiol.-Gastroint. Liver Physiol.*, **292**, g829–g838.
- Bourgougnon, N., Lahaye, M., Chermann, J.C. and Kornprobst, J.M. (1993) Composition and antiviral activities of a sulfated polysaccharide from *Schizymenia dubyi* (Rhodophyta, Gigartinales). *Bioorg. Med. Chem. Lett.*, **3**, 1141–1146.
- Buck, C.B., Thompson, C.D., Roberts, J.N., *et al.* (2006) Carrageenan is a potent inhibitor of papillomavirus infection. *PLOS Pathogens*, **2**, 671–680.
- Burgermeister, J., Paper, D.H., Vogl, H., Linhardt, R.J. And Franz, G. (2002) Lapsvs1, a (1→3)-beta-galactan sulfate and its effect on angiogenesis in vivo and in vitro. *Carbohydr. Res.*, **337**, 1459–1466.
- Caceres, P.J., Carlucci, M.J., Damonte, E.B., Matsuihiro, B. And Zuniga, E.A. (2000) Carrageenans from Chilean samples of *Stenogramme interrupta* (Phyllophoraceae): structural analysis and biological activity. *Phytochemistry*, **53**, 81–86.
- Castro, M.O., Pomin, V.H., Santos, L.L., *et al.* (2009) A unique 2-sulfated beta-galactan from the egg jelly of the sea urchin *Glyptocidaris crenularis* conformation flexibility versus induction of the sperm acrosome reaction. *J. Biol. Chem.*, **284**, 18790–18800.
- Catanzar, P.J, Schwartz, H.J. and Graham, R.C. (1971) Spectrum and possible mechanism of carrageenan cytotoxicity. *Am. J. Pathol.*, **64**, 387.
- Chattopadhyay, K., Ghosh, T., Pujol, C.A., Carlucci, M.J., Damonte, E.B. and Ray, B. (2008) Polysaccharides from *Gracilaria corticata*: sulfation, chemical characterization and anti-HSV activities. *Int. J. Biol. Macromol.*, **43**, 346–351.
- Chevolot, L., Foucault, A., Chaubet, F., *et al.* (1999) Further data on the structure of brown seaweed fucans: relationships with anticoagulant activity. *Carbohydr. Res.*, **319**, 154–165.
- Chevolot, L., Mulloy, B., Ratiskol, J., Foucault, A. and Collic-Jouault, S. (2001) A disaccharide repeat unit is the major structure in fucoidans from two species of brown algae. *Carbohydr. Res.*, **330**, 529–535.
- Chizhov, A.O., Dell, A., Morris, H.R., *et al.* (1999) A study of fucoidan from the brown seaweed chorda filum. *Carbohydr. Res.*, **320**, 108–119.
- Choi, J.I., Raghavendran, H.R.B., Sung, N.Y., *et al.* (2010). Effect of fucoidan on aspirin-induced stomach ulceration in rats. *Chemico-biological Interactions*, **183**, 249–254.
- Clark, D.L., Su, S.D. and Davidson, E.A. (1997) Saccharide anions as inhibitors of the malaria parasite. *Glycoconjugate Journal*, **14**, 473–479.
- Cumashi, A., Ushakova, N.A., Preobrazhenskaya, M.E., *et al.* (2007) A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiology*, **17**, 541–552.
- Daniel, R., Chevolot, L., Carrascal, M., Tissot, B., Mourao, P.A.S. and Abian, J. (2007) Electrospray ionization mass spectrometry of oligosaccharides derived from fucoidan of *Ascophyllum nodosum*. *Carbohydr. Res.*, **342**, 826–834.
- Del Bigio, M.R., Yan, H.J., Campbell, T.M. and Peeling, J. (1999) Effect of fucoidan treatment on collagenase-induced intracerebral hemorrhage in rats. *Neurol. Res.*, **21**, 415–419.
- Deux, J.F., Meddahi-Pelle, A., Le Blanche, A.F., *et al.* 2002. Low molecular weight fucoidan prevents neointimal hyperplasia in rabbit iliac artery in-stent restenosis model. *Arterioscler. Thromb. Vasc. Biol.*, **22**, 1604–1609.
- Dische, Z. (1947) A new specific color reaction of hexuronic acids. *J. Biol. Chem.*, **167**, 189–198.
- Doty, M.S. (1988). Prodrum Ad Systematica Eucheumatoideorum: A Tribe of Commercial Seaweeds Related to *Eucheuma* (Solieriaceae, Gigartinales). In: *Taxonomy of Economic Seaweeds with Reference to some Pacific and Caribbean Species*, Volume II (ed. I.A. Abbott). California Sea Grant, La Jolla, CA, pp. 159–207.
- Duarte, M.E.R., Cauduro, J.P., Nosedá, D.G., *et al.* (2004) The structure of the agaran sulfate from *Acanthophora*

- spicifera* (Rhodomelaceae, Ceramiales) and its antiviral activity. Relation between structure and antiviral activity in agarans. *Carbohydr. Res.*, **339**, 335–347.
- Duarte, M.E.R., Nosedá, D.G., Nosedá, M.D., Tulio, S., Pujol, C.A. and Damonte, E.B. (2001) Inhibitory effect of sulfated galactans from the marine alga *Bostrychia montagnei* on herpes simplex virus replication in vitro. *Phytomedicine*, **8**, 53–58.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350–356.
- Fareed, J., Hoppensteadt, D.A. and Bick, R.L. (2000) An update on heparins at the beginning of the new millennium. *Semin. Thromb. Hemost.*, **26**, 5–21.
- Farias, E.H.C., Pomin, V.H., Valente, A.P., Nader, H.B., Rocha, H.A.O. and Mourao, P.A.S. (2008) A preponderantly 4-sulfated, 3-linked galactan from the green alga *Codium isthmocladum*. *Glycobiology*, **18**, 250–259.
- Farias, W.R.L., Valente, A.P., Pereira, M.S. and Mourao, P.A.S. (2000) Structure and anticoagulant activity of sulfated galactans - isolation of a unique sulfated galactan from the red algae botryocladia occidentalis and comparison of its anticoagulant action with that of sulfated galactans from invertebrates. *J. Biol. Chem.*, **275**, 29299–29307.
- Feldman, S.C., Reynaldi, S., Stortz, C.A., Cerezo, A.S. and Damonte, E.B. (1999) Antiviral properties of fucoidan fractions from leathesia difformis. *Phytomedicine*, **6**, 335–340.
- Fonseca, R.J.C., Oliveira, S., Melo, F.R., Pereira, M.G., Benavides, N.M.B. and Mourao, P.A.S. (2008) Slight differences in sulfation of algal galactans account for differences in their anticoagulant and venous antithrombotic activities. *Thromb. Haemost.*, **99**, 539–545.
- Freile-Pelegrin, Y., Robledo, D. and Azamar, J.A. (2006) Carrageenan of eucheuma isiforme (solieriaceae, rhodophyta) from yucatan, mexico. 1. Effect of extraction conditions. *Bot. Mar.*, **49**, 65–71.
- Giraux, J.L., Tapon-Bretaudière, J., Matou, S. and Fischer, A.M. 1998. Fucoidan, as heparin, induces tissue factor pathway inhibitor release from cultured human endothelial cells. *Thromb. Haemost.*, **80**, 692–695.
- Guerrini, M., Beccati, D., Shriver, Z., et al. (2008) Over-sulfated chondroitin sulfate is a contaminant in heparin associated with adverse clinical events. *Nat. Biotechnol.*, **26**, 669–675.
- Haroun-Bouhedja, F., Ellouali, M., Sinquin, C. and Boisson-Vidal, C. (2000) Relationship between sulfate groups and biological activities of fucans. *Thromb. Res.*, **100**, 453–459.
- Hayashi, K., Nakano, T., Hashimoto, M., Kanekiyo, K. and Hayashi, T. 2008. Defensive effects of a fucoidan from brown alga *Undaria pinnatifida* against herpes simplex virus infection. *Int. Immunopharmacol.*, **8**, 109–116.
- Honya, M., Morim, M., Anzai, M., Araki, Y. and Nisizawa, K. (1999) Monthly changes in the content of fucans their constituent sugars and sulphate in cultured *Laminaria japonica*. *Hydrobiologia*, **398**, 411–416.
- Imbs, T.I., Shevchenko, N.M., Sukhoverkhov, S.V., Semenova, T.L., Skriptsova, A.V. and Zvyagintseva, T.N. 2009. Seasonal variations of the composition and structural characteristics of polysaccharides from the brown alga *Costaria costata*. *Chem. Nat. Comp.*, **45**, 786–791.
- Irhimeh, M.R., Fitton, J.H. and Lowenthal, R.M. (2007) Fucoidan ingestion increases the expression of CXCR4 on human CD34(+) cells. *Exp. Hematol.*, **35**, 989–994.
- Jin, J.O., Song, M.G., Kim, Y.N., Park, J.I. and Kwak, J.Y. 2010. The mechanism of fucoidan-induced apoptosis in leukemic cells: involvement of erk1/2, jnk, glutathione, and nitric oxide. *Molec. Carcinogen.*, **49**, 771–782.
- Killing, H. (1913) Zur biochemie der meersalgen. *Physiol. Chem.*, **83**, 171–197.
- Kishimoto, T.K., Viswanathan, K., Ganguly, T., et al. (2008) Contaminated heparin associated with adverse clinical events and activation of the contact system. *N. Engl. J. Med.*, **358**, 2457–2467.
- Kloareg, B. (1984) Isolation and analysis of cell-walls of the brown marine-algae *Pelvetia canaliculata* and *Ascophyllum nodosum*. *Physiologie vegetale*, **22**, 47–56.
- Kloareg, B., Demarty, M. and Mabeau, S. (1986) Polyanionic characteristics of purified sulfated homofucans from brown-algae. *Int. J. Biol. Macromol.*, **8**, 380–386.
- Knutsen, S.H., Myslabodski, D.E., Larsen, B. and Usov, A.I. (1994) A modified system of nomenclature for red algal galactans. *Bot. Mar.*, **37**, 163–169.
- Koyanagi, S., Tanigawa, N., Nakagawa, H., Soeda, S. and Shimeno, H. (2003) Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities. *Biochem. Pharmacol.*, **65**, 173–179.
- Lahaye, M. (2001) Developments on gelling algal galactans, their structure and physico-chemistry. *J. Appl. Phycol.*, **13**, 173–184.
- Lake, A.C., Vassy, R., Di Benedetto, M., et al. (2006) Low molecular weight fucoidan increases VEGF(165)-induced endothelial cell migration by enhancing VEGF(165) binding to VEGFR-2 and NRP1. *J. Biol. Chem.*, **281**, 37844–37852.
- Larsen, B., Haug, A. and Painter, T.J. (1966) Sulphated polysaccharides in brown algae .1. Isolation and preliminary characterisation of 3 sulphated polysaccharides from ascophyllum nodosum (l) le jol. *Acta Chem. Scand.*, **20**, 219.
- Lee, J.B., Hayashi, K., Hashimoto, M., Nakano, T. and Hayashi, T. (2004) Novel antiviral fucoidan from

- sporophyll of *Undaria pinnatifida* (mekabu). *Chem. Pharm. Bull.*, **52**, 1091–1094.
- Lee, S.H., Athukorala, Y., Lee, J.S. and Jeon, Y.J. (2008) Simple separation of anticoagulant sulfated galactan from marine red algae. *J. Appl. Phycol.*, **20**, 1053–1059.
- Leite, E.L., Medeiros, M.G.L., Rocha, H.A.O., *et al.* (1998) Structure and pharmacological activities of a sulfated xylofucoglucuronan from the alga *Spatoglossum schroederi*. *Plant Sci.*, **132**, 215–228.
- Li, B., Lu, F., Wei, X.J. and Zhao, R.X. (2008) Fucoidan: structure and bioactivity. *Molecules*, **13**, 1671–1695.
- Lin, Y.L., Zhang, L.N., Chen, L., *et al.* (2004) Molecular mass and antitumor activities of sulfated derivatives of alpha-glucan from *Poria cocos* mycelia. *Int. J. Biol. Macromol.*, **34**, 289–294.
- Logeart, D., Prigentrichard, S., Boissonvidal, C., *et al.* (1997) Fucans, sulfated polysaccharides extracted from brown seaweeds, inhibit vascular smooth muscle cell proliferation .2. Degradation and molecular weight effect. *Eur. J. Cell Biol.*, **74**, 385–390.
- Love, J. and Percival, E. (1964) Polysaccharides of green seaweed codium fragile .3. Beta-1,4-linked mannan. *J. Chem. Soc.*, 3345.
- Luyt, C.E., Meddahi-Pelle, A., Ho-Tin-Noe, B., *et al.* (2003) Low-molecular-weight fucoidan promotes therapeutic revascularization in a rat model of critical hindlimb ischemia. *J. Pharmacol. Exp. Ther.*, **305**, 24–30.
- Mabeau, S., Kloareg, B. and Joseleau, J.P. (1990) Fractionation and analysis of fucans from brown-algae. *Phytochemistry*, **29**, 2441–2445.
- Maeda, H., Yamamoto, R., Hirao, K. and Tochikubo, O. (2005) Effects of agar (kanten) diet on obese patients with impaired glucose tolerance and type 2 diabetes. *Diabetes Obesity Metab.*, **7**, 40–46.
- Makarenkova, I.D., Derianbin, P.G., L'vov, D.K., Zviagintseva, T.N. and Besendnova, N.N. (2010) Antiviral activity of sulfated polysaccharide from the brown algae *Laminaria japonica* against avian influenza A (H5N1) virus infection in the cultured cells. *Voprosy Virusologii*, **55**, 41–45.
- Mandal, P., Mateu, C.G., Chattopadhyay, K., Pujol, C.A., Damonte, E.B. and Ray, B. (2007) Structural features and antiviral activity of sulphated fucans from the brown seaweed *Cystoseira indica*. *Antiviral Chem. Chemother.*, **18**, 153–62.
- Martinez-Rumayor, A. and Januzzi, J.L. (2006) Non-ST segment elevation acute coronary syndromes: a comprehensive review. *S. Med. J.*, **99**, 1103–1110.
- Maruyama, H., Tamauchi, H., Hashimoto, M. and Nakano, T. (2005) Suppression of th2 immune responses by mekabu fucoidan from *Undaria pinnatifida* sporophylls. *Int. arch. Allergy Immunol.*, **137**, 289–294.
- Maruyama, H., Tamauchi, H., Iizuka, M. and Nakano, T. (2006) The role of nk cells in antitumor activity of dietary fucoidan from *Undaria pinnatifida* sporophylls (mekabu). *Planta Med.*, **72**, 1415–1417.
- Matou, S., Helley, D., Chabut, D., Bros, A. and Fischer, A.M. (2002) Effect of fucoidan on fibroblast growth factor-2-induced angiogenesis in vitro. *Thromb. Res.*, **106**, 213–221.
- Matsubara, K., Matsuura, Y., Bacic, A., Liao, M.L., Hori, K. and Miyazawa, K. (2001) Anticoagulant properties of a sulfated galactan preparation from a marine green alga, *Codium cylindricum*. *Int. J. Biol. Macromol.*, **28**, 395–399.
- Matsubara, K., Xue, C., Zhao, X., Mori, M., Sugawara, T. and Hirata, T. (2005) Effects of middle molecular weight fucoidans on in vitro and ex vivo angiogenesis of endothelial cells. *Int. J. Molec. Med.*, **15**, 695–699.
- Matsuhiro, B., Conte, A.F., Damonte, E.B., *et al.* (2005) Structural analysis and antiviral activity of a sulfated galactan from the red seaweed *Schizymenia binderi* (Gigartinales, Rhodophyta). *Carbohydr. Res.*, **340**, 2392–2402.
- Mazumder, S., Ghosal, P.K., Pujol, C.A., Carlucci, M.J., Damonte, E.B. and Ray, B. (2002) Isolation, chemical investigation and antiviral activity of polysaccharides from *Gracilaria corticata* (Gracilariaceae, Rhodophyta). *Int. J. Biol. Macromol.*, **31**, 87–95.
- Mccormick, C.J., Newbold, C.I. and Berendt, A.R. (2000) Sulfated glycoconjugates enhance CD36-dependent adhesion of *Plasmodium falciparum*-infected erythrocytes to human microvascular endothelial cells. *Blood*, **96**, 327–333.
- Mccormick, C.J., Tuckwell, D.S., Crisanti, A., Humphries, M.J. and Hollingdale, M.R. (1999) Identification of heparin as a ligand for the a-domain of plasmodium falciparum thrombospondin-related adhesion protein. *Molec. Biochem. Parasitol.*, **100**, 111–124.
- Medcalf, D.G., Schneider, T.L. and Barnett, R.W. (1978) Structural features of a novel glucuronogalactofucan from *Ascophyllum nodosum*. *Carbohydr. Res.*, **66**, 167–171.
- Medeiros, V.P., Queiroz, K.C.S., Cardoso, M.L., *et al.* (2008) Sulfated galactofucan from *Lobophora variegata*: anticoagulant and anti-inflammatory properties. *Biochemistry-Moscow*, **73**, 1018–1024.
- Mian, A.J. and Percival, E. 1973. Carbohydrates of brown seaweeds *Himanthalia lorea*, *Bifurcaria bifurcata*, and *Padina pavonia* .1. Extraction and fractionation. *Carbohydr. Res.*, **26**, 133–146.
- Mollnes, T.E. and Fosse, E. (1994) The complement-system in trauma-related and ischemic tissue-damage – a brief review. *Shock*, **2**, 301–310.

- Mourao, P.A.S. (2004) Use of sulfated fucans as anticoagulant and antithrombotic agents: future perspectives. *Curr. Pharm. Design*, **10**, 967–981.
- Mulloy, B., Ribeiro, A.C., Vieira, R.P. and Mourao, P.A.S. (1994) Structural analysis of sulfated fucans by high-field nmr. *Brazilian J. Med. Biol. Res.*, **27**, 515–521.
- Murano, E., Toffanin, R., Cecere, E., Rizzo, R. and Knutsen, S.H. (1997) Investigation of the carrageenans extracted from *Solieira filiformis* and *Agardhiella subulata* from Mar Piccolo, Taranto. *Mar. Chem.*, **58**, 319–325.
- Narazaki, M., Segarra, M. and Tosato, G. (2008) Sulfated polysaccharides identified as inducers of neuropilin-1 internalization and functional inhibition of VEGF165 and semaphorin3a. *Blood*, **111**, 4126–4136.
- Nishino, T., Aizu, Y., Nagumo, T. (1991) The influence of sulfate content and molecular weight of a fucan sulfate from the brown seaweed *Ecklonia kurome* on its antithrombin activity. *Thromb. Res.*, **15**, 723–731.
- Nishino, T., Takabe, Y. and Nagumo, T. (1994) Isolation and partial characterization of a novel beta-d-galactan sulfate from the brown seaweed *Laminaria angustata* var *longissima*. *Carbohydr. Polym.*, **23**, 165–173.
- Nishino, T., Ura, H. and Nagumo, T. (1995) The relationship between the sulfate content and the antithrombin activity of an alpha(1–2)-fucoidan purified from a commercial fucoidan fraction. *Bot. Mar.*, **38**, 187–193.
- Norman, P.M., Kjellbom, P., Bradley, D.J., Hahn, M.G. and Lamb, C.J. (1990) Immunoaffinity purification and biochemical-characterization of plasma-membrane arabino-galactan-rich glycoproteins of *Nicotiana glutinosa*. *Planta*, **181**, 365–373.
- Ostergaard, C., Yieng-Kow, R.V., Benfield, T., Frimodt-Moller, N., Espersen, F. and Lundgren, J.D. (2000) Inhibition of leukocyte entry into the brain by the selectin blocker fucoidin decreases interleukin-1 (IL-1) levels but increases il-8 levels in cerebrospinal fluid during experimental pneumococcal meningitis in rabbits. *Infect. Immun.*, **68**, 3153–3157.
- Patankar, M.S., Oehninger, S., Barnett, T., Williams, R.L. and Clark, G.F. (1993) A revised structure for fucoidan may explain some of its biological-activities. *J. Biol. Chem.*, **268**, 21770–21776.
- Patel, M.K., Mulloy, B., Gallagher, K.L., O'Brien, L. and Hughes, A.D. (2002) The antimitogenic action of the sulphated polysaccharide fucoidan differs from heparin in human vascular smooth muscle cells. *Thromb. Haemost.*, **87**, 149–154.
- Pavao, M.S.G., Albano, R.M., Lawson, A.M. and Mourao, P.A.S. (1989) Structural heterogeneity among unique sulfated L-galactans from different species of ascidians (tunicates). *J. Biol. Chem.*, **264**, 9972–9979.
- Pavao, M.S.G., Mourao, P.A.S. and Mulloy, B. (1990) Structure of a unique sulfated alpha-L-galactofucan from the tunicate *Clavelina*. *Carbohydr. Res.*, **208**, 153–161.
- Percival, E. 1968. Glucuronoxylfucan a cell-wall component of *Ascophyllum nodosum* .I. *Carbohydr. Res.*, **7**, 272.
- Percival, E. and McDowell, R.H. (1967) *Chemistry and Enzymology of Marine Algal Polysaccharides*. Academic Press, New York, pp. 157–175.
- Percival, E.G.V. and Ross, A.G. (1950) Fucoidin .I. The isolation and purification of fucoidin from brown seaweeds. *J. Chem. Soc.*, 717–720.
- Pereira, M.G., Benevides, N.M.B., Melo, M.R.S., Valente, A.P., Melo, F.R. and Mourao, P.A.S. (2005) Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? *Carbohydr. Res.*, **340**, 2015–2023.
- Pereira, M.S., Mulloy, B. and Mourao, P.A.S. (1999) Structure and anticoagulant activity of sulfated fucans – comparison between the regular, repetitive, and linear fucans from echinoderms with the more heterogeneous and branched polymers from brown algae. *J. Biol. Chem.*, **274**, 7656–7667.
- Pereira, M.S., Melo, F.R. and Mourao, P.A.S. (2002) Is there a correlation between structure and anticoagulant action of sulfated galactans and sulfated fucans? *Glycobiology*, **12**, 573–580.
- Pomin, V.H. (2009) An overview about the structure-function relationship of marine sulfated homopolysaccharides with regular chemical structures. *Biopolymers*, **91**, 601–609.
- Pomin, V.H. (2010) Structural and functional insights into sulfated galactans: a systematic review. *Glycoconjugate Journal*, **27**, 1–12.
- Pomin, V.H. and Mourao, P.A.S. (2008) Structure, biology, evolution, and medical importance of sulfated fucans and galactans. *Glycobiology*, **18**, 1016–1027.
- Pomin, V.H., Pereira, M.S., Valente, A.P., Tollefsen, D.M., Pavao, M.S.G. and Mourao, P.A.S. (2005a) Selective cleavage and anticoagulant activity of a sulfated fucan: stereospecific removal of a 2-sulfate ester from the polysaccharide by mild acid hydrolysis, preparation of oligosaccharides, and heparin cofactor ii-dependent anticoagulant activity. *Glycobiology*, **15**, 369–381.
- Pomin, V.H., Valente, A.P., Pereira, M.S. and Mourao, P.A.S. (2005b) Mild acid hydrolysis of sulfated fucans: a selective 2-desulfation reaction and an alternative approach for preparing tailored sulfated oligosaccharides. *Glycobiology*, **15**, 1376–1385.

- Rogerson, S.J., Chaiyaroj, S.C., Ng, K., Reeder, J.C. and Brown, G.V. (1995) Chondroitin sulfate is a cell-surface receptor for plasmodium-falciparum-infected erythrocytes. *J. Exp. Med.*, **182**, 15–20.
- Rumjanek, V.M., Watson, S.R. and Sljivic, V.S. (1977) Re-evaluation of role of macrophages in carrageenan-induced immunosuppression. *Immunology*, **33**, 423–432.
- Saitoh, Y., Nagai, Y. and Miwa, N. (2009) Fucoidan-vitamin C complex suppresses tumor invasion through the basement membrane, with scarce injuries to normal or tumor cells, via decreases in oxidative stress and matrix metalloproteinases. *Int. J. Oncol.*, **35**, 1183–1189.
- Santos, J.A., Mulloy, B. and Mourao, P.A.S. (1992) Structural diversity among sulfated alpha-L-galactans from ascidians (tunicates) – studies on the species *Ciona intestinalis* and *Herdmania monus*. *Eur. J. Biochem.*, **204**, 669–677.
- Scudder, P., Tang, P.W., Hounsell, E.F., Lawson, A.M., Mehmet, H. and Feizi, T. (1986) Isolation and characterization of sulfated oligosaccharides released from bovine corneal keratan sulfate by the action of endo-beta-galactosidase. *Eur. J. Biochem.*, **157**, 365–373.
- Sen, A.K., Das, A.K., Banerji, N., *et al.* (1994) A new sulfated polysaccharide with potent blood anti-coagulant activity from the red seaweed *Grateloupia indica*. *Int. J. Biol. Macromol.*, **16**, 279–280.
- Shashkov, A.S., Senchenkova, S.N., Vinogradov, E.V., *et al.* (2000) Full structure of the O-specific polysaccharide of *Proteus mirabilis* O24 containing 3,4-O-(S)-L-carboxyethylidene-D-galactose. *Carbohydr. Res.*, **329**, 453–457.
- Silva, T.M.A., Alves, L.G., Queiroz, K.C.S., *et al.* (2005) Partial characterization and anticoagulant activity of a heterofucan from the brown seaweed *Padina gymnospora*. *Brazilian J. Med. Biol. Res.*, **38**, 523–533.
- Sinha, S., Astani, A., Ghosh, T., Schnitzler, P. and Ray, B. (2010) Polysaccharides from sargassum tenerrimum: structural features, chemical modification and anti-viral activity. *Phytochemistry*, **71**, 235–242.
- Soeda, S., Kozako, T., Iwata, K. and Shimeno, H. (2000) Oversulfated fucoidan inhibits the basic fibroblast growth factor-induced tube formation by human umbilical vein endothelial cells: its possible mechanism of action. *Biochim. Biophys. Acta-Molec. Cell Res.*, **1497**, 127–134.
- Talarico, L.B., Pujol, C.A., Zibetti, R.G.M., *et al.* (2005) The antiviral activity of sulfated polysaccharides against dengue virus is dependent on virus serotype and host cell. *Antiviral Res.*, **66**, 103–110.
- Talarico, L.B., Zibetti, R.G.M., Faria, P.C.S., *et al.* (2004) Anti-herpes simplex virus activity of sulfated galactans from the red seaweeds *Gymnogongrus griffithsiae* and *Cryptonemia crenulata*. *Int. J. Biol. Macromol.*, **34**, 63–71.
- Taoda, N., Shinji, E., Nishi, K., *et al.* (2008) Fucoidan inhibits parainfluenza virus type 2 infection to LLCMK2 cells. *Biomed. Res.-Tokyo*, **29**, 331–334.
- Tissot, B. and Daniel, R. (2003) Biological properties of sulfated fucans: the potent inhibiting activity of algal fucoidan against the human complement system. *Glycobiology*, **13**, 29g–30g.
- Tobacman, J.K. (2001) Review of harmful gastrointestinal effects of carrageenan in animal experiments. *Environmental Health Perspectives*, **109**, 983–994.
- Turville, S.G., Aravantinou, M., Miller, T., *et al.* (2008) Efficacy of carraguard (R)-based microbicides in vivo despite variable in vitro activity. *PLOS One*, **3**.
- Usov, A.I. (1998) Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloids*, **12**, 301–308.
- Van de Velde, F., Pereira, L. and Rollemans, H.S. (2004) The revised NMR chemical shift data of carrageenans. *Carbohydr. Res.*, **339**, 2309–2313.
- Vogl, H., Paper, D.H. and Franz, G. (2000) Preparation of a sulfated linear (1→4)-beta-D-galactan with variable degrees of sulfation. *Carbohydr. Polym.*, **41**, 185–190.
- Whittaker, H. (1969) New concepts of kingdoms of organisms. *Science*, **163**, 150.
- Yamasaki-Miyamoto, Y., Yamasaki, M., Tachibana, H. and Yamada, K. (2009) Fucoidan induces apoptosis through activation of caspase-8 on human breast cancer MCF-7 cells. *J. Agric. Food Chem.*, **57**, 8677–8682.
- Ye, J., Li, Y.P., Teruya, K., *et al.* (2005) Enzyme-digested fucoidan extracts derived from seaweed mozuku of *Cladosiphon novae-caledoniae* Kylin inhibit invasion and angiogenesis of tumor cells. *Cytotechnology*, **47**, 117–126.
- Ying, P., Shakibaei, M., Patankar, M.S., *et al.* (1997) The malaria circumsporozoite protein: interaction of the conserved regions i and ii-plus with heparin-like oligosaccharides in heparan sulfate. *Exp. Parasitol.*, **85**, 168–182.
- Yoon, S.J., Pyun, Y.R., Hwang, J.K. and Mourao, P. A. S. (2007) A sulfated fucan from the brown alga *Laminaria cichorioides* has mainly heparin cofactor II-dependent anticoagulant activity. *Carbohydr. Res.*, **342**, 2326–2330.
- Zhou, G.F., Sheng, W.X., Yao, W.H. and Wang, C.H. (2006) Effect of low molecular lambda-carrageenan from *Chondrus ocellatus* on antitumor H-22 activity of 5-FU. *Pharmacol. Res.*, **53**, 129–134.
- Zhou, G.F., Sun, Y.P., Xin, H., Zhang, Y.N., Li, Z. and Xu, Z.H. (2004) In vivo antitumor and immunomodulation activities of different molecular weight

- lambda-carrageenans from *Chondrus ocellatus*. *Pharmacol. Res.*, **50**, 47–53.
- Zhou, G.F., Xin, H., Sheng, W.X., Sun, Y.P., Li, Z. and Xu, Z.H. (2005) In vivo growth-inhibition of S180 tumor by mixture of 5-FU and low molecular lambda-carrageenan from *Chondrus ocellatus*. *Pharmacol. Res.*, **51**, 153–157.
- Zvyagintseva, T.N., Shevchenko, N.M., Nazarova, I.V., Scobun, A.S., Luk'yanov, P.A. and Elyakova, L.A. (2000) Inhibition of complement activation by water-soluble polysaccharides of some far-eastern brown seaweeds. *Comp. Biochem. Physiol. C-Toxicol. Pharmacol.*, **126**, 209–215.

# 12

## Bioactive Metabolites from Seaweeds

Jing Hu, Bin Yang, Xiuping Lin, Xue-Feng Zhou, Xian-Wen Yang, and Yonghong Liu

*Key Laboratory of Marine Bio-resources Sustainable Utilization/Guangdong Key Laboratory of Marine Materia Medica/Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China*

### 12.1 Introduction

Marine macroalgae, commonly known as seaweeds, are conspicuous and dominant features in marine ecosystems. They are not only an important part of marine primary productivity, but also potential sources of bioactive compounds with immense pharmaceutical, biomedical, and nutraceutical importance (Kumari *et al.*, 2010; Cardozo *et al.*, 2007; Kim *et al.*, 2008). Some species of marine macroalgae can be directly consumed as nutritional human food, while some others can be used as ingredients in both medicinal and food preparations. According to the differences of the pigmentation, marine macroalgae are commonly classified into three main phyla: Phaeophyta, or brown seaweeds, are predominantly brown due to the presence of the xanthophyll pigment – fucoxanthin, in addition to chlorophyll *a* and *c*. Chlorophyta, or green seaweeds, are dominated by chlorophyll *a* and *b*, along with various characteristic xanthophylls (yellowish or brownish pigments). Rhodophyta, or red seaweeds, the principal pigments in which are phycoerythrin and phycocyanin (O'Sullivan *et al.*, 2010).

Seaweeds are directly exposed in marine ecological system and are supposed to be susceptible to ambient microorganisms. However, they have amazing capability of survival due to the existence of an inherently available chemical defense mechanism. Thus, novel bioactive compounds which, in some cases, unparalleled by their terrestrial counterparts could be found. Investigations on the biochemical constitution and general phytochemistry of marine macroalgae have been widely carried out. To date, numerous structurally unusual secondary metabolites, such as sesquiterpenes (Tori *et al.*, 1994; Guella *et al.*, 1997), diterpenes (Gedara *et al.*, 2003; Goetz *et al.*, 1994), meroterpenoids (Areche *et al.*, 2009), C<sub>15</sub>-acetogenins (Kladi *et al.*, 2008), phlorotannins (Xu *et al.*, 2004b), and steroids (Fleury *et al.*, 1994; Kamenarska *et al.*, 2002), have been frequently reported from various species of seaweeds. It is noteworthy that many of the compounds have demonstrated various biological activities, widely ranging from antitumor (Xu *et al.*, 2004a; Matloub and Awad, 2009), antibacterial (del Val *et al.*, 2001; Vairappan *et al.*, 2010; Nylund *et al.*, 2010), antioxidant (Li *et al.*, 2007), anti-inflammatory properties (Chatter

*et al.*, 2009), to anticoagulant (Sen *et al.*, 1994), antifeedant (Jormalainen and Ramsay, 2009; Marques *et al.*, 2006; Valim *et al.*, 2007), and antiviral activities (Sen *et al.*, 1994). These bioactive natural products have provided essential substances for human nutrition, promising drug leads, offered targets for synthetic organic chemists, and afforded opportunities for elucidation of unusual biosynthetic pathways. In this chapter, some of the major research achievement of chemical investigations and biological activities of seaweeds, are presented.

## 12.2 Chemical constituents

### 12.2.1 Sesquiterpenes

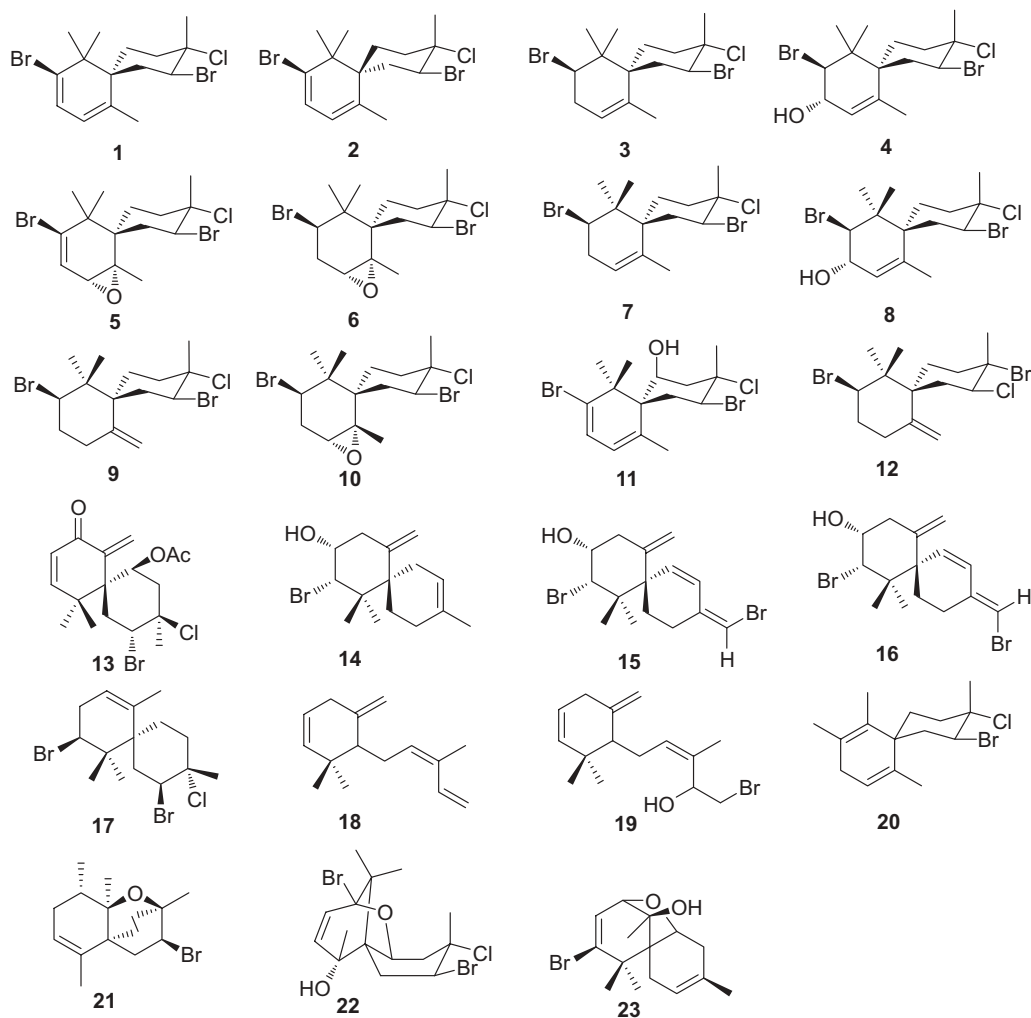
Sesquiterpenes are primary components of marine biologically active substances. Some of them act as semiochemicals, such as defensive agents or pheromones, and play a key role for the organisms' survival. The sesquiterpenoids are C<sub>15</sub> compounds formed by the assembly of three isoprenoid units. They may be acyclic or contain rings, including many unique combinations. The large number of sesquiterpenoid carbon skeletons, all arise from the common precursor, farnesyl pyrophosphate, by various modes of cyclizations followed, in many cases, by skeletal rearrangement. Among all marine macroalgae, red algae, especially the genus *Laurencia* (class Rhodophyceae, order Ceramiales, family Rhodomelaceae), are the most attractive sources of sesquiterpenes. For this, the reasons are twofold: first, algae belonging to genus *Laurencia* are extremely widespread, being found in all oceans and seas at all latitudes; second, they have unique ability to biosynthesize an astonishing variety of structurally diverse sesquiterpenes, with either new skeletons, like (seco)- or (9,10-friedo)-chamigrane, guimarane, (cyclo) perforane and poitane. Many of the sesquiterpenes

from red algae are characterized by their relatively high degree of halogenations. Their functions are in defense against herbivores, fouling organisms and pathogens and they are also of importance in reproduction, protection from UV radiation and as allelopathic agents. Species from brown algae and green algae also partly contribute to marine sesquiterpenes. However, the occurrence of halogenated compounds is unusual. According to the carbon skeletons, the sesquiterpenes isolated from seaweeds, can generally be divided into the following groups: chamigrane, laurene, cuparene, brasi-lene, bisabolene, and other skeletons.

#### *Sesquiterpenes from red algae*

##### *Chamigrane skeleton*

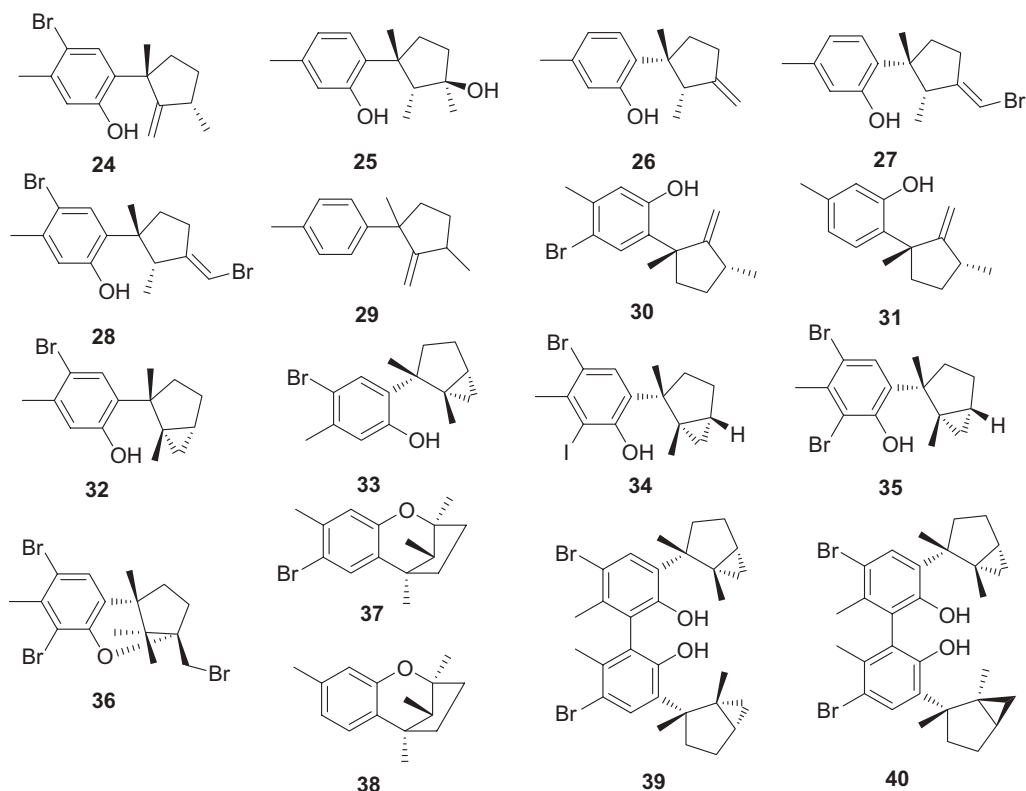
Eleven halogenated sesquiterpenes **1–6** (Ji *et al.*, 2007), **7–11** (Ji *et al.*, 2008), isolated from red algae *Laurencia okamurai* and *L. composite*, possess the same 2,10-dibromo-3-chloro-chamigrane structure skeleton. Having the similar structure, compound **12** (Ji *et al.*, 2008) differs only in the sub-positions of chlorine and bromine atoms. Five sesquiterpenes **13–16** (Vairappan *et al.*, 2001b), **17** (Wright *et al.*, 1991), also belonging to the halogenated chamigrane-type, were obtained from *Laurencia mariannensis*, *L. majuscula*, and *L. implicata*. Marginal antibacterial activity was observed in **15** and **16**, while prominent activity was seen in **14**. The MIC (minimum inhibitory concentration) values of **14** were in the range of 10–30 µg/disk against five species of bacteria, *Alcaligenes aquamarinus*, *Azomonas agilis*, *Azotobacter beijerinckii*, *Erwinia amylovora*, and *Escherichia coli* (Vairappan *et al.*, 2001b). Compounds **18–23** (Wright *et al.*, 1991; Ji *et al.*, 2008; Cassano *et al.*, 2008; Roviro-sa *et al.*, 1999) are new rearranged chamigrane sesquiterpenes, among which **24** and **25** are monocyclic rather than bicyclic, while **21–23** contain novel oxygen bridges.



### Laurane skeleton

Compounds **24** (Vairappan *et al.*, 2001b), **25** (Kladi *et al.*, 2006), **26** and **27** (Kladi *et al.*, 2006), **28** (Kladi *et al.*, 2006), **29** (Kladi *et al.*, 2007), **30** and **31** (Cassano *et al.*, 2008), possessing the laurane skeleton, were isolated from different species of the genus *Lauremia*. **32** (Vairappan *et al.*, 2001b), **33** (Kladi *et al.*, 2006), **34** and **35** (Kladi *et al.*, 2007) are four cyclolaurane-type sesquiterpenes. Of these compounds, **34** is a rare iodinated one. **34** and **35** exhibited

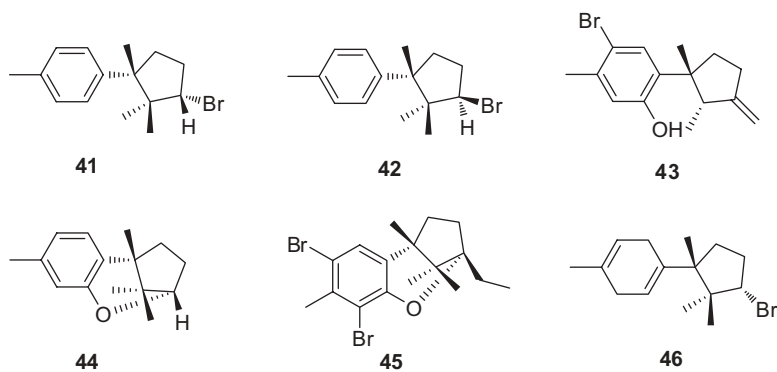
significant cytotoxicity against five human tumor cell lines, and it seems that the presence of the aromatic hydroxyl group can increase the cytotoxicity to the tested cell lines. Three laurane cyclic ethers **36** (Kladi *et al.*, 2007), **37** and **38** (Cassano *et al.*, 2008), isolated from *Laurencia microcladia* and *L. caduciramulosa*, contain a six-membered oxide ring rather than the five-membered ring of the aplysin series. In addition, two dimeric sesquiterpenes of the cyclolaurane-type **39** (Kladi *et al.*, 2006) and **40** (Kladi *et al.*, 2007) were isolated from *L. microcladia*.



### Cuparane skeleton

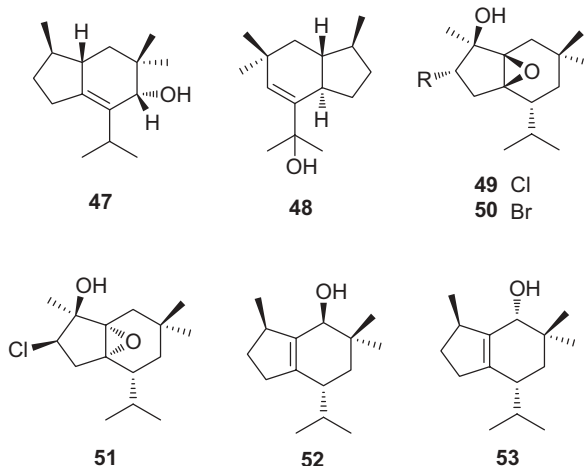
Most of the cuparane sesquiterpenes isolated from seaweeds have an aromatic ring, such as compounds **41** and **42** (Kladi *et al.*, 2007), **43** (Koenig and Wright, 1997), **44** (Kladi *et al.*, 2006), **45** (Kladi *et al.*, 2007). **41** and **42** were found to be non-significantly cytotoxic against a series of human cancer cell lines, yet **43** showed prominent activity towards

the bacterium *Bacillus megaterium* with MIC of 4  $\mu$ g/ml. In combination with results obtained with similar metabolites it seems that the presence of the aromatic hydroxyl group increases the cytotoxicity (Koenig and Wright, 1997). Besides, an unusual sesquiterpene **46**, which is lack of aromaticity, was obtained from *Lauremia majusrnka* (Wright *et al.*, 1993).

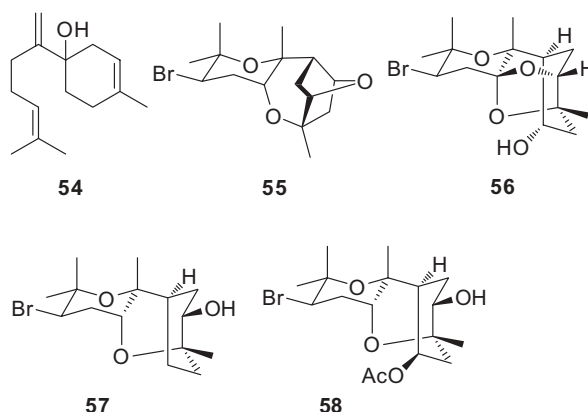


*Brasilane skeleton*

Red alga *Laurencia implicate* afforded two brasilane sesquiterpenes **47** and **48** (Wright *et al.*, 1991; Tori *et al.*, 1994). And from red alga *L. obtuse*, five halogenated rearranged sesquiterpenes **49–53** were isolated. Compounds **49–51** contain the unprecedented 1,6-epoxy moiety (Iliopoulou *et al.*, 2002b).

*Bisabolane skeleton*

Compound **54** is the hydroxyl derivative of  $\beta$ -bisabolene from *Laurencia microcladia* (Kladi *et al.*, 2007). Compounds **55–58** are four sesquiterpenes isolated from the red alga *L. aldingensis*. They belong to a novel oxacyclic class of bisabolane-type derivatives (Brito *et al.*, 2002; de Carvalho *et al.*, 2006).

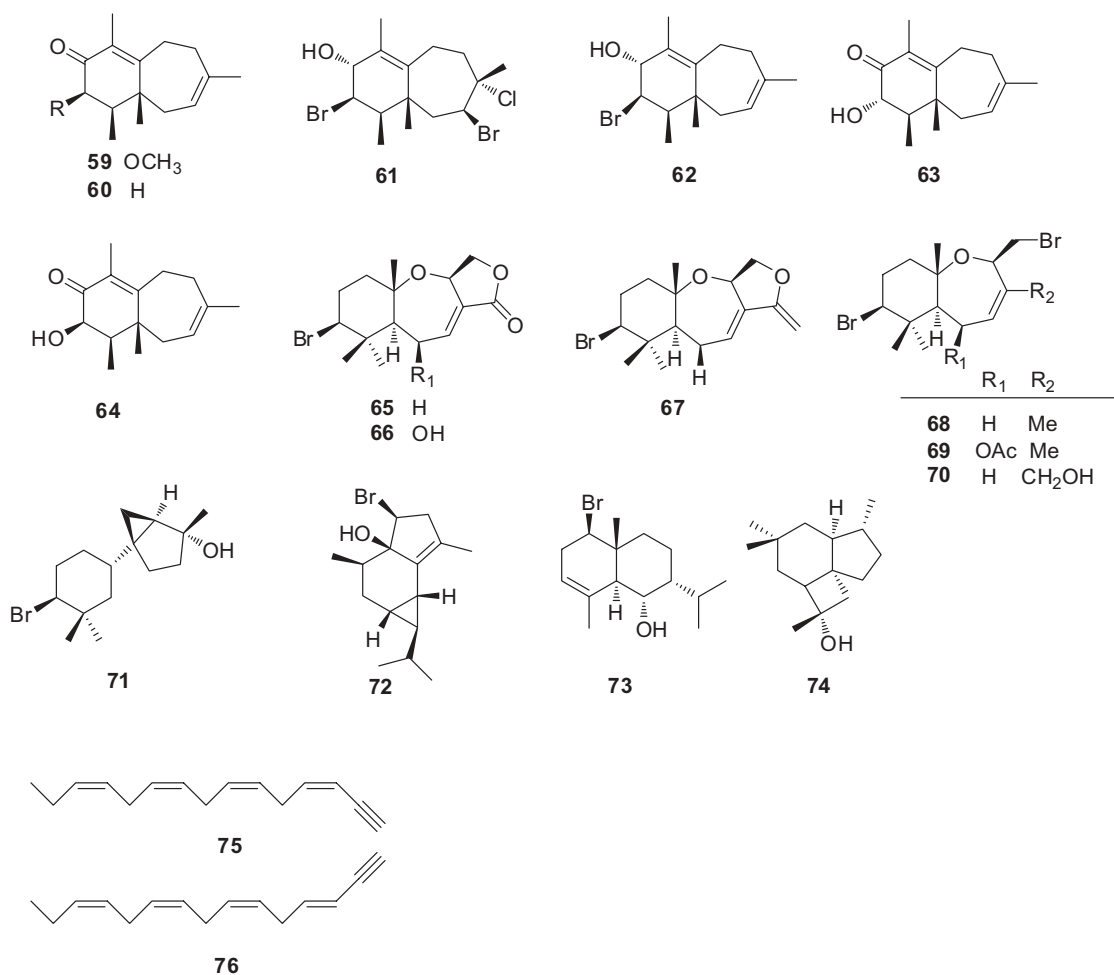
*Other skeletons*

In addition to the skeletons mentioned above, there are some other types of sesquiterpenes reported from species

of red algae. Six perforane-type sesquiterpenes **59–61** (Iliopoulou *et al.*, 2002a), **62** (Kladi *et al.*, 2006), **63–64** (Kladi *et al.*, 2006), were isolated from red algae *Laurencia obtuse* and *L. microcladia*. **65–70** are sesquiterpene ethers isolated from *L. implicate* and *L. karlae* (Wright *et al.*, 1991; Su *et al.*, 1995). A bromo cyclococane-type sesquiterpene **71**, containing fused cyclopropane–cyclopentane rings, was

obtained from the red alga *L. obtuse* (de Carvalho *et al.*, 2003). Calenzanol **72**, the major metabolite of the red seaweed *L. microcladia*, established a new class of sesquiterpenes with a novel skeleton, calenzanane (Guella *et al.*, 2001). A rearranged sesquiterpene **73** (Norte *et al.*, 1994) from *L. viridis* possesses an uncommon fused 4,5,6 tricyclic carbon skeleton. Furthermore, **74** is a halogenated selinane-

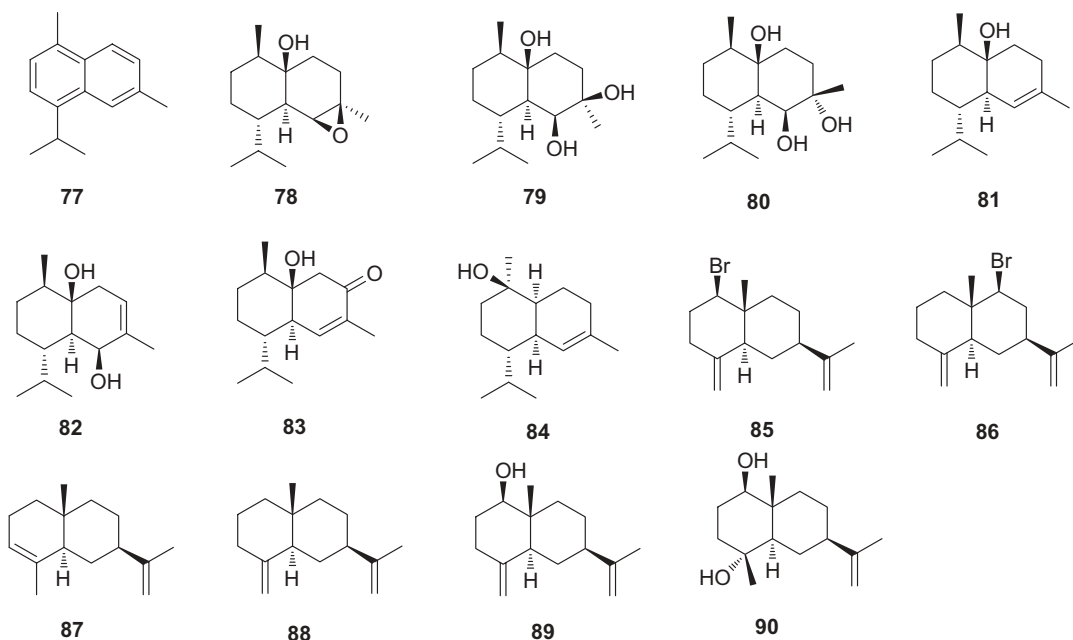
type bromos sesquiterpenoid sesquiterpene isolated from the red alga *L. intricate* (Suzuki *et al.*, 2002). And a linear laurencenyne **75** and a *trans*-laurencenyne **76** were reported from Chinese marine red alga *L. composita* (Ji *et al.*, 2008).



### Sesquiterpenes from brown algae

Selinane and cadinane are the two main types of sesquiterpenes reported from brown algae. **77–84** are eight cadinane sesquiterpenes isolated from brown alga *Dictyopteris*

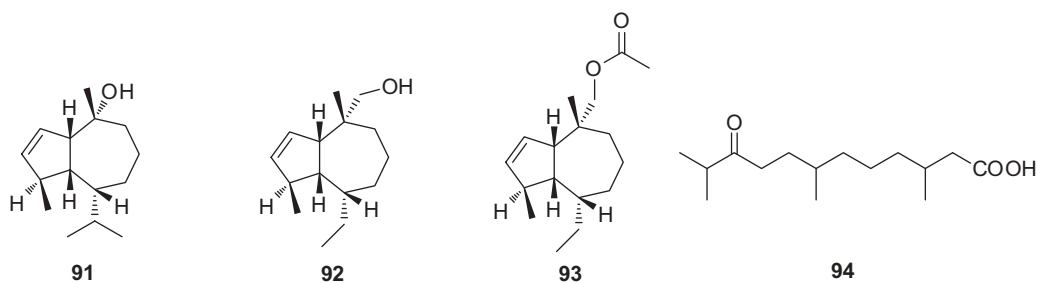
*divaricata*, among which compound **78** is a novel epoxy-cadinene (Qiao *et al.*, 2009). From this same species, six selinane sesquiterpenes **85–90** were obtained (Ji *et al.*, 2009).



### Sesquiterpenes from green algae

Three guaiane sesquiterpene derivatives **91–93** were obtained from seaweed *Ulva fasciata* (Chakraborty *et al.*, 2010a). And a linear sesquiterpene **94** was reported from *Caulerpa racemosa* (Anjaneyulu *et al.*, 1991).

(Gross and Koenig, 2006). Among all marine algal genera, the brown alga *Dictyota* sp. are the most significant producers of different structural classes of diterpenoids. They are one of the most abundant seaweeds in tropical marine habitats represented by more than 40 species. Many members of



### 12.2.2 Diterpenes

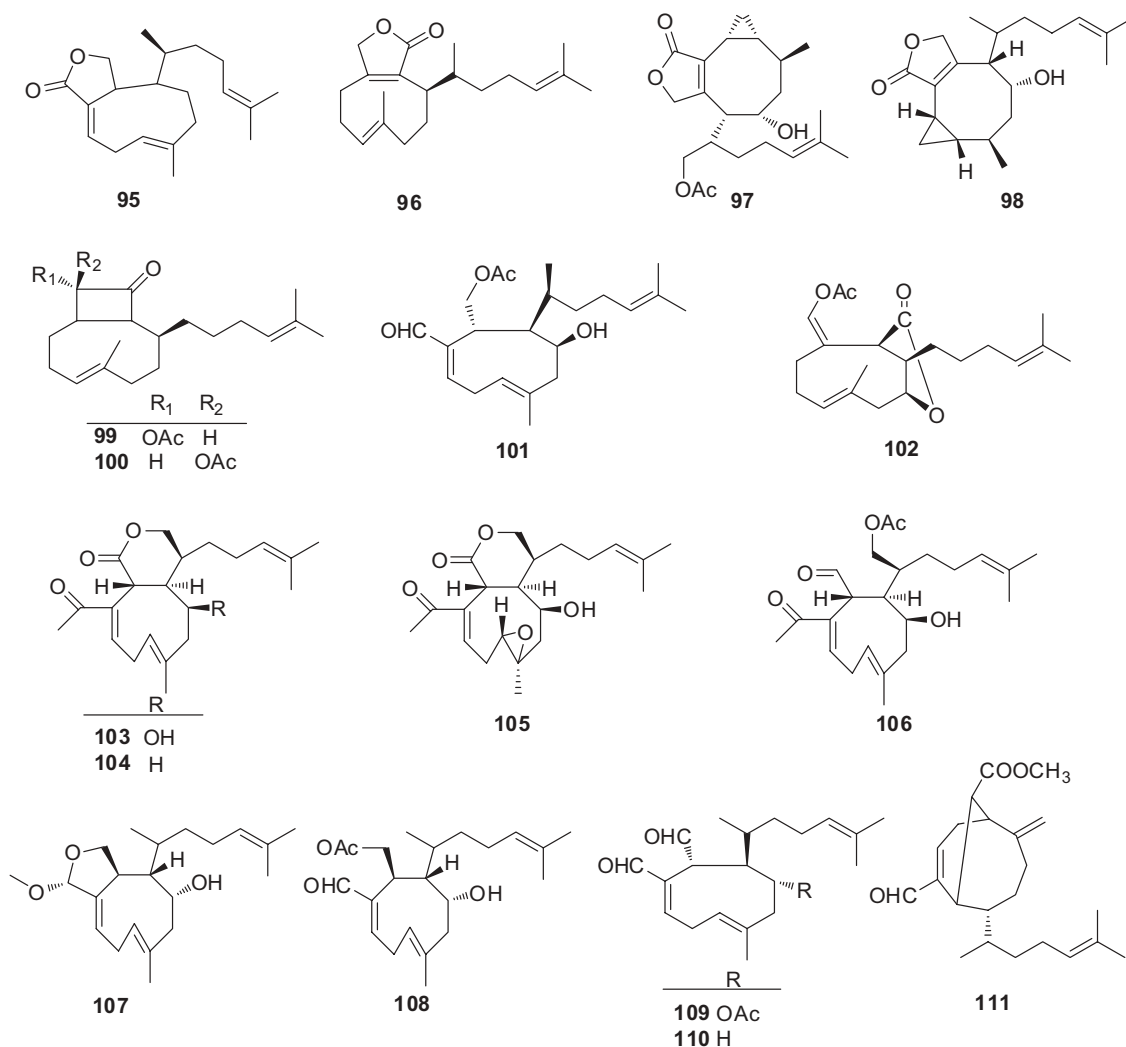
Diterpenoids are a large group of natural compounds with a backbone of 20 carbon atoms derived from geranylgeranyl pyrophosphate. They are found in higher plants, fungi, insects, and marine organisms. Many of the diterpenoids are known to be antimicrobial and anti-inflammatory

this genus produce cyclic diterpenes, unique in the structural variety of marine natural products though, the presence of a 6-methyl-5-hepten-2-yl side chain A, is very typical. Generally, diterpenes reported from seaweeds have three predominant types of carbon skeletons: xenicenes, dolabelenes (including dolastenes), and “extended sesquiterpenes”.

**Diterpenes from brown algae***Xenicane skeleton*

Compounds **95** (Kim *et al.*, 2006), **96** (Siamopoulou *et al.*, 2004), **97** (Konig *et al.*, 1991), **98** (Viano *et al.*, 2009) are xenicene lactones isolated from different species of *Dictyota*. **95** showed high (95%) algicidal activity against *H. akashiwo* and *K. mikimotoi* at a dose of 10–20  $\mu\text{g/ml}$  and moderate activity ( $41.5 \pm 8.2\%$  at 10  $\mu\text{g/ml}$ ) against dinoflagellate *Alexandrium catenella* (Kim *et al.*, 2006). **99–102** are four xenicane-type diterpenes isolated from the brown alga *Dilopbus ligulatus*, among which **102** is a novel bicyclic diterpene derivative. The cytotoxicity tests of these compounds against a series of mammalian cells showed that **101–102**

have significant activity ( $\text{ED}_{50} < 4 \mu\text{g/ml}$ ) against KB (human nasopharynx carcinoma) cells and P-388 (murine leukemia) cells. Meanwhile, compound **102** showed activity ( $\text{ED}_{50} = 3.30 \mu\text{g/ml}$ ) against NSCLC-N6 (human lung carcinoma) cells (Bouaicha *et al.*, 1993). Diterpenes of the xenicane class **103–106** were isolated from *Dictyota divaricata*. Of these compounds, **103–105** are three xeniolide derivatives with the carboxylic function at C-18 of the xenicane ring system (Konig *et al.*, 1991). Moreover, compounds **107** (Viano *et al.*, 2009), **108** (Viano *et al.*, 2009), **109** (Siamopoulou *et al.*, 2004), **110** (Siamopoulou *et al.*, 2004), **111** (Siamopoulou *et al.*, 2004) are xenicane diterpenoids isolated from different species of brown algae.

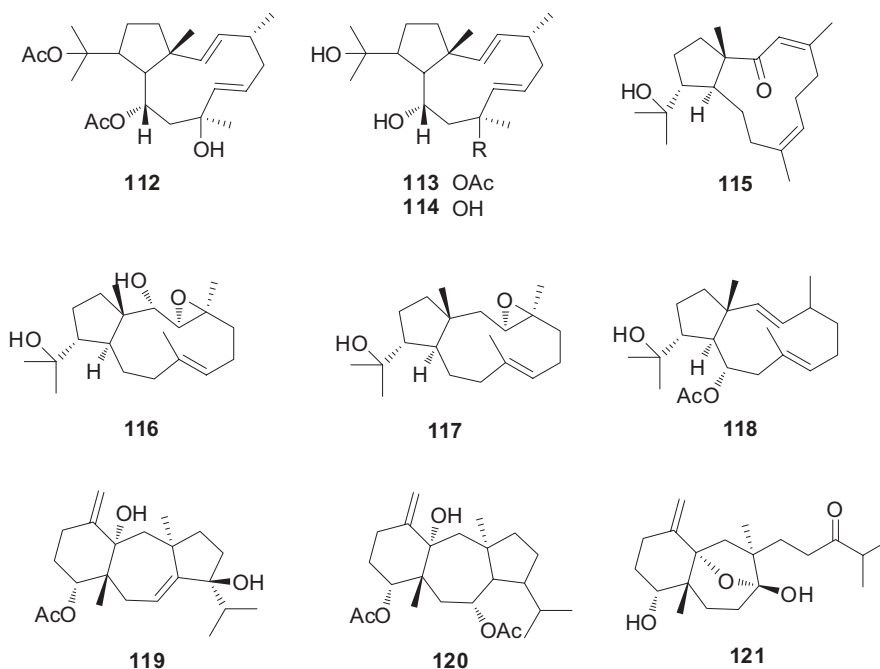


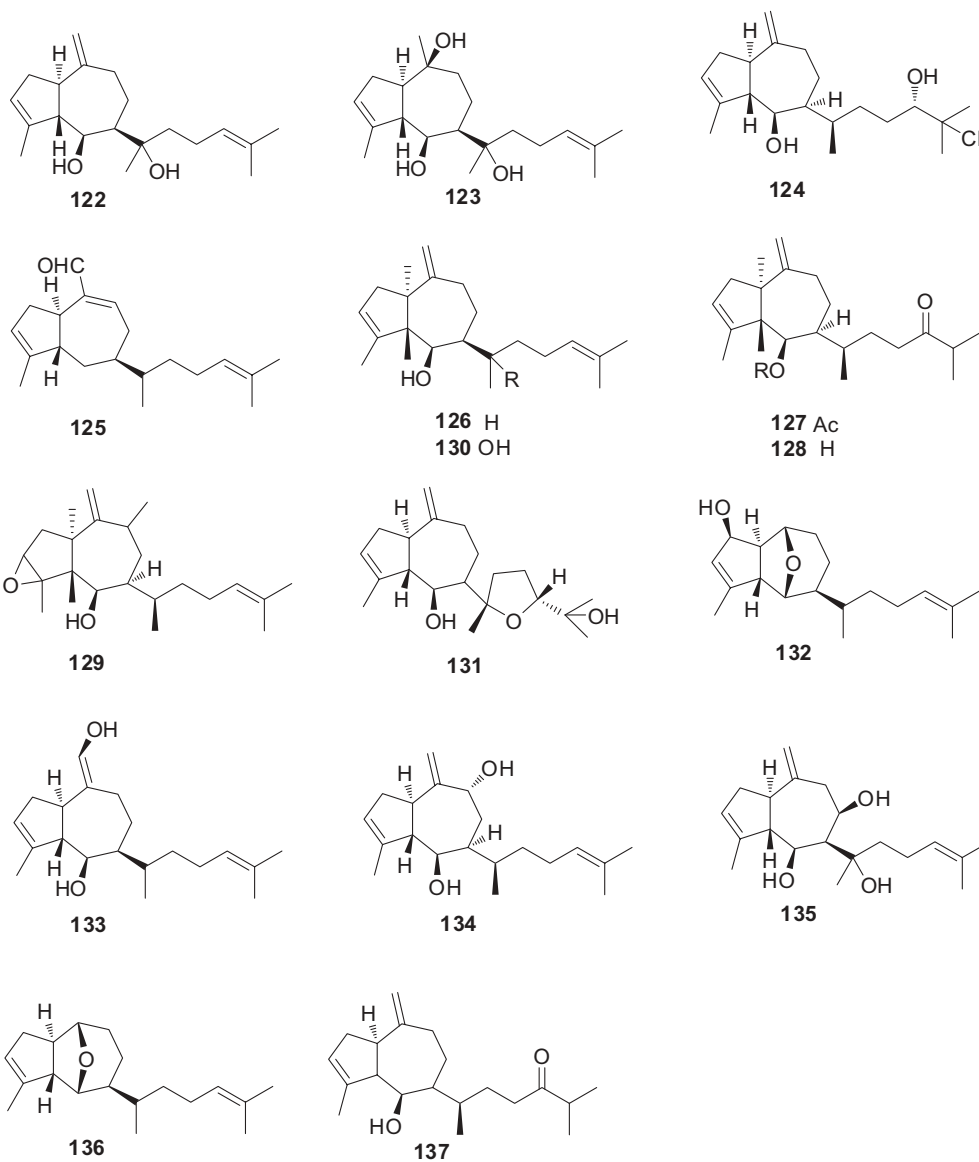
*Dolabellane and dolastane skeletons*

Diterpenes possessing a dolabellane skeleton are also frequently encountered in seaweeds of the *Dictyota* genus. Dolabellane diterpenes **112–114** (Barbosa *et al.*, 2003, 2004) were isolated from brown alga *Dictyota pfaffii*, **112** exhibited significant feeding deterrent properties against the herbivorous sea urchin *Lytechinus variegatus* as well as a strong *in vitro* anti-herpes simplex virus-1 activity (Barbosa *et al.*, 2007). **115–118** are four novel dolabellane diterpenoids from brown alga *Dictyota* sp. (Viano *et al.*, 2009). Dolastane diterpenes are tricyclic compounds, which are structurally similar to dolabellane dolabellanes. They may be biosynthetically derived from the dolabellanes. Bioassay-guided fraction of *Canistrocarpus cervicornis* resulted in the isolation of two dolastanes **119–120** and a seco-dolastane **121** diterpene (Bianco *et al.*, 2009).

*Extended sesquiterpenes (hydroazulenoids)*

Diterpenes based on perhydroazulane skeleton are a class of compounds combining guaiane sesquiterpene with an isoprene group. Thus, they are also named as extended sesquiterpenes. The algae of the family Dictyotaceae, within the order Dictyotales, have afforded a wide range of this type of diterpenes. Compounds **122–123** (Viano *et al.*, 2009), **124** (Kim *et al.*, 2006), **125** (Siamopoulou *et al.*, 2004), **126–130** (Ayyad *et al.*, 2003a), **131–133** (Konig *et al.*, 1991) are perhydroazulene diterpenes isolated from different species of genus *Dictyota*, and compounds **134–136** (De Nys *et al.*, 1993, Arroyo *et al.*, 1991) were isolated from the brown alga *Glossophora kuntii*. **130** exhibited moderate cytotoxic activity on the cancer cell line KA3IT ( $IC_{50} = 10 \mu\text{g/ml}$ ) and showed reduced cytotoxicity towards the normal cells NIH3T3 (Ayyad *et al.*, 2003a). Moreover, from brown alga *Sargassum asperifolium*, a diterpene **137** with a hydroazulene skeleton was obtained (Ayyad *et al.*, 2003b).

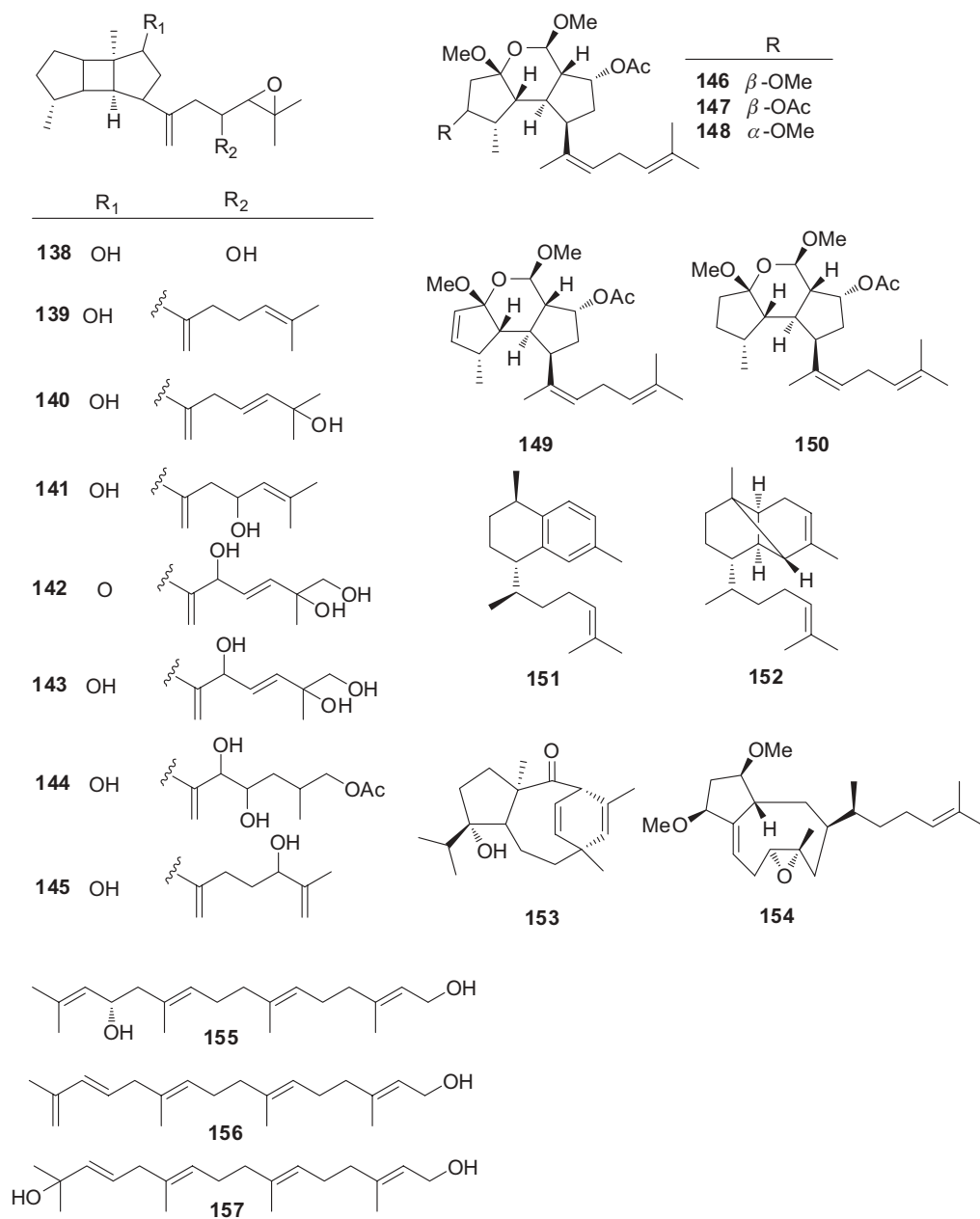




### 12.2.3 Other skeletons

Apart from the above structural skeletons, eight epoxy spatane derivatives **138–144** were isolated from *Stoechospermum marginatum* (Venkateswarlu and Biabani, 1995). **146–150** are five secospatane diterpenoids obtained from *Dilophus okamurai* Dawson (Yamase *et al.*, 1999). Two tricyclic diterpenoids **151–152**, possessing a serrulatane skeleton system, were isolated from the Far-eastern brown alga *Dictyota dichotoma* (Kolesnikova *et al.*, 2006). A new class

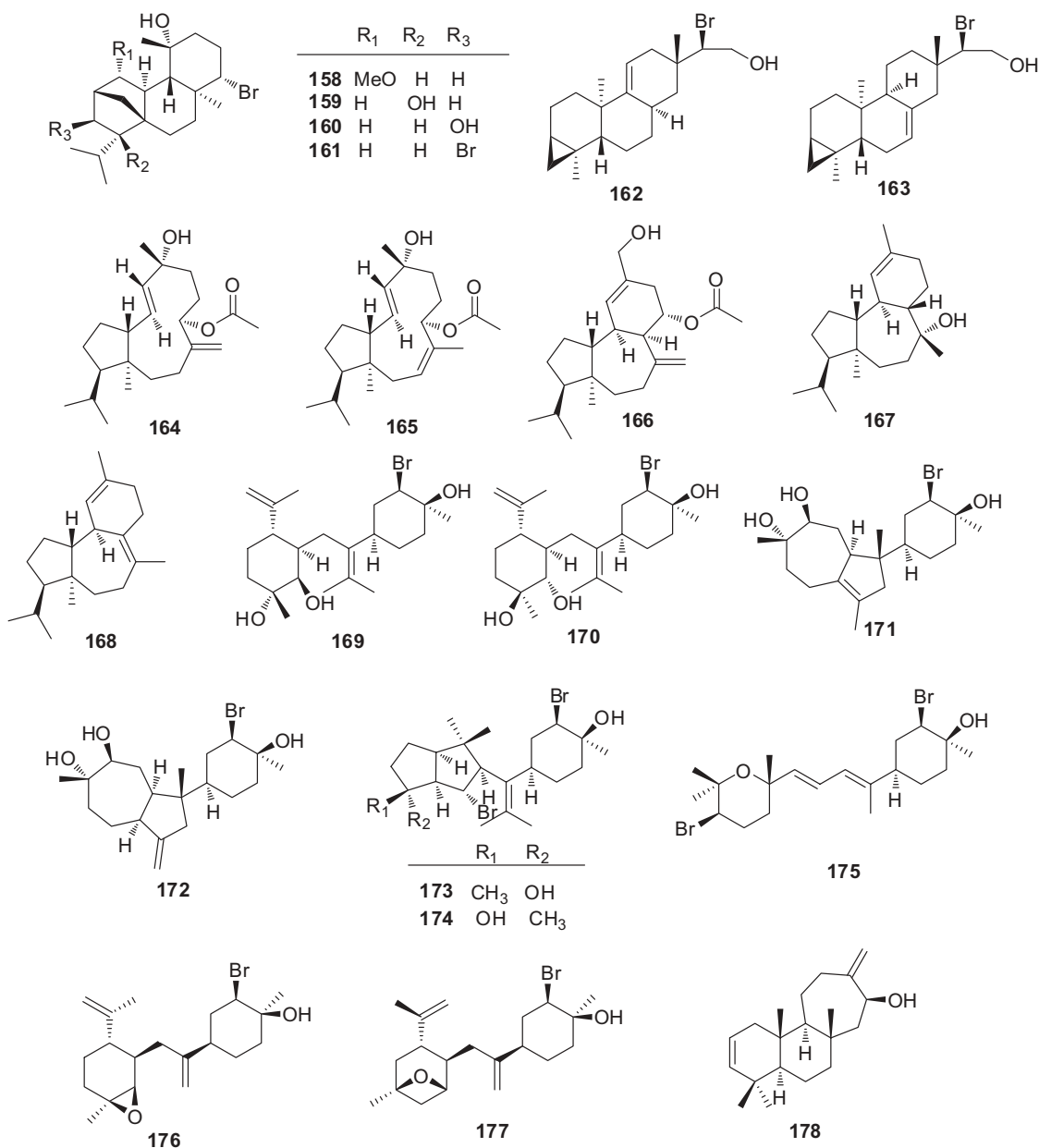
of diterpene **153**, having a novel tricarboxylic skeleton, was isolated Indian Ocean specimen of *D. divaricata* (Trimurtulu *et al.*, 1992). **154** is a diterpene epoxide from brown alga of the genus *Dictyota*, it demonstrated significant vasopressin receptor antagonist activity *in vitro* (Patil *et al.*, 1993). In addition, three linear diterpenes **155–157** were reported from the brown alga *Bijiacaria bijiacata*, and they revealed a potent cytotoxicity to fertilized sea urchin eggs *Paracentrotus lividus* with an ED<sub>50</sub> of 4 µg/ml (Valls *et al.*, 1993).



### Diterpenes from red algae

In all species of red algae, *Sphaerococcus coronopifolius* is an unusual prolific source of interesting diterpenes having di-, tri-, or tetracyclic skeletons, often rearranged, most of which contain one or more Br-atoms. Compounds **158–161** are four new tetracyclic brominated diterpenes isolated from *Sphaerococcus coronopifolius*. Their antibacterial activity against a panel of *Staphylococcus aureus* strains were evaluated. The MICs of them were found to be in

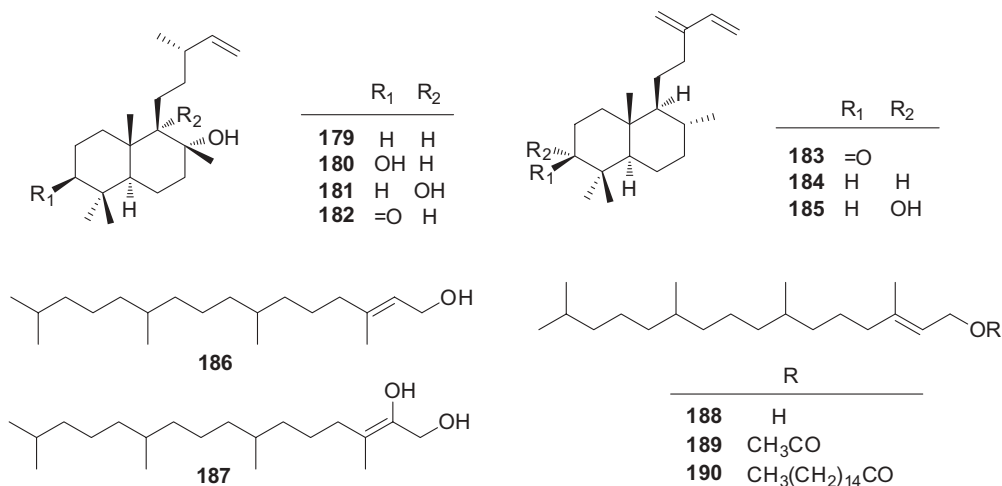
the range of 16–128 µg/ml. The activities exhibited were 4 to 16 times stronger than the standard antibiotic, norfloxacin, against EMRSA-16. Compounds possessing additional OH groups, like **159** and **160**, showed higher activity than compounds bearing an additional bromine (i.e., **161**), or a MeO group (i.e., **158**; Smyrniotopoulos *et al.*, 2010). From the red alga *Laurencia nipponica*, two pargueranes **162**, **163** were obtained (Lyakhova *et al.*, 2004). Two diterpenes with neodolabellane carbon skeletons **164**, **165**, and three sphaeroane diterpenes **166–168** were



isolated from *Sphaerococcus coronopifolius* (Smyrniotopoulos *et al.*, 2009). Nine brominated diterpenes **169–177** were isolated from the organic extract of the red alga *Laurencia obtuse*. The cytotoxic activity test against five human cell lines showed that compounds **170** and **174** were potent as cytotoxic agents for the majority of the cell lines tested and demonstrated an inhibitory activity at doses lower than 100  $\mu$ M (Iliopoulou *et al.*, 2003, Mihopoulos *et al.*, 2001). Additionally, a novel labdane-derived diterpenoid **178** was isolated from *L. karlae* (Su *et al.*, 1995).

### Diterpenes from green algae

Seven labdane diterpenoids **179–185** were isolated from green alga *Ulva fasciata* as major constituents. Antimicrobial assay showed that compounds **180** and **182** were inhibitory to the growth of *Vibrio parahaemolyticus* (MICs 30 and 40  $\mu$ g/ml, respectively) and *V. alginolyticus* (MICs 30 and 80  $\mu$ g/ml, respectively) (Chakraborty *et al.*, 2010b). Moreover, five linear diterpenoids **186–190** were isolated from green algae *Caulerpa* spp. and *Codium fragile* (Aliya and Shameel, 2003; Yin *et al.*, 2005).

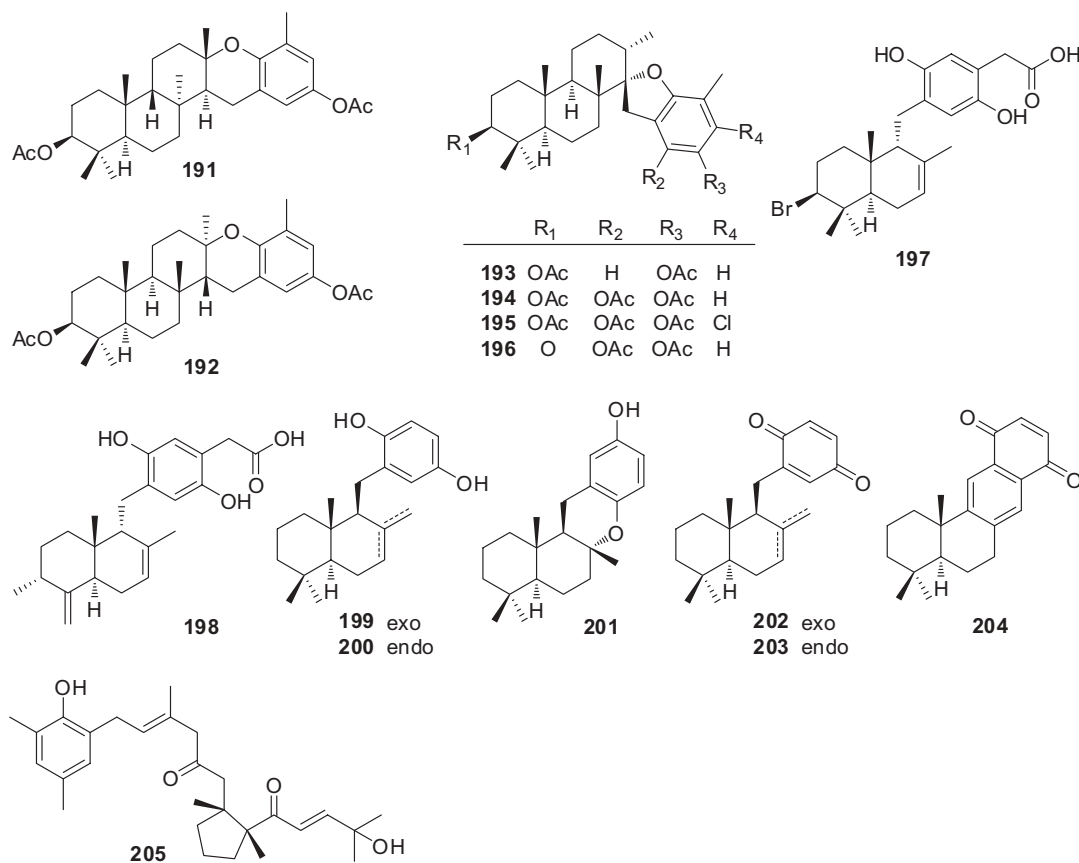


### 12.2.4 Meroterpenoids

Meroterpenoids, also called prenylated aromatic compounds, are of mixed biogenesis containing terpenoid and non-terpenoid derived fragments. They are natural products combining acyclic, monocyclic, bicyclic terpenes with aromatic or substituted aromatic groups possessing different degrees of oxidation. Many marine meroterpenoids have interesting biological activities such as antibacterial, antiviral and antifeeding activities.

Polycyclic meroditerpenoids **191–196** were isolated from brown alga *Styopodium flabelliforme*, among which compound **195** is an unusual halogenated meroditerpenoid (Areche *et al.*, 2009). Bioassay-guided fractionation of *Peyssonnelia* sp. yielded two novel antimicrobial

sesquiterpene hydroquinones **197–198** (Lane *et al.*, 2010). Five sesquiterpene-substituted benzoquinone derivatives **199–204** were obtained from brown alga *Dictyopteris undulate*. They exhibited potent feeding-deterrent activity (electivity index (EI) were 0.85, 0.78, 0.80, 0.92, 0.85, and 0.93, respectively) against the young abalone *Haliotis discus hananai* at a concentration of 75  $\mu$ g of each sample (Kurata *et al.*, 1996). Additionally, A meroditerpenoid **205** possessing antifungal and antibacterial activity was isolated from the brown alga *Cystoseira tamariscifolia*. It could inhibit the growth of three tomato pathogenic fungi: *Botrytis cinerea*, *Fusarium oxysporum*, *mycopersici* sp. and *Verticillium albo-atrum*, and the inhibitory activities against *Agrobacterium tumefaciens* and *Escherichia coli* were comparable to tetracycline (Bennamara *et al.*, 1999).

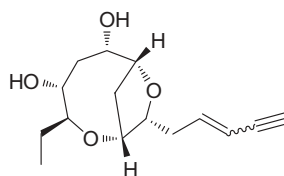
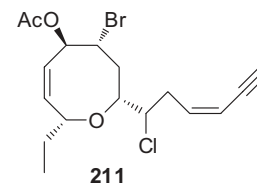
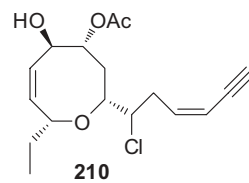
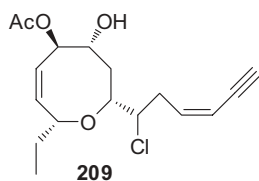
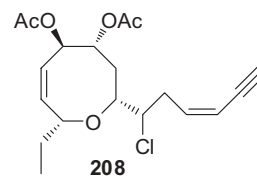
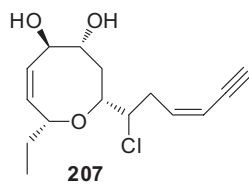
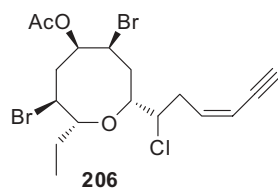


### 12.2.5 C<sub>15</sub>-acetogenins

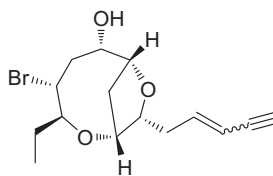
Acetogenins are a class of biosynthetic compounds formed from ethyl acetate or acetylcoenzyme A. From seaweeds, a series of halogenated C<sub>15</sub>-acetogenins, containing oxygen heterocycles, acetylenes and allenes were obtained.

Compounds **206–211** are C<sub>15</sub> eight-membered cyclic ethers with a characteristic terminal cis ene-yne moiety. They exhibited significant antistaphylococcal activity with MICs in the range of 8–256 µg/ml. Among them, compound **208** was found to be the most active (MIC = 8–16 µg/ml) and the presence of the two acetyl groups in **208** may play a key role by improving its cellular bioavailability by being more lipophilic (Kladi *et al.*, 2008). Three new diastereomeric pairs of cyclic ether acetogenins **212–217** were isolated from the aqueous extract of the red alga *Laurencia* sp. Cytotoxic tests showed that **215** exhibited moderate non-selective activity against three solid tumors (murine colon 38, human colon H116 and human lung H125), leukemia L1210 and human normal cells CFU-GM. However, its isomer **214**, exhibited very weak activity against murine colon 38 only. Similarly, compound **212** showed moderate non-selective activity against leukemia as well as

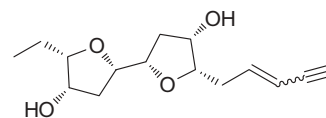
solid tumors, but its isomer, **215**, showed no significant activity against murine colon 38 (Abdel-Mageed *et al.*, 2010). Compounds **218–223** are halogenated C<sub>15</sub>-acetogenins with a terminal bromoallene moiety isolated from the red alga *Laurencia intricate*, *L. implicate*, and *L. majusrnka* (Suzuki *et al.*, 2002; Wright *et al.*, 1991; Wright *et al.*, 1993). **218** possesses a novel 2,10-dioxabicyclo [7.3.0] dodecene skeleton. Also from red alga *L. majusrnka*, three C<sub>15</sub>-acetogenins **224–226**, possessing the rare 2,5-dioxabicyclo [2.2.1] heptane ring system, were obtained (Wright *et al.*, 1993). Prominent antibacterial activity was seen in compound **227** isolated from *L. mariannensis*. It showed activity against *Alcaligenes aqua-marinus*, *Azomonas agilis*, *Erwinia amylovora*, and *Escherichia coli*. Their MIC values are in the range of 20–30 µg/disk (Vairappan *et al.*, 2001b). Two halogenated C<sub>15</sub> acetogenins **228–229** were reported from the Malaysian *Laurencia* sp., compound **228** showed weaker antibacterial activity with MIC values in the range of 20–60 µg/disk against 13 species of marine bacteria (Vairappan *et al.*, 2001a). Moreover, compounds **230–234** (Ji *et al.*, 2007; Wright *et al.*, 1991; Suzuki *et al.*, 1999) are halogenated C<sub>15</sub>-acetogenins reported from red algae of genus *Laurencia*.



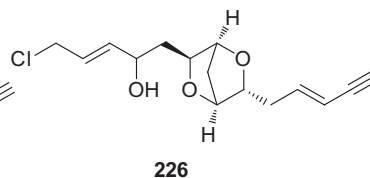
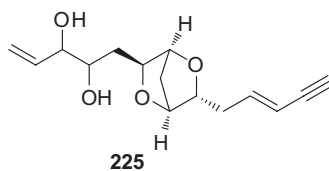
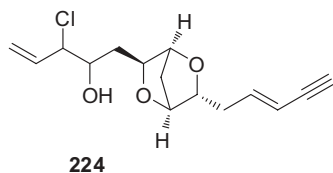
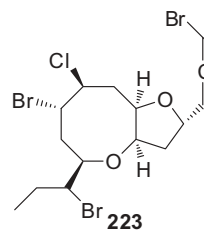
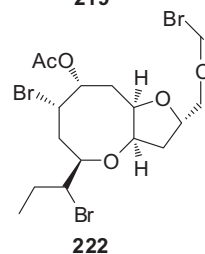
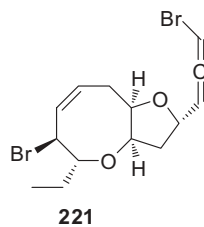
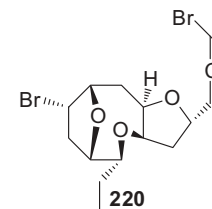
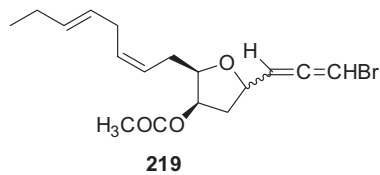
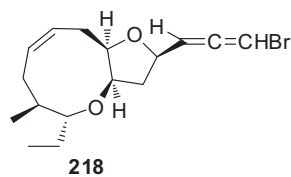
**213** 3,4 trans

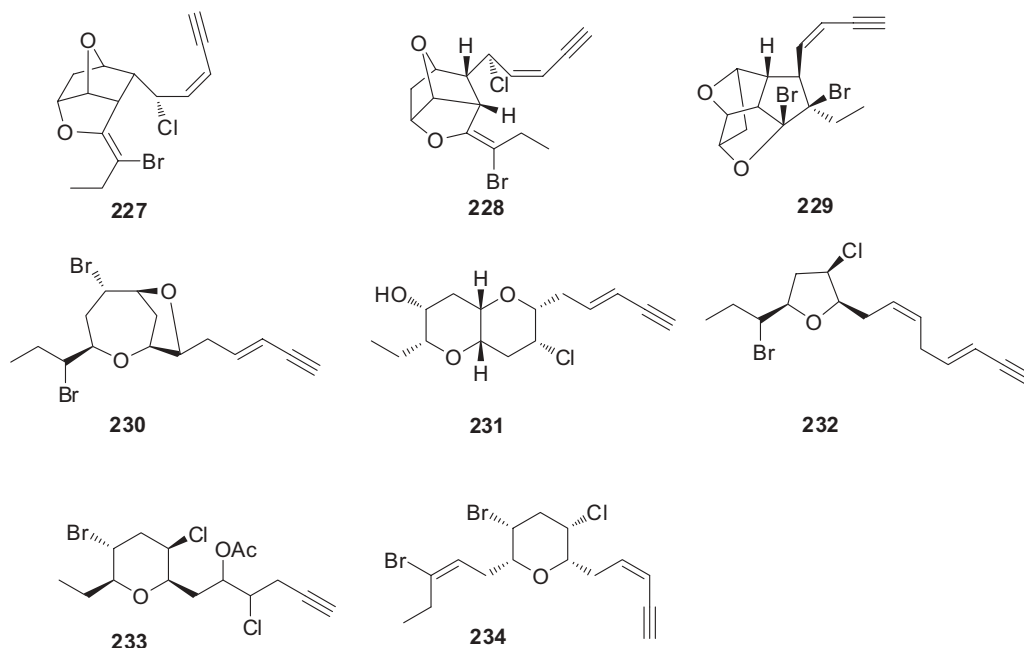


**215** 3,4 trans



**217** 3,4 trans



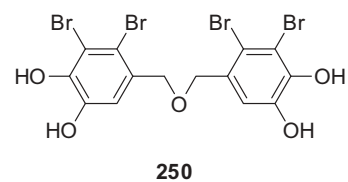
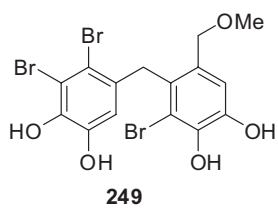
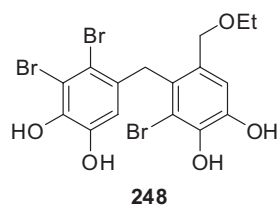
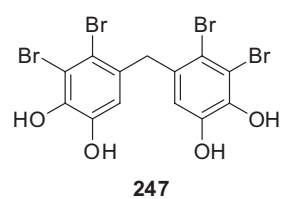
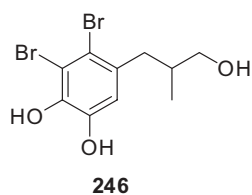
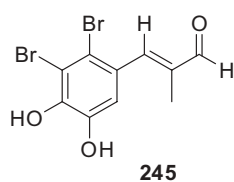
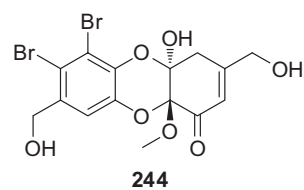
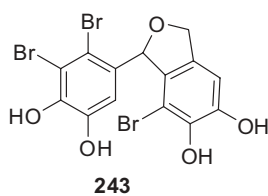
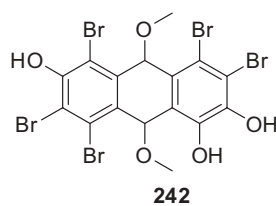
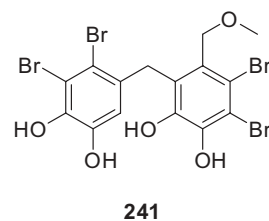
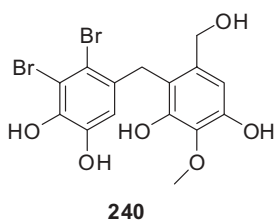
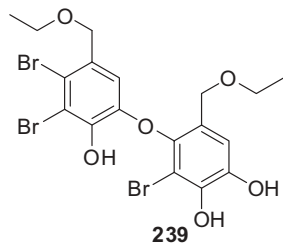
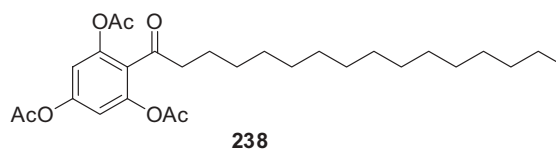
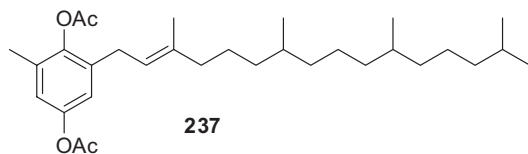
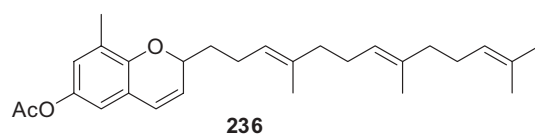
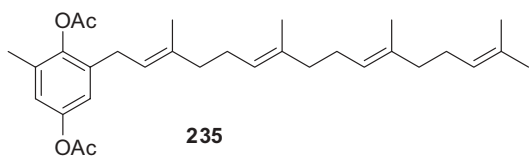


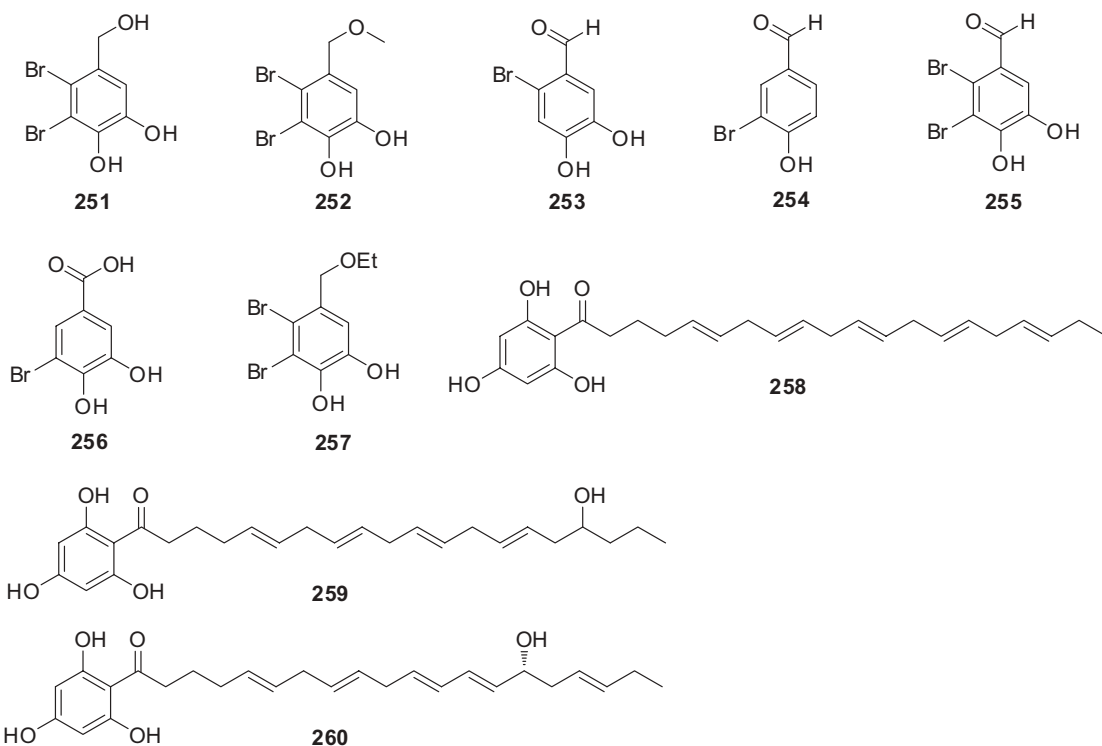
### 12.2.6 Phlorotannins

Phlorotannins are common secondary metabolites from marine brown algae based on units of phloroglucinol. Most of them could act as efficient antioxidants and feeding deterrents. They could inhibit consumption or diminish absorption efficiency of some herbivores through combining with the protein.

Tetraprenylhydroquinones **235–236**, one chromene **237** and one polyketide **238**, were isolated from *Stypopodium flabelliforme* (Areche *et al.*, 2009). The ethanolic extract of the brown alga *Leathesia nana* afforded six novel dibenzyl bromophenols **239–244** with different dimerization patterns and two propyl bromophenol derivatives **245** and **246**, together with eleven bromophenol derivatives

**247–257**. *In vitro* biological screening indicated that **241** and **247–250** were cytotoxic against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), breast cancer (MCF-7), hepatoma (Bel7402), and human colon cancer (HCT-8) cell lines (Xu *et al.*, 2004b). Compounds **258–260** are three phloroglucinols with a C-20 acyl side chain isolated from brown alga *Zonaria diesingiana*. All three compounds were toxic to brine shrimp, a rice-land shrimp and a guppy and showed cytotoxicity activity by inhibiting cell division of fertilized sea urchin eggs. In addition, **260** showed potent activity against *B. subtilis* and *S. aureus*, suggesting its potential for development as an antibiotic (Wispongpan and Kuniyoshi, 2003).

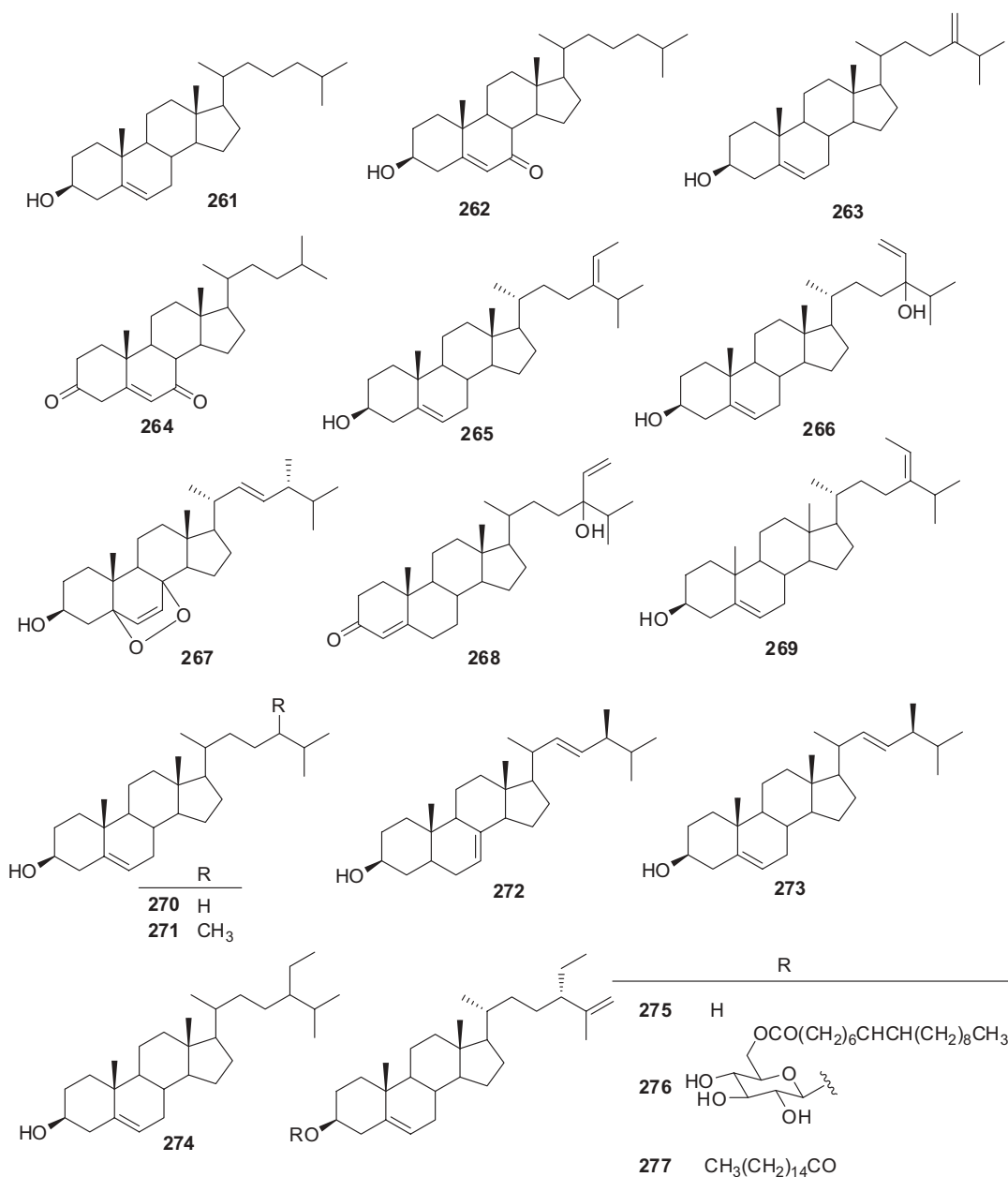




### 12.2.7 Steroids

Steroids, a class of functionally important triterpenoid lipids, are typical metabolites occurring in marine algae. Red algae predominantly make  $C_{27}$  steroids and produce  $C_{28}$  in large quantities while  $C_{29}$  in small quantities. The green algae are more variable. They show a strong clustering in the region of  $C_{29}$  (Kodner *et al.*, 2008). Two  $C_{27}$  steroids **261**, **262**, along with a  $C_{28}$  steroid **263** and a rare  $C_{26}$  steroid **264** were isolated from red alga *Melanothamnus so-*

*malensis* (Ahmad *et al.*, 1996). Four steroids **265**–**268** were isolated from genus *Sargassum* of brown alga (Liu *et al.*, 2009; Ayyad *et al.*, 2003b). From the brown alga *Dictyota dichotoma*, **269** was isolated. It displayed potent cytotoxic activity against mouse P388 leukemia cells with  $IC_{50}$  of 0.6  $\mu\text{g/ml}$  (Ayyad *et al.*, 2003a). Five steroids **270**–**274** were isolated from green algae *Caulerpa* spp. (Aliya and Shameel, 2003). Clerosterol **275**, its acylglycosyl derivative **276**, and its palmityl ester **277** were isolated from green alga *Codium fragile* (Yin *et al.*, 2005).



## 12.3 Conclusions

It is well known that marine macroalgae are at the bottom of food chain in marine ecosystem. They possess invaluable potential economic impact in food science, pharmaceutical industry and public health. Due to their special defense strategies, a tremendous diversity of compounds with high complexity and unlimited diversity of pharmacological and/or biological properties have been reported. Many of them are unlike those found in terrestrial species. How-

ever, phytochemical investigations and biological studies of seaweeds have tended so far to concentrate on only a few genera. For red algae, emphasis has been placed on members of families Ceramiaceae and Rhodomelaceae. Their metabolites are characterized by relatively high degree of halogenations. Dictyotales and Fucales are the most thoroughly investigated orders of the brown algae. On the contrary to those from red algae, compounds derived from brown algae are in general not halogenated. This interesting phenomenon may lead to further research on biosynthetic

pathways for organohalogen production and their chemotaxonomic significance. And with the improved bioassay techniques allowing the screening of the crude materials and the subsequent activity-guided fraction for the active principles, future work should therefore aim to explore the chemistry and associated biological activities of lesser known species.

## References

- Abdel-Mageed, W.M., Ebel, R., Valeriote, F.A. and Jaspars, M. (2010) Laurefurenynes A-F, new cyclic ether acetogenins from a marine red alga *Laurencia* sp. *Tetrahedron*, **66**, 2855–2862.
- Ahmad, V.U., Memon, A.H., Ali, M.S., Perveen, S. and Shameel, M. 1996. Somalenone, a C-26 sterol from the marine red alga *Melanothamnus somalensis*. *Phytochemistry (Oxford)*, **42**, 1141–1143.
- Aliya, R. and Shameel, M. (2003) Marine natural products of *Caulerpa* (Siphonocladophyceae). *Pakistan J.Bot.*, **35**, 659–669.
- Anjaneyulu, A.S.R., Prakash, C.V.S. and Mallavadhani, U.V. 1991. Two caulerpin analogues and a sesquiterpene from *Caulerpa racemosa*. *Phytochemistry (Oxford)*, **30**, 3041–3042.
- Areche, C., San-Martin, A., Roviroso, J., Soto-Delgado, J. and Contreras, R. 2009. An unusual halogenated meroditerpenoid from *Stypopodium flabelliforme*: Studies by NMR spectroscopic and computational methods. *Phytochemistry (Amsterdam)*, **70**, 1315–1320.
- Arroyo, P., Norte, M., Vazquez, J.T. and Nakanishi, K. 1991. Absolute configuration of hydroazulenoid diterpenes based on circular dichroism. *J. Org. Chem.*, **56**, 2671–2675.
- Ayyad, S.-E. N., Abdel-Halim, O.B., Shier, W.T. and Hoye, T.R. (2003a) Cytotoxic hydroazulene diterpenes from the brown alga *Cystoseira myrica*. *Zeitschrift fuer Naturforschung Section C Journal of Biosciences*, **58**, 33–38.
- Ayyad, S.E.N., Sowellim, S.Z.A., El-Hosini, M.S. and Abo-Atia, A. (2003b) The structural determination of a new steroidal metabolite from the brown alga *Sargassum asperifolium*. *Zeitschrift Fur Naturforschung C-a Journal of Biosciences*, **58**, 333–336.
- Barbosa, J.P., Fleury, B.G., Da Gama, B.A.P., Teixeira, V.L. and Pereira, R.C. (2007) Natural products as antifoulants in the Brazilian brown alga *Dictyota pfaffii* (Phaeophyta, Dictyotales). *Biochem. Syst. Ecol.*, **35**, 549–553.
- Barbosa, J.P., Pereira, R.C., Abrantes, J.L., et al. (2004) In vitro antiviral diterpenes from the Brazilian brown alga *Dictyota pfaffii*. *Planta Med.*, **70**, 856–860.
- Barbosa, J.P., Teixeira, V.L., Villaca, R., Pereira, R.C., Abrantes, J.L. and Palmer Da Paixao Frugulhetti, I.C. (2003) A dolabellane diterpene from the Brazilian brown alga *Dictyota pfaffii*. *Biochemical Systematics and Ecology*, **31**, 1451–1453.
- Bennamara, A., Abourriche, A., Berrada, M., et al. (1999) Methoxybifurcarenone: an antifungal and antibacterial meroditerpenoid from the brown alga *Cystoseira tamariscifolia*. *Phytochemistry*, **52**, 37–40.
- Bianco, E.M., Rogers, R., Teixeira, V.L. and Pereira, R.C. (2009) Antifoulant diterpenes produced by the brown seaweed *Canistrocarpus cervicornis*. *Journal of Applied Phycology*, **21**, 341–346.
- Bouaicha, N., Pesando, D., Puel, D. and Tringali, C. (1993) Cytotoxic diterpenoids from the brown alga *Dilophus ligulatus*. *J. Nat. Prod. (Lloydia)*, **56**, 1747–1752.
- Brito, I., Cueto, M., Dorta, E. and Darias, J. (2002) Bromocyclococanol, a halogenated sesquiterpene with a novel carbon skeleton from the red alga *Laurencia obtusa*. *Tetrahedron Lett.*, **43**, 2551–2553.
- Cardozo, K.H.M., Guaratini, T., Barros, M.P., et al. (2007) Metabolites from algae with economical impact. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.*, **146**, 60–78.
- Cassano, V., De-Paula, J.C., Fujii, M.T., Perez Da Gama, B.A. and Teixeira, V.L. (2008) Sesquiterpenes from the introduced red seaweed *Laurencia caduciramulosa* (Rhodomelaceae, Ceramiales). *Biochem. Syst. Ecol.*, **36**, 223–226.
- Chakraborty, K., Lipton, A.P., Paulraj, R. and Chakraborty, R.D. (2010a) Guaiane sesquiterpenes from seaweed *Ulva fasciata* Delile and their antibacterial properties. *Eur. J. Med. Chem.*, **45**, 2237–2244.
- Chakraborty, K., Lipton, A.P., Raj, R.P. and Vijayan, K.K. (2010b) Antibacterial labdane diterpenoids of *Ulva fasciata* Delile from southwestern coast of the Indian Peninsula. *Food Chem.*, **119**, 1399–1408.
- Chatter, R., Kladi, M., Tarhouni, S., et al. (2009) Neorgioltriol: A brominated diterpene with analgesic activity from *Laurencia glandulifera*. *Phytochem. Lett.*, **2**, 25–28.
- De Carvalho, L.R., Fujii, M.T., Roque, N.F., Kato, M. J. and Lago, J.H.G. (2003) Aldingenin A, new brominated sesquiterpene from red algae *Laurencia aldingensis*. *Tetrahedron Lett.*, **44**, 2637–2640.
- De Carvalho, L.R., Fujii, M.T., Roque, N.F. and Lago, J. H. G. (2006) Aldingenin derivatives from the red alga *Laurencia aldingensis*. *Phytochemistry (Amsterdam)*, **67**, 1331–1335.
- De Nys, R., Wright, A.D., Konig, G.M. and Sticher, O. (1993) A diterpene from the marine alga *Glossophora kunthii*. *Phytochemistry (Oxford)*, **32**, 463–465.
- Del Val, A.G., Platas, G., Basilio, A., et al. (2001) Screening of antimicrobial activities in red, green and brown

- macroalgae from Gran Canaria (Canary Islands, Spain). *Int. Microbiol.*, **4**, 35–40.
- Fleury, B.G., Pereira, M.V.G., Da Silva, J.R.P., Kaisin, M., Teixeira, V.L. and Kelecom, A. (1994) Sterols from Brazilian marine brown algae. *Phytochemistry (Oxford)*, **37**, 1447–1449.
- Gedara, S.R., Abdel-Halim, O.B., El-Sharkawy, S.H., Salama, O.M., Shier, T.W. and Halim, A.F. (2003) Cytotoxic hydroazulene diterpenes from the brown alga *Dictyota dichotoma*. *Zeitschrift fuer Naturforschung Section C Journal of Biosciences*, **58**, 17–22.
- Goez, C.E., Wright, A.D., Koenig, G.M. and Sticher, O. (1994) Diterpenes from the brown alga *Dilophus mediterraneus*. *Phytochem. Anal.*, **5**, 68–73.
- Gross, H. and Koenig, G.M. 2006. Terpenoids from marine organisms: unique structures and their pharmacological potential. *Phytochemistry Reviews*, **5**, 115–141.
- Guella, G., Oztunc, A., Mancini, I. and Pietra, F. (1997) Stereochemical features of sesquiterpene metabolites as a distinctive trait of red seaweeds in the genus *Laurencia*. *Tetrahedron Lett.*, **38**, 8261–8264.
- Guella, G., Skropeta, D., Breuils, S., Mancini, I. and Pietra, F. (2001) Calenzanol, the first member of a new class of sesquiterpene with a novel skeleton isolated from the red seaweed *Laurencia microcladia* from the Bay of Calenzana, Elba Island. *Tetrahedron Lett.*, **42**, 723–725.
- Iliopoulou, D., Roussis, V., Pannecouque, C., De Clercq, E. and Vagias, C. (2002a) Halogenated sesquiterpenes from the red alga *Laurencia obtusa*. *Tetrahedron*, **58**, 6749–6755.
- Iliopoulou, D., Vagias, C., Galanakis, D., Argyropoulos, D. and Roussis, V. (2002b) Brasilane-type sesquiterpenoids from *Laurencia obtusa*. *Org. Lett.*, **4**, 3263–3266.
- Iliopoulou, D., Mihopoulos, N., Vagias, C., Papazafiri, P. and Roussis, V. (2003) Novel cytotoxic brominated diterpenes from the red alga *Laurencia obtusa*. *J. Org. Chem.*, **68**, 7667–7674.
- Ji, N.Y., Li, X.M., Zhang, Y. and Wang, B.G. (2007) Two new halogenated chamigrane-type sesquiterpenes and other secondary metabolites from the marine red alga *Laurencia okamurai* and their chemotaxonomic significance. *Biochem. Syst. Ecol.*, **35**, 627–630.
- Ji, N.Y., Li, X.M., Li, K., Gloer, J.B. and Wang, B.G. (2008) Halogenated sesquiterpenes and non-halogenated linear C-15-acetogenins from the marine red alga *Laurencia composita* and their chemotaxonomic significance. *Biochem. Syst. Ecol.*, **36**, 938–941.
- Ji, N.Y., Wen, W., Li, X.M., Xue, Q.Z., Xiao, H.L. and Wang, B.G. (2009) Brominated selinane sesquiterpenes from the marine brown alga *Dictyopteris divaricata*. *Marine Drugs*, **7**, 355–360.
- Jormalainen, V. and Ramsay, T. (2009) Resistance of the brown alga *Fucus vesiculosus* to herbivory. *Oikos*, **118**, 713–722.
- Kamenarska, Z., Gasic, M.J., Zlatovic, M., et al. (2002) Chemical composition of the brown alga *Padina pavonia* (L.) Gaill. from the Adriatic Sea. *Bot. Mar.*, **45**, 339–345.
- Kim, J.Y., Alamsjah, M.A., Hamada, A., Fujita, Y. and Ishibashi, F. (2006) Algicidal diterpenes from the brown alga *Dictyota dichotoma*. *Biosci. Biotechnol. Biochem.*, **70**, 2571–2574.
- Kim, S., Ravichandran, Y.D., Khan, S.B. and Kim, Y.T. 2008. Prospective of the Cosmeceuticals Derived from Marine Organisms. *Biotechnology and Bioprocess Engineering*, **13**, 511–523.
- Kladi, M., Xenaki, H., Vagias, C., Papazafiri, P. and Roussis, V. (2006) New cytotoxic sesquiterpenes from the red algae *Laurencia obtusa* and *Laurencia microcladia*. *Tetrahedron*, **62**, 182–189.
- Kladi, M., Vagias, C., Papazafiri, P., Furnari, G., Serio, D. and Roussis, V. (2007) New sesquiterpenes from the red alga *Laurencia microcladia*. *Tetrahedron*, **63**, 7606–7611.
- Kladi, M., Vagias, C., Stavri, M., Rahman, M., Gibbons, S. and Roussis, V. (2008) C<sub>15</sub> acetogenins with antistaphylococcal activity from the red alga *Laurencia glandulifera*. *Phytochem. Lett.*, **1**, 31–36.
- Kodner, R.B., Pearson, A., Summons, R.E. and Knoll, A.H. (2008) Sterols in red and green algae: quantification, phylogeny, and relevance for the interpretation of geologic steranes. *Geobiology*, **6**, 411–420.
- Koenig, G.M. and Wright, A.D. (1997) Sesquiterpene content of the antibacterial dichloromethane extract of the marine red alga *Laurencia obtusa*. *Planta Med.*, **63**, 186–187.
- Kolesnikova, S.A., Kalinovskiy, A.I., Fedorov, S.N., Shubina, L.K. and Stonik, V.A. (2006) Diterpenes from the Far-Eastern brown alga *Dictyota dichotoma*. *Phytochemistry (Amsterdam)*, **67**, 2115–2119.
- Konig, G.M., Wright, A.D. and Sticher, O. (1991) New xenicane and hydroazulenoid diterpenes from an Australian collection of *Dictyota divaricata*. *Tetrahedron*, **47**, 1399–1410.
- Kumari, P., Kumar, M., Gupta, V., Reddy, C.R.K. and Jha, B. (2010) Tropical marine macroalgae as potential sources of nutritionally important PUFAs. *Food Chem.*, **120**, 749–757.
- Kurata, K., Taniguchi, K. and Suzuki, M. (1996) Cyclozonarone, a sesquiterpene-substituted benzoquinone derivative from the brown alga *Dictyopteris undulata*. *Phytochemistry (Oxford)*, **41**, 749–752.
- Lane, A.L., Mular, L., Drenkard, E.J., et al. (2010) Ecological leads for natural product discovery: novel sesquiterpene

- hydroquinones from the red macroalga *Peyssonnelia* sp. *Tetrahedron*, **66**, 455–461.
- Li, K., Li, X.M., Ji, N.Y. and Wang, B.G. (2007) Natural bromophenols from the marine red alga *Polysiphonia urceolata* (Rhodomelaceae): Structural elucidation and DPPH radical-scavenging activity. *Bioorg. Med. Chem.*, **15**, 6627–6631.
- Liu, X., Wang, C.Y., Shao, C.L., *et al.* (2009) Chemical constituents from *Sargassum pallidum* (Turn.) C. Agardh. *Biochem. Syst. Ecol.*, **37**, 127–129.
- Lyakhova, E.G., Kalinovsky, A.I., Kolesnikova, S.A., Vaskovsky, V.E. and Stonik, V.A. (2004) Halogenated diterpenoids from the red alga *Laurencia nipponica*. *Phytochemistry (Amsterdam)*, **65**, 2527–2532.
- Marques, L.V., Villaca, R. and Pereira, R.C. (2006) Susceptibility of macroalgae to herbivorous fishes at Rocas Atoll, Brazil. *Botanica Marina*, **49**, 379–385.
- Matloub, A.A. and Awad, N.E. 2009. Chemical composition of some Sargassum species and their cytotoxic and antimicrobial activities. *Planta Med.*, **75**, 974.
- Mihopoulos, N., Vagias, C., Mikros, E., Scoullou, M. and Roussis, V. (2001) Prevezols A and B: New brominated diterpenes from the red alga *Laurencia obtusa*. *Tetrahedron Lett.*, **42**, 3749–3752.
- Norte, M., Fernandez, J.J. and Souto, M.L. (1994) Viridianol, a rearranged sesquiterpene with a novel carbon skeleton from *Laurencia viridis*. *Tetrahedron Lett.*, **35**, 4607–4610.
- Nylund, G.M., Persson, F., Lindegarth, M., Cervin, G., Hermansson, M. and Pavia, H. 2010. The red alga *Bonnemaisonia asparagoides* regulates epiphytic bacterial abundance and community composition by chemical defence. *Fems Microbiology Ecology*, **71**, 84–93.
- O'sullivan, L., Murphy, B., Mcloughlin, P., *et al.* (2010) Prebiotics from marine macroalgae for human and animal health applications. *Marine Drugs*, **8**, 2038–2064.
- Patil, A.D., Berry, D., Brooks, D.P., *et al.* (1993) A diterpene epoxide from the marine brown alga *Dictyota* sp.: Possible vasopressin V1 receptor antagonist. *Phytochemistry (Oxford)*, **33**, 1061–1064.
- Qiao, Y.Y., Ji, N.Y., Wen, W., Yin, X.L. and Xue, Q.Z. 2009. A new epoxy-cadinane sesquiterpene from the marine brown alga *Dictyopteris divaricata*. *Marine Drugs*, **7**, 600–604.
- Rovirosa, J., Soto, H., Cueto, M., Darias, J., Herrera, J. and San-Martin, A. (1999) Sesquiterpenes from *Laurencia claviformis*. *Phytochemistry (Oxford)*, **50**, 745–748.
- Sen, A.K., Das, A.K., Banerji, N., *et al.* (1994) A new sulfated polysaccharide with potent blood anti-coagulant activity from the red seaweed *Grateloupia indica* *Int. J. Biol. Macromol.*, **16**, 279–280.
- Siamopoulou, P., Bimplakis, A., Iliopoulou, D., *et al.* (2004) Diterpenes from the brown algae *Dictyota dichotoma* and *Dictyota linearis*. *Phytochemistry (Amsterdam)*, **65**, 2025–2030.
- Smyrniotopoulos, V., Vagias, C., Rahman, M.M., Gibbons, S. and Roussis, V. (2010) Structure and antibacterial activity of brominated diterpenes from the red alga *Sphaerococcus coronopifolius*. *Chem. Biodiv.*, **7**, 186–195.
- Smyrniotopoulos, V., Vagias, C. and Roussis, V. (2009) Sphaeroane and neodolabellane diterpenes from the red alga *Sphaerococcus coronopifolius*. *Marine Drugs*, **7**, 184–195.
- Su, J.Y., Zhong, Y.L., Zeng, L.M., Wu, H.M. and Ma, K. (1995) Terpenoids from *Laurencia karlae*. *Phytochemistry (Oxford)*, **40**, 195–197.
- Suzuki, M., Nakano, S., Takahashi, Y., Abe, T. and Masuda, M. (1999) Bisezakyne-A and -B, halogenated C15 acetogenins from a Japanese *Laurencia* species. *Phytochemistry (Oxford)*, **51**, 657–662.
- Suzuki, M., Takahashi, Y., Mitome, Y., Itoh, T., Abe, T. and Masuda, M. (2002) Brominated metabolites from an Okinawan *Laurencia intricata*. *Phytochemistry (Oxford)*, **60**, 861–867.
- Tori, M., Nakashima, K., Seike, M., *et al.* (1994) Revised structure of a brasilane-type sesquiterpene isolated from the red alga *Laurencia implicata* and its absolute configuration. *Tetrahedron Lett.*, **35**, 3105–3106.
- Trimurtulu, G., Kushlan, D.M., Faulker, D.J. and Rao, C.B. (1992) Divarinone, a novel diterpene from the brown alga *Dictyota divaricata* of the Indian Ocean. *Tetrahedron Lett.*, **33**, 729–732.
- Vairappan, C.S., Anangdan, S.P., Tan, K.L. and Matsunaga, S. (2010) Role of secondary metabolites as defense chemicals against ice-ice disease bacteria in biofouler at carrageenophyte farms. *J. Appl. Phycol.*, **22**, 305–311.
- Vairappan, C.S., Daitoh, M., Suzuki, M., Abe, T. and Masuda, M. (2001a) Antibacterial halogenated metabolites from the Malaysian *Laurencia* species. *Phytochemistry (Oxford)*, **58**, 291–297.
- Vairappan, C.S., Suzuki, M., Abe, T. and Masuda, M. (2001b) Halogenated metabolites with antibacterial activity from the Okinawan *Laurencia* species. *Phytochemistry (Oxford)*, **58**, 517–523.
- Vallim, M.A., Teixeira, V.L. and Pereira, R.C. (2007) Feeding-deterrent properties of diterpenes of *Dictyota mertensii* (Phaeophyceae, Dictyotales). *Brazilian J. Oceanogr.*, **55**, 223–229.
- Valls, R., Banaigs, B., Pioveti, L., Archavlis, A. and Artaud, J. (1993) Linear diterpene with antimitotic activity from the brown alga *Bifurcaria bifurcata*. *Phytochemistry (Oxford)*, **34**, 1585–1588.

- Venkateswarlu, Y. and Biabani, M.A.F. (1995) A spatane diterpene from the brown alga *Stoechospermum marginatum*. *Phytochemistry (Oxford)*, **40**, 331–333.
- Viano, Y., Bonhomme, D., Camps, M., *et al.* (2009) Diterpenoids from the Mediterranean brown alga *Dictyota* sp. evaluated as antifouling substances against a marine bacterial biofilm. *J. Nat. Prod.*, **72**, 1299–1304.
- Wisnespongpan, P. and Kuniyoshi, M. (2003) Bioactive phloroglucinols from the brown alga *Zonaria diesingiana*. *J. Appl. Phycol.*, **15**, 225–228.
- Wright, A.D., Konig, G.M. and Sticher, O. (1991) New sesquiterpenes and C-15 acetogenins from the marine red alga *Laurencia implicata*. *J. Nat. Prod. (Lloydia)*, **54**, 1025–1033.
- Wright, A.D., Konig, G.M., Nys, R.D. and Sticher, O. (1993) Seven new metabolites from the marine red alga *Laurencia majuscula*. *J. Nat. Prod. (Lloydia)*, **56**, 394–401.
- Xu, N., Fan, X., Yan, X. and Tseng, C.K. (2004a) Screening marine algae from China for their antitumor activities. *J. Appl. Phycol.*, **16**, 451–456.
- Xu, X.L., Song, F.H., Wang, S.J., *et al.* (2004b) Dibenzyl bromophenols with diverse dimerization patterns from the brown alga *Leathesia nana*. *J. Nat. Prod.*, **67**, 1661–1666.
- Yamase, H., Umemoto, K., Ooi, T. and Kusumi, T. (1999) Structures and absolute stereochemistry of five new secospatanes and a spatane isolated from the brown alga *Dilophus okamurai* Dawson. *Chem. Pharm. Bull. (Tokyo)*, **47**, 813–818.
- Yin, S.W., Wang, C.Y., Li, X.M. and Wang, B.G. (2005) A new clerosterol derivative, trans-phytol, and related metabolites from marine green alga *Codium fragile* (Codiaceae) and their chemotaxonomic significance. *Biochem. Syst. Ecol.*, **33**, 1288–1292.

# 13

## Seaweed Digestibility and Methods Used for Digestibility Determination

**Ladislava Mišurcová**

*Tomas Bata University in Zlín, Faculty of Technology, Department of Food Technology and Microbiology, Czech Republic*

### 13.1 Digestibility

Digestibility is a very important factor showing a utilization rate of significant nutritive factors. Therefore, it should be a part of the defined nutritive value of food. Seaweed is used as food due to its high content of many bioactive compounds, but their bioavailability by the human body is poorly documented.

The term digestibility is generally used for expressing the food utilization level. Digestion is a set of mechanical, and subsequently chemical, processes of food being resolved into smaller chemical compounds. These compounds can be absorbed into the bloodstream and consequently utilized by the body. Digestibility is most frequently determined as protein digestibility. This idea is based on the hypothesis that if the food protein is digested, the other food nutrients will be digested too. Consequently, protein digestibility is one of several qualitative indexes for the validation of protein nutritional values. These indexes are subsequently used for the food quality determination.

Searching for the current definition of a protein digestibility has been implemented by many scientists, physicians and research institutes such as the World Health Organization (WHO) and the Food and Agriculture Organization for many years. It has also been discussed in many scientific papers (Sarwar and Peace, 1994; Sarwar, 1997; Young and Pellett, 1991; WHO, 2002; Moughan, 2003).

#### 13.1.1 Protein digestibility

Protein digestibility is usually defined as the difference between nitrogen intake and fecal nitrogen output. This provides the information about the extent of food protein digestion and absorption by gastrointestinal tract of human or animal body. However, a measurement of nitrogen balance is insufficient because this value gives incomplete information about the protein digestibility and also about the true amino acids bioavailability from dietary proteins (Reeds and Garlick, 2003). Development of the definition of “protein digestibility” has been based on many factors that more or less influence values of ileal or fecal digestibility and consequently the value of true bioavailability of dietary protein (Rowan *et al.*, 1994; Hogkinson, 2006). For many years the question of protein digestibility has been argued and many factors influencing protein digestibility have been described. The results of these studies have led to the development of laboratory methods with the aim of gaining more accurate information about protein digestibility. That was followed by the evaluation of many methods for protein digestibility determination.

On the grounds of many factors affecting the methods for protein digestibility determination, apparent and true digestibilities have started to be distinguished. The term apparent digestibility represents the protein digestibility value assessed from the measurement differences of nitrogen

intake and nitrogen content after the entire digestion processes. Its calculation is shown in Equation 13.1.

$$APD = \frac{(I_N - F_N) \times 100}{I_N} \quad (13.1)$$

where APD is apparent protein (N) digestibility,  $I_N$  is nitrogen intake, and  $F_N$  is fecal nitrogen loss on the test diet.

However, the value of apparent digestibility is overestimated due to the presence of certain amounts of exogenous and endogenous nitrogen, which is made by the colon microflora from indigestible dietary protein and further by hydrolytic enzymes, which are secreted from salivary glands and pancreas.

In addition, the source of endogenous nitrogen is also mucus and epithelial cells sloughed from the intestinal mucosa (Cummings and Macfarlane, 1991; Hodgkinson, 2006; Rowan *et al.*, 1994). Hence, true nitrogen digestibility should be corrected in relation to the amount of non-dietary nitrogen and should be assessed as ileal digestibility (Rowan *et al.*, 1994; Boisen and Moughan, 1996). Its calculation is shown in Equation 13.2.

$$TPD = \frac{I_N - (F_N - F_{NF}) \times 100}{I_N} \quad (13.2)$$

Where TPD is true protein (N) digestibility,  $I_N$  is nitrogen intake,  $F_{NF}$  is fecal nitrogen loss in a protein-free diet.

Determination of the true digestibility from ileal digesta should be provided using different animals. Rats were mostly used, but due to their dissimilarity of metabolism in comparison with human, rats are not suitable models for the determination of protein digestibility by human body (Rowan *et al.*, 1994). True digestibility could be mainly conducted in humans who had previously undergone an ileostomy operation (Hodgkinson, 2006).

Moreover, protein digestibility does not exactly express the biological value of proteins. Biological value (BV) of proteins represents the value of retained nitrogen by animal or human body for the purpose of maintenance and growth (Levesque *et al.*, 2010) rather than absorbed nitrogen. Biological value of proteins is assessed as a difference in urinary and fecal nitrogen excretion between a nitrogen-free diet and a diet with test protein. The biological value of high-quality proteins, such as egg proteins, approaches 100. The biological value of protein or nitrogen could be expressed as apparent or true biological value by Equations 13.3 and 13.4.

$$ABV = \frac{(I_N - F_N - U_N) \times 100}{I_N - F_N} \quad (13.3)$$

Where ABV is apparent protein (N) biological value,  $U_N$  is urinary nitrogen loss on the tested diet.

$$TBV = \frac{I_N - (F_N - F_{NF}) - (U_N - U_{NF}) \times 100}{I_N - (F_N - F_{NF})} \quad (13.4)$$

Where TBV is true protein (N) biological value,  $U_{NF}$  is urinary nitrogen loss on a protein-free diet.

Equations 13.1 to 13.4 are modified according to WHO (2002).

With regard to the fact that proteins are the source of essential amino acids needed for synthesis of human proteins and for many metabolic pathways, amino acid score and essential amino acid index is often used for the protein quality determination.

### Amino acid score (AAS)

Protein quality depends on the concentration and distribution of specific amino acids. Proteins which have greater ratio of essential amino acids show greater biological value in contrast to proteins which contain some amino acids in lower concentrations than the required level. Amino acid score (AAS) represents the level of effectiveness of fulfilling the essential amino acid requirements at a safe intake of protein by absorbed dietary nitrogen. AAS is expressed as a ratio of essential amino acid content in tested protein to content of the same essential amino acid in the required pattern. The limiting amino acid of the tested protein represents the essential amino acid that is required by preschool-age children. The value of AAS refers to individual amino acid thus it is needed to assess all essential amino acids according to the generally known equation 13.5.

$$AAS = \frac{A_p}{A_{RP}} \quad (13.5)$$

Where  $A_p$  is mg amino acid in 1 g of tested protein,  $A_{RP}$  is amino acid content in standard protein.

### Essential amino acid index (EAAI)

EAAI predicts a more accurate value of protein quality than AAS. The EAAI is calculated by the well-known equation 13.6 as the geometric mean of the individual amino acid scores and it is equal to the antilogarithm of the individual scores.

$$EAAI = \sqrt[n]{\frac{100A_1}{A_{1S}} \times \frac{100A_2}{A_{2S}} \times \dots \times \frac{100A_n}{A_{nS}}} \quad (13.6)$$

Where EAAI is essential amino acid index,  $A_1, A_2 \dots A_n$  is amino acid contents in tested protein, and  $A_{1S}, A_{2S} \dots A_{nS}$  is amino acid content in standard protein.

For high value proteins such as casein and beef the EAAI value is 100. The value of EAAI of soybean protein tends to be approximately 100%; it obviously ranges between 90–99 % (Seligson and Mackey, 1984).

The limiting amino acid for each dietary protein can be evaluated from the values of AAS. It shows the greatest difference in the concentration of the same amino acid in the reference or high value protein.

## 13.2 Methods of seaweed digestibility assessment

With respect to a wide range of significant nutritive compounds it is not easy to determine a uniform method for digestibility determination of biological matter and therefore it is very problematic to compare the results. *In vivo*, *in situ* and *in vitro* methods can be used for digestibility determination. The first two methods have been primarily used for digestibility determination of plant materials intended for animal nutrition. The results are used for the calculation of optimal feeding ratio, then.

### 13.2.1 In vivo methods of digestibility assessment

*In vivo* methods are biological assays that determine digestibility as the amount of consumed nitrogen in relation to nitrogen absorbed and excreted by a model organism. The choice of the model organism is vital. Previously, rats were used for the evaluation of feedstuff protein quality. Pigs, due to their physiological and anatomical similarity of the human digestive tracts, were proposed as appropriate animals for the evaluation of protein quality of either food or feed (Rowan *et al.*, 1994; Miller and Ullrey, 1987; Tuan *et al.*, 1999; Moughan, 2003). Some authors suggested that the measurement of protein digestibility should be conducted in human body (Darragh and Hodgkinson, 2000; Marriotti *et al.*, 2000), but this way is expensive and difficult with regards to sampling suitable volunteers.

The nutritional value of proteins can be determined by several methods such as protein efficiency ratio (PER), net protein utilization (NPU), AAS, protein digestibility corrected amino acid score (PDCAAS) and indicator amino acid oxidation (IAAO) method.

### Protein efficiency ratio (PER)

The PER method was used for the evaluation of protein quality for many years. This factor expressed the ratio of weight gain of test rats to protein intake during a specific time period. However, usage of PER for protein quality evaluation for human was disclaimed by many authors. That is due to differences in essential amino acid requirement and growth rates between rats and humans (Sattterlee *et al.*, 1979; Wolzak *et al.*, 1981; Seligson and Mackey, 1984; Boza *et al.*, 1999). In addition, the PER overestimates the value of animal proteins while the value of vegetable proteins is underestimated (Elango *et al.*, 2009).

### Net protein utilization (NPU)

NPU was also used as a method for the measurement of food protein quality, which expresses the percentage ratio of retained body nitrogen to consumed nitrogen. Two groups of rats were used for this assignment. The first was fed with the tested diet and the second with the basal non-protein diet for appointed time period. The value NPU 1 means that 100% of dietary protein was utilized (Sattterlee *et al.*, 1979; Friedman, 1996). High-value proteins such as casein or egg protein approximate to this value. Unfortunately, the value of NPU profoundly depends on the conditions of tested rats and conducted assay. Further disadvantage is the impossibility of application of obtained results for the evaluation of protein utilization by human due to different utilization of some amino acids between tested animals and human (Bodwell, 1977; Tagle and Donoso, 1965; Boza *et al.*, 1999).

In addition to this fact PER and NPU are limited by time and cost demands. It is also very difficult to keep the defined conditions of experiments. Thus, methods using amino acids determination are more often used for the evaluation of protein quality and can be used for determination of seaweed digestibility.

### Amino acid score (AAS)

AAS is a method that uses the first limiting essential amino acid in comparison to the essential amino acid profile of whole egg protein or standard protein according to WHO (2002) for quality protein valorization. This method has also a limitation due to the problems associated with the accuracy of amino acid assays, but also due to different utilization of some amino acids from different protein sources by rats compared to humans (Bodwell, 1977; Sattterlee *et al.*, 1979; Sarwar and Peace, 1986; Friedman, 1996; Seligson and Mackey, 1984).

### **Protein digestibility corrected amino acid score (PDCAAS)**

The PDCAAS is a modern method of protein quality measurement and may also be used for establishing seaweed digestibility. It is a modified amino acid score method and has been recommended by WHO (2002) as the preferred method for protein quality validation. PDCAAS method is recommended for a routine determination of food protein quality. Values of PDCAAS are calculated by multiplying amino acid scores and fecal true digestibility of the test protein from studies undertaken on animals (Tuan *et al.*, 1999; El and Kavas, 1996; WHO, 2002; Darragh and Hodgkinson, 2000; Schaafsma, 2000; Kannan *et al.*, 2001; Elango *et al.*, 2009).

Nevertheless, the PDCAAS method has also several disadvantages. First, a PDCAAS value higher than 100% should be rounded to 100% as values of PDCAAS exceeding 100% do not provide additional nutritional benefit (Schaafsma, 2000). Hence, the PDCAAS method cannot distinguish protein qualities of high nutritional value proteins such as milk or soya (Sarwar, 1997; Elango *et al.*, 2009). Further, the PDCAAS method does not take into consideration the bioavailability of individual amino acids (Sarwar, 1997).

It is known that it is very difficult to assess an accurate value of true digestibility. In many scientific papers there have been discussions about the methodology of fecal versus ileal amino acid digestibility and other various aspects, including endogenous and exogenous sources of nitrogen influencing digestibility value (Hodgkinson, 2006; Rowan *et al.*, 1994; Millward *et al.*, 1996). It was discussed that it is significant to consider the utilization of ileal instead of fecal digestibility for the true digestibility evaluation because the measurement of fecal digestibility tends to overestimate the actual absorption of dietary amino acids (Rowan *et al.*, 1994; Hodgkinson, 2006; Moughan, 2003). With respect to that, ileal digest also contains a significant amount of endogenous nitrogen compounds, which are derived from the secretions of digestive enzymes produced by the gastrointestinal tract (Hodgkinson, 2006). These endogenous nitrogen compounds may influence the digestibility value so they should be taken into account for the calculation of true digestibility value (Sarwar, 1997; Hodgkinson, 2006; Tuan *et al.*, 1999). The values of endogenous nitrogen compounds are obtained from the protein-free diet. However, this approach has been criticized because of physiologically abnormal metabolism, which leads to lower results of ileal endogenous protein losses compared with the protein-containing diet (Deglaire *et al.*, 2007, 2008).

Finally, it was reported by Sarwar (1997) and Friedman (1996) that PDCAAS value of treated food products was

influenced by losses in nutritional values of food protein during heat processing.

### **Indicator amino acid oxidation (IAAO) method**

The improvement of methods for protein quality validation has been still developing. Nowadays a new stable isotope IAAO method has been evolving. This method is based on the concept that amino acids which are not utilized for protein synthesis have to be oxidized (Elango *et al.*, 2009; Levesque *et al.*, 2010). In contrast to the methods mentioned above, the IAAO method has several advantages. In comparison to the method of the true digestibility determination, the IAAO method is able to determine the metabolic availability of amino acids in food and seaweed. Unlike PDCAAS, the IAAO method takes into account bioavailability of individual amino acids. The IAAO method was originally evolved for determination of bioavailability of amino acids gained from pig feedstuff (Moehn *et al.*, 2004, 2005; Levesque *et al.*, 2010). Its advantages of shorter assay time and non-invasive procedure recommend IAAO as well-suited method to determine amino acid bioavailability by vulnerable populations, including adults, school-age children (Humayun *et al.*, 2007; Elango *et al.*, 2008, 2009). Studies that would establish amino acid requirements in school-age children are limited. Their amino acids needs are evaluated mostly from the current protein requirements in adults (Rodriguez, 2005). The application of the IAAO method was reported by several authors who used IAAO method for amino acids needs determination in school-age children (Mager *et al.*, 2003; Turner *et al.*, 2006; Elango *et al.*, 2007). The IAAO method has also been used for the assessment of branched-chain amino acids needs in children with chronic liver disease, which makes them more vulnerable to progression of anorexia (Mager *et al.*, 2006).

### **13.2.2 In situ methods of digestibility assessment**

*In situ* method is another way to determine digestibility. It uses a laboratory animal enzyme system. It requires inserting a carrier with bags containing tested samples directly into the animal's bowel or duodenum. After a fixed time period the amount of absorbed nitrogen is calculated by the difference in nitrogen compounds in the original samples and in the samples after the incubation in the animal's body. This methodology has been used mainly for the evaluation of food (Gosselink *et al.*, 2004; Třináctý *et al.*, 2003, 2005; Carvalho *et al.*, 2005).

### 13.2.3 *In vitro* methods of digestibility assessment

Since biological assays are very expensive and time-consuming, *in vitro* methods are used for food digestibility studies where *in vivo* methods are simulated in laboratory conditions. Many studies regarding *in vitro* methods for digestibility evaluation of different food and feed have been reported. Hur *et al.* (2011) reviewed more than 80 studies conducted to evaluate *in vitro* methods for food digestibility prediction. *In vitro* models for digestibility determination are defined as static models simulating a method of the true digestibility process either in separate parts or in series of gastrointestinal tract (Golding and Wooster, 2010). It is possible to simulate digestion of individual nutritional factors by choosing correct laboratory conditions but *in vivo* conditions can never be absolutely imitated under *in vitro* conditions (Boisen and Eggum, 1991; Hur *et al.*, 2011; Minekus *et al.*, 1999). The *in vitro* digestibility measurement may be implemented in different assays and thus it is important to select appropriate conditions.

Obviously, digestibility has been established from the difference between the content of determined compounds before and after enzyme hydrolysis of food products. Further, for the validation of *in vitro* method it is difficult to determine which enzymes or mixture of enzymes is preferable to specific food and seaweed types. In agreement with the type of investigated matters, different enzymes for evaluation of *in vitro* digestibility should be used. Pepsin, trypsin, chymotrypsin, pancreatin, peptidase and  $\alpha$ -amylase are used most frequently and further bile salt and mucin might be used for the food digestion simulation (Hur *et al.*, 2011). Digestibility determination could be conducted as a single-enzyme, two or multi-enzyme system due to the character of investigated biological matter (Boisen and Eggum, 1991; Eggum *et al.*, 1989; Galland-Irmouli *et al.*, 1999; Mišurcová *et al.*, 2010). The length of enzymatic hydrolysis is also important regarding to different types of food and seaweed (Hur *et al.*, 2011).

As well as *in vivo* method, nitrogen compounds are mostly considered as useful indicators of *in vitro* digestibility. Thus, digestibility may be estimated from the difference between the nitrogen compounds content before and after enzyme hydrolysis being applied. And prediction of further nutritional factors such as minerals and lipids can be also solved by *in vitro* methods (Au and Reddy, 2000; Golding and Wooster, 2010).

Methods for the *in vitro* digestibility measurement could be classified according to different principles to dialysis cells methods, pH-drop and pH-stat methods, gravimetric methods and to the method by using Caco-2 cell culture (Boisen and Eggum, 1991; Savoie and Gauthier, 1986;

Satterlee *et al.*, 1979; Wolzak *et al.*, 1981; Adesogan, 2005; Wortley *et al.*, 2005; Navarro *et al.*, 2000; Mišurcová *et al.*, 2010).

Each of them has the specific requirements for assignment conditions and has to be conducted with using special equipment.

#### Dialysis cell methods

Dialysis cell methods are based on enzymatic hydrolysis of protein samples with subsequent dialysis of protein hydrolysate in a dialysis cell (Hamilton and Archibald, 1944; Savoie and Gauthier, 1986). The course of enzymatic hydrolysis could be conducted as one-step, two-step or multi-step enzymatic hydrolysis. The choice of enzyme kinds depends on investigated samples (Savoie and Charbonneau, 1990). This method was used for seaweed protein digestibility determination by Galland-Irmouli *et al.* (1999). It was expressed as a relative nitrogen digestibility of 56% in comparison with casein as 100% reference digestibility gained from red seaweed *Palmaria palmata* (Table 13.1).

#### pH-stat and pH-drop methods

These methods are based on the reaction medium acidification by release of hydrogen protons after enzymatic hydrolysis of peptide bonds. pH is kept constant by automatic titration with sodium hydroxide in the pH-stat method. The quantity of sodium hydroxide is proportional to the amount of dissociated peptide bonds (Martínez and Moyano, 2003; Rozan *et al.*, 1997). Decrease in pH is recorded in the pH-drop method (Sultana *et al.*, 2010). Hydrolysis of tested protein by different enzyme systems might be used. It could be either a mixture of trypsin, chymotrypsin, and peptidase (Hsu *et al.*, 1977; Oshodi *et al.*, 1997), especially for prediction of vegetable protein digestibility, or an enzyme system of trypsin, chymotrypsin, and peptidase from *Streptomyces griseus* for more accurate prediction of protein digestibility of meat or egg products (Satterlee *et al.*, 1979; Wolzak *et al.*, 1981). For the evaluation of feed protein digestibility a direct enzyme extract from animals such as a shrimp *Litopenaeus vannamei* (Lemos *et al.*, 2009) might be used.

According to Hsu *et al.* (1977), the pH-drop method showed the high correlation with the *in vivo* apparent digestibility of rats. Boisen and Eggum (1991) noted that both the pH-drop and pH-stat methods have given poor agreement with *in vivo* values for a great variety of foods. Owing to that fact, different regression equations should be used to obtain reliable digestibility results of different kinds of food. However, it is indisputable that the pH-drop and pH-stat methods have a significant advantage for their rate of

**Table 13.1** Protein digestibility

Author seaweed	Mean %	Used <i>in vitro</i> method	Enzyme system	Condition of hydrolysis time/temperature/pH
<b>Galland-Irmouli <i>et al.</i> (1999)</b>		Dialysis cells method	Pepsin-predigestion	30 min
<i>Palmaria palmata</i>	R 56.0 <sup>C</sup>		Pancreatin	6 h
<b>Wong and Cheung (2001)</b>		pH stat method	Multienzyme system	10 min/ 37 °C/ pH 8
<i>Hypnea charoides</i>	R 88.7 <sup>SC</sup>		Porcine pancreatic trypsin	
<i>Hypnea japonica</i>	R 88.9 <sup>SC</sup>		Bovine pancreatic chymotrypsin	
<i>Ulva lactuca</i>	G 85.7 <sup>SC</sup>		Porcine intestinal peptidase	
<b>Goñi, Urbano and Saura-Calixto (2000)</b>		not shown	Pepsin	30 min / 37 °C/ pH 1.9
<i>Porphyra tenera</i>	R 69.0		Pancreatin	24 h / 37 °C/ pH 7.5
<i>Chondrus crispus</i>	R 45.0			
<i>Fucus vesiculosus</i>	B 15.0			
<i>Laminaria digitata</i>	B 17.0			
<i>Undaria pinnatifida</i>	B 28.0			
<b>Mišurcová <i>et al.</i> (2010)</b>		gravimetric method	Pepsin	24 h / 39 °C/ pH 1.5
<i>Palmaria palmata</i>	R 87.3 <sup>C</sup>		Pancreatin	24 h / 39 °C/ pH 7.5
<i>Porphyra tenera</i>	R 70.2 <sup>C</sup>			
<i>Eisenia bicyclis</i>	B 57.1 <sup>C</sup>			
<i>Hizikia fusiformis</i>	B 51.8 <sup>C</sup>			
<i>Laminaria japonica</i>	B 72.1 <sup>C</sup>			
<i>Undaria pinnatifida</i>	B 60.1 <sup>C</sup>			

R, red seaweed; G, green seaweed; B, brown seaweed.

<sup>C</sup> Value is expressed to 100 % digestibility of casein.

<sup>SC</sup> Value is expressed to 100 % digestibility of sodium caseinate.

assignment and also for a high degree of sensitivity, as they are able to detect some factors such as trypsin inhibitor and chlorogenic acid that influence protein digestibility values (Hsu *et al.*, 1977).

### Gravimetric and filtering methods

Gravimetric and filtering methods offer vast possibilities of digestibility determination of diverse nutritional factors. They have been widely used for routine analyses of nutrient digestibility of feed but also of food. In the gravimetric method digestibility is determined by a weight decrease of the original samples after their enzyme hydrolysis and subsequent drying (dry matter digestibility – DMD) and burning (organic matter digestibility – OMD) (Adesogan, 2005; Mišurcová *et al.*, 2010).

In filtering methods nutrients are assessed after enzymatic hydrolysis either as dissolved or insoluble matter. In both methods, enzymatic hydrolysis is provided under specific conditions by different enzymes as simple-enzyme or

multiple-enzyme systems (Boisen and Eggum, 1991). Further, the specific nylon bags are mostly used for enclosing tested samples, which are consequently exposed to digestive enzymatic systems (Adesogan, 2005).

Gravimetric and filtering methods could be used to evaluate different nutrient factor digestibility in some seaweed by choosing the right enzyme systems and suitable conditions for imitating digestion in human or animal digestive tracts. Thus, this enables the establishment of the digestibility of nitrogen compounds and some other components that are left in the food matter after various technology processes.

### Gas production methods

This method can be used as a simple technique for the evaluation of *in vitro* digestion. *In vitro* gas production technique is based on the measurement of gas production during anaerobic fermentation process of tested food or feed by mixtures of purified enzymes or by inoculum from

human or animal feces in special batches (Coles *et al.*, 2005; Negesse *et al.*, 2009). The gas production technique could be used to evaluate the fermentation process range of dietary fiber in the human colon (Cummings and Macfarlane, 1991).

### **Caco-2 cell culture method**

The Caco-2 cell culture model, which has been routinely applied in the pharmaceutical industry to determine the absorption potential of new drugs, might be used for the prediction of food and seaweed digestibility (Crespi *et al.*, 1996; Hu *et al.*, 1999). As a rapid and low-cost method it has been suggested for the determination of bioavailability of some minerals, especially iron and zinc from different kinds of food (Glahn *et al.*, 1998; Wortley *et al.*, 2005; Navarro *et al.*, 2000). Caco-2 cells are a human colon adenocarcinoma cell line demonstrating numerous morphological and biochemical characteristics of enterocytes (Au and Reddy, 2000). This cell method is based on the measurement of iron solubility and availability by the assignment of metal uptake through Caco-2 cell monolayer under the conditions simulating *in vivo* digestion in the gastrointestinal tract (Glahn *et al.*, 1998).

The Caco-2 cell culture model for food digestibility measurement has been developed on base of a good correlation of results from *in vivo* and *in vitro* studies of iron uptake by Caco-2 in humans (Mahler *et al.*, 2009). However, the disadvantage of the Caco-2 cell culture model is the lack or underexpression of important oxidative metabolic enzymes of the intestinal tract that contribute to metabolism of many drugs and xenobiotics (Hu *et al.*, 1999). The assignment by this method can be influenced on the grounds of another factor such as interference of zinc with iron uptake (Glahn *et al.*, 1998), and further by the different range of iron uptake inhibition by phytic acid and tannic acid. According to Glahn and Wortley (2002) iron uptake depends partly on its origin. Heme iron was less inhibited by phytic acid than non-heme iron. Also phenolic compounds are inhibitors of iron absorption thanks to the binding of iron and make it unavailable.

## **13.3 Factors influencing digestibility of seaweed and seaweed products**

Evidently, it is very difficult to compare results of digestibility values from different research studies. The reason is the changeable composition of diverse seaweed species and different methods applied for various nutritional factors determination and also the weak possibility to keep identical

accurate assay conditions (Hur *et al.*, 2011). Generally, the true digestibility value observed in *in vitro* methods was higher than in *in vivo* methods. This fact can be explained by more complicated release of amino acids from food proteins and follow-up absorption by gastrointestinal tract of humans or animals under *in vivo* conditions (Eggum *et al.*, 1989). According to Boisen and Eggum (1991) the value of *in vivo* digestibility might be influenced by variable amounts of endogenous and bacterial matter and thus should be rather considered as the apparent digestibility. In contrast to this, *in vitro* method for digestibility determination better corresponds to the true digestibility values. In agreement with Boisen and Eggum (1991) has been observed that the apparent digestibility of 26 ingredients for *Litopenaeus vannamei* feed significantly correlated with the extent of *in vitro* protein hydrolysis using shrimp hepatopancreatic enzymes (Lemos *et al.*, 2009). Nevertheless, some authors have suggested that it is necessary also to evaluate *in vivo* methods because *in vitro* methods offer only information about presumptive nutritional value of food or feed.

Moreover, digestibility values may be generally influenced by the considerable ranges of endogenous and exogenous factors that differ in many ways in *in vivo* and *in vitro* methods, and which could cause digestive losses and structural changes of nutrients (Millward *et al.*, 2008). The digestibility evaluation and availability of individual nutrients might be also influenced by factors affecting the biological needs such as growth and protein deposition (Reeds and Garlick, 2003).

### **13.3.1 Endogenous factors influencing seaweed digestibility**

Endogenous factors influencing digestibility determination are defined by particular model organisms and their specific metabolic reactions in digestive processes.

#### **Digestibility model organism**

Human or animal bodies as different types of model organisms are the main endogenous factors of digestibility assignment in *in vivo* methods. Apart from different methods of food or feed digestion by human or animal body due to their diverse structures, digestibility value could be considerably influenced also by age, sex, genetic make-up, and health conditions (Boisen and Eggum, 1991; Levesque *et al.*, 2010).

The gut is responsible for digestion, absorption and metabolism of dietary nutrients. A lot of research studies have documented the influence of numerous endogenous antinutritional factors which are able to affect true digestive process in gut (Wang *et al.*, 2009). One of them is microbial

activity, principally in the large intestine, which influences the content of nitrogen compounds in digesta. Digestibility is determined as protein or amino acid apparent digestibility due to digestibility value overestimation. The overestimation is based on nitrogen increase in feces because of exogenous and endogenous nitrogen, which is a subject for digestion and metabolism by the colon microflora (Hodgkinson, 2006; Rowan *et al.*, 1994). The main sources of exogenous and endogenous nitrogen are indigestible dietary proteins and hydrolytic enzymes such as amylase, lipase, proteases, and peptidases that are secreted by pancreas (Cummings and Macfarlane, 1991; Hodgkinson, 2006). According to Mariotti *et al.* (2000) the amount of endogenous nitrogen in humans has not been influenced by the kind of food.

For long time rats were used as model organisms for the simulation of *in vivo* conditions for the evaluation of digestibility and availability of different food. Because of the different metabolism of some nutrients, other alternatives for a model organism have been proposed. Pigs have comparable metabolism to humans of significant nutrients and consequently as simple-stomach animals they are preferred as a testing model for the evaluation of digestibility and availability of food (Cooper *et al.*, 1997; Tuan *et al.*, 1999). But some authors have suggested and preferred to evaluate digestibility processes directly in humans.

### 13.3.2 Exogenous factors influencing seaweed digestibility

All external conditions within *in vitro* digestion methods, such as the origin of enzymes and their specificity or activity, can be included in the group of exogenous factors. Further, the chemical composition of tested seaweed or food and feed in general is another factor influencing digestibility value. Moreover, tested seaweed or food consists of many compounds that influence digestibility by the formation of stable complexes with some nutrients and making them unexploitable for digestion enzymes. The nature of tested matter determines the part of the gastrointestinal tract in which the digestibility measurement by an *in vivo* method should be established. The character of tested matter is also significant for the choice of the suitable enzymes or enzyme systems for an *in vitro* method.

#### Digestive enzyme systems

Enzyme systems are powerful factors affecting the real process of digestion and its extent in both *in vivo* and *in vitro* methods. *In vivo* methods can be carried out to determine the apparent seaweed fecal digestibility after their digestion in the whole gastrointestinal tract with the participation of

all digestive enzymes. If the true digestibility is assessed, only ileal digestibility is measured and the enzyme systems of the stomach and ileum take part in the digestion process (Rowan *et al.*, 1994). Moreover, proteolysis is performed by the mixture of enzymes whose proportion and concentration vary along the gastrointestinal tract (Savoie and Charbonneau, 1990).

The choice of enzymes for *in vitro* digestibility determination is derived from the type of tested diet so as to have individual nutrients digested by the single enzyme. However, the food mixture consisting of various nutrients is decomposed better by multienzyme processes (Boisen and Eggum, 1991). The extent of enzymatic hydrolysis could be influenced by using enzymes according to the concentration of commercial enzyme products. As seaweed consists of various chemical compounds, seaweed hydrolysis may be provided by the extract of multienzyme mixtures gained directly from human or animal digestive tracts. This method is mostly used for the analyses of fish diets (Eid and Matty, 1989; Geurden *et al.*, 2009; Lemos *et al.*, 2009; Sultana *et al.*, 2010). Preparing the extract of multienzyme mixture directly from the human digestive tract has not been realized for the purpose of seaweed digestibility determination, but it has been conducted very rarely to investigate some diseases.

Savoie and Charbonneau (1990) reported that the choice of proteolytic enzymes in *in vitro* assay might be guided according to the source of tested protein. They suggested a predigestion step with pepsin especially for vegetable proteins whose digestibility is generally low. Brown seaweed shows a strong inhibition effect of the soluble fibers on *in vitro* pepsin activity, which may decrease the digestibility results (Fleurence, 1999; Horie *et al.*, 1995). Further, the influence of some commercial enzymes based on xylanases on the extent of the solubility of cell wall polysaccharides of the red seaweed *Palmaria palmata* was reported. These xylanases could be a tool to increase the algal digestibility (Lahaye and Vigouroux 1992). Moreover, Savoie and Charbonneau (1990) recommended using a complete enzyme mixture, particularly chymotrypsin, because pure enzymes are less stable in the mixture. In addition, the correct enzyme system choice for digestibility evaluation depends on the conditions of different substrates of hydrolysis in diverse part of gastrointestinal tract. So it is necessary to know the process of the postprandial metabolism in human (Millward *et al.*, 1996; Coleman *et al.*, 1996).

#### Conditions of enzyme hydrolysis

The range of enzyme hydrolysis depends on the activity of used enzymes. It is generally known that enzyme reactions are very specific and require special conditions such as a

certain temperature, pH, incubation time, particular enzyme concentration and also presence of enzyme activators or inhibitors.

The hydrolysis time is important to complete digestion of food nutrients. The true time period of food remain in the adult human colon is 20–80 h (Coles *et al.*, 2005). *In vitro* hydrolysis process is obviously conducted as a follow-up process when the incubation with pepsin simulating digestion in stomach is followed by the incubation of pancreatin simulating digestion processes in small intestine (Boisen and Eggum, 1991; Hur *et al.*, 2011). The hydrolysis time could be influenced by several factors, for example, the nature of tested samples (individual nutrients or mixtures) and size of the particle. Mixture of large particles will have longer retention period in stomach than a single nutrient or small particles. The hydrolysis time used for samples hydrolysis in *in vitro* methods ranges from several minutes to 24 h in dependence on parameters of measurement, the origin of sample and chosen analytic methods (Rowan *et al.*, 1992; Hur *et al.*, 2011).

According to Rowan *et al.* (1992) the value of amino acid content might be influenced by incorrect results of hydrolysis process and this can result in the assessment of underestimated or overestimated digestibility value.

#### **Chemical composition of seaweed and presence of antinutritional factors**

Digestibility of seaweed is influenced by many factors whose efficiency depends directly on the seaweed composition. The seaweed chemical composition is very variable even within a species. It depends on numerous factors such as a location and time of harvest, salinity and purity of the seawater as it has been mentioned above. Beside many compounds with health benefit, seaweed contains also antinutrients reducing the nutrients availability and causing different ailments (Thompson, 1993). The presence of different macronutrients in a diet may induce a distinct pattern of seaweed passing through gastrointestinal tract, which may lead to diverse protein digestibility (Mackie and Macierzanka, 2010). Besides, it is necessary to consider antinutrients as endogenous factors, which are derived by reciprocal interactions between chemical compounds presented in seaweed matter. Synergic and antagonistic behavior of seaweed matter may significantly affect the utilization of individual nutritional factors. The compounds, which are able to decrease the digestibility value, are tannin, some parts of dietary fiber, phytic acids, polyphenolic substances and enzyme inhibitors (Martínez and Moyano, 2003; Horie *et al.*, 1995; Burtin, 2003). It is generally known that high content of dietary fiber could cause deficient utilization of trace elements (Santoso *et al.*, 2006). Seaweed is an

abundant source of soluble and insoluble fibers. Different effects on the apparent digestibility of minerals after the addition of red and brown seaweed *Porphyra tenera* and *Laminaria pinnatifida* to the rat diet was determined by Urbano and Goñi (2002). The apparent digestibility values were mostly higher using the diet with *Laminaria pinnatifida* than *Porphyra tenera*, except from iron whose decrease was almost equal by 21.3% and 22.8%, respectively. The highest decrease of magnesium apparent digestibility compared to a control diet without seaweeds was established at 35.4% and 18.3% in the case of *Laminaria pinnatifida* and *Porphyra tenera*, respectively. Similar trend was assessed in the case of zinc (24.7% and 5.2%, respectively). However, direct relation between the content of dietary fiber in individual seaweed and decrease of mineral availability has not been observed.

Further, presence of fiber and antinutritional factors will underestimate true protein digestibility (Hodgkinson, 2006). The significant part of dietary protein may not be available for digestive enzymes in the gastrointestinal tract because some proteins could be kept in complexes with non-starch polysaccharides or other components, such as polyphenols (Cummings and Macfarlane, 1991).

Seaweed cell walls are rich in many different polysaccharides, which are able to form stable complexes with seaweed protein, whereby seaweed proteins become inaccessible for proteolytic enzymes. Consequently, the value of seaweed protein digestibility decreases. The apparent protein digestibility was decreased by the addition of red and brown seaweed *Porphyra tenera* and *Undaria pinnatifida* to the rat diet equally by 6.5% (Urbano and Goñi, 2002). Furthermore, Horie *et al.* (1995) observed the effects of a different part of dietary fiber gained from several brown seaweed food products (kombu, wakame and hijiki) on pepsin *in vitro* activity. They reported that the inhibition by soluble dietary fiber was higher than by insoluble part of dietary fiber in all investigated samples. The highest inhibition effect of almost 100% had kombu soluble dietary fiber. The inhibition effect of soluble dietary fiber ranged approximately between 60% to almost 100% while the inhibition effect of insoluble dietary fiber ranged somewhere between 20 and 40%. Finally, the high inhibition effect of soluble dietary fiber could be explained by their higher viscous properties.

In many research studies there has been reported that brown seaweed polysaccharides such as alginates, fucoidans, laminarins and phlorotannins have a lot of biological functions. Many of them have health benefit properties (Shan *et al.*, 1999). Their effect on the microbial activity has also been studied. The addition of extract from brown seaweed *Ascophyllum nodosum*, *Laminaria hyperborea* and *L. digitata* in piglet diet suppressed the growth of anaerobes, especially *E. coli* in gut (Dierick *et al.*, 2010; Reilly *et al.*, 2008).

On the other hand, dietary fiber has an important role in digestive processes due to their resistance to digestion and due to bulking of feces, water retention and mediation of ion exchange (Rupérez and Saura-Calixto, 2001; Jiménez-Estrig and Sánchez-Muniz, 2000). Moreover, dietary fiber stimulates microbial activity in gastrointestinal tract and reduces the transit time of the digesta (Boisen and Eggum, 1991). According to Cummings and Macfarlane (1991) at least 50% of cellulose and 80% of non-cellulosic polysaccharides from different vegetable sources are digested by microorganisms in human gut. Finally, this fact can cause increase in the apparent digestibility value.

The fucoidans influence on reducing digestibility of starches has been reported. Sulfated polysaccharides are water-soluble non-starch polysaccharides and are commonly founded in brown seaweed such as *Ascophyllum nodosum*, *Fucus vesiculosus*, *Fucus evanescens*, *Laminaria japonica*, *Ecklonia stolonifera* and *Ecklonia cava* (You *et al.*, 2010; Yang *et al.*, 2008). The inhibition effect of different sulfated fucans of brown seaweed *Undaria pinnatifida* on starch hydrolysis has been observed. Cho *et al.* (2010) reported that diverse sulfated fucoidans had different inhibition effects of amyloglucosidase and  $\alpha$ -amylase. Oversulfated fucoidan with a content of 51.1% of sulfates showed an inhibitory activity to amyloglucosidase in contrast to native fucoidan with a content of 41.5% of sulfates. However, in both native and oversulfated fucoidans the inhibitory effect to  $\alpha$ -amylase has not been determined. Lignin, as a component of dietary fiber, may also decrease the seaweed protein digestibility because its phenol units can be complexed with proteins and this fact could explain the lower digestibility of vegetable proteins (Rozañ *et al.*, 1997).

Moreover, values of seaweed or plant protein digestibility are influenced by their occurrence in different parts of plant tissue and by different distribution of digestible protein and unavailable part of seaweed or plant protein (Sarwar *et al.*, 1989). Great differences in protein digestibility and availability have been established by *in vitro* method after pepsin and pancreatin incubation due to the presence of non-digestive part of seaweed proteins and their different distribution of total protein between different species of brown seaweed. The content of digestible protein was 69% in *Porphyra tenera* in contrast to *Fucus vesiculosus* and *Laminaria digitata* with 15 and 17% of digestible protein (Table 13.1), but the last two seaweed showed the highest content of unavailable protein 24 and 23%, respectively (Goñi, *et al.*, 2002).

Further, protein digestibility or utilization could be influenced by the presence of phenolic compounds in seaweed matter. These compounds are able to break protein structure by covalent binding in oxidative conditions which complicates protein extraction (Moure *et al.*, 2001; Wang

*et al.*, 2009). Tannins are natural polyphenols found in plants. They may reduce protein digestibility due to their ability to form strong insoluble complexes with carbohydrates and proteins, which are resistant to digestive enzymes of human and other monogastric animals (Rehman and Shah, 2005; Martínez and Moyano, 2003; De Oliveira *et al.*, 2009). The interactions between tannins and proteins are highly influenced by pH (Martínez and Moyano, 2003). The concentration of tannins is different within different species. In red seaweed *Palmaria palmata* there were 59.0 mg of tannins in 100 g sample (De Oliveira *et al.*, 2009) while in green seaweed *Enteromorpha* spp. tannins were determined in the range of 62–97 mg in 100 g sample (Aguilera-Morales *et al.*, 2005).

Polyphenols from brown seaweed *Ascophyllum nodosum* and *Fucus vesiculosus* chelate divalent metal ions, such as  $\text{Sr}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Be}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  in weak acidic aqueous solutions (Ragan *et al.*, 1979). But according to Wang *et al.* (2009) polyphenols did not appear to be effective metal chelators because no clear correlation was found between the total phenolic content and chelating ability in extracts from different eight seaweed species, including brown seaweed from genera *Fucus*, *Laminaria*, and *Alaria* and red seaweed from genera *Palmaria* and *Chondrus*.

Phytic acid is also an antinutritional factor of seaweed which is able to reduce the bioavailability of some essential minerals (Likuski and Forbes, 1964). Phytic acid forms stable chelates with almost all multivalent cations insoluble at pH 6 to 7. These complexes are most likely to be responsible for decreased bioavailability of complexed minerals and also for decreased protein digestibility due to the resistance of these complexes to proteolytic digestion at low pH value (Cheryan, 1980).

In addition, apart from the basal nutrient component of seaweed there could be a lot of secondary metabolites that are formed for their protection from consumers or environmental conditions (Hay, 1996). Some of these compounds can be either food and feed nutrient or the factors positively or negatively influencing digestibility processes (Targett *et al.*, 1995).

### Food processing

Increase of digestibility and palatability is the reason why food is processed in different ways.

Chemical composition of seaweed products depends on the way of raw material preparation, for example processes of washing, drying and storage and also food processing. The step of washing could influence digestibility and availability of some nutrients of seaweed. That is due to the removal of the part of soluble dietary fiber, which can form

stable complexes with different nutrients (Marion *et al.*, 2003). This author reported that the seaweed digestibility value can be increased by insertion of seaweed washing process before digestion process as it eliminates compounds inhibiting proteolysis. Nevertheless, in that study with red seaweed *Palmaria palmata* it was observed that only 50% of total nitrogen was released by this treatment. The losses were probably caused by the interaction of remaining xylan with protein or peptides.

During culinary processing of food and seaweed secondary metabolites might be evolved, which improve seaweed palatability but also could lead to the formation of antinutritional compounds due to enzymatic or nonenzymatic reactions caused by pH and heat effect (Friedman, 1996).

Seaweed is often used as an ingredient in vegetable salads, but mostly it is prepared by heating. It is generally known that heating induces structural changes in proteins. The denaturing process increases protein accessibility for digestive enzymes and consequently increases digestibility. Food processing might affect the activation of enzyme inhibitors such as  $\alpha$ -amylase and trypsin inhibitors. The influence of dry heat process on the activation of  $\alpha$ -amylase inhibitors and trypsin inhibitors in seaweed extracts has been reported. Low heat stability of  $\alpha$ -amylase inhibitors has been confirmed because after 10-minute heating process at 200 °C  $\alpha$ -amylase inhibitor was inactivated, whereas trypsin inhibitors have been observed as heat-resistant. The activity of trypsin inhibitors decreased only by 3% after 30-minute heating process at 200 °C (De Oliveira *et al.*, 2009). Trypsin inhibitors reduce trypsin availability and are connected with growth inhibition and with pancreatic hypertrophy (Liener, 1979). Heating of seaweed may induce some denaturation changes in trypsin inhibitors whereby the amino acids near the active sites become unapproachable for the trypsin–trypsin inhibitor complex (Rozan *et al.*, 1997). Heating could influence also the solubility of minerals as well as pH. Solubility of minerals is necessarily required for their bioavailability. Thus, both seaweed composition determining pH of seaweed products and pH values inside the gastrointestinal tract might fundamentally affect the extent of mineral solubility. Bioavailability of minerals depends strongly on better solubility in stomach and small intestine (Yoshie *et al.*, 1999). In samples of red – Nori, green – Aonori and brown – Hijiki seaweed higher amounts of soluble zinc at pH 2 than at pH 6 were measured, and also after pepsin treatment than after pepsin–pancreatin treatment. However, iron solubility was lower than zinc solubility and was higher after complex hydrolysis by pepsin and pancreatin than after simple hydrolysis by pepsin. Moreover, chemical forms of minerals predestine their solubility and consequent availability. Iron

and zinc exist in the low molecular weight fraction (Yoshie *et al.*, 1999). Higher content of proteins and dietary fiber in seaweed can lead to lower solubility of seaweed minerals. Santoso *et al.* (2006) mentioned the influence of boiling and diverse pH value on mineral solubility in different green, brown, and red Indonesian seaweed. A significant increase of calcium and magnesium solubility was established by boiling for 20 minutes, especially with the addition of 0.5% acetic acid.

In the case of tannins and phytic acid there were also observed some effects of heating on their composition and subsequently their negative influence on the nutritional value of seaweed or plant food. Pressure cooking of tannins at 121 °C was more effective in improvement of protein and starch digestibility in food legumes than cooking in boiling water (Rehman and Salariya, 2005). Unfortunately, the effect of different processing methods of seaweed products on the change of tannin content has not yet been investigated. Further, influence of cooking on the content of phytic acid in legumes was observed. It was reported that bean treatment by cooking and by soaking plus cooking incurred 0.38% and 0.76% decrease of in phytic acid content (Lathia *et al.*, 1987). On the other hand Davies and Reid (1979) published that cooking had no effect on the zinc, copper, iron, manganese, or phytate contents. Even if the content of phytic acid in seaweed is relatively low (0.45%) with regard to an unfavorable impact on mineral bioavailability, it could be used as an indicator of mineral bioavailability (De Oliveira *et al.*, 2009).

Fermentation processing is widely used in the Japanese food industry to improve meal flavor and appearance. Further, fermentation facilitates destruction of undesirable materials and improves keeping quality and finally enhances nutritional value because of improved digestibility (Hesseltine and Wang, 1980). Fermentation of some vegetable protein such as soybean by *Rhizopus oligosporus* can damage the complexes in which trypsin inhibitors exist. The fermentation effect on red seaweed *Palmaria palmata* digestibility was documented by Marion *et al.* (2003) who reported different improvements of seaweed digestibility after the fermentation by various molds – *Rhizopus microsporus* var. *chinensis*, *Aspergillus oryzae* and *Trichoderma pseudokongii* – due to different enzymes produced by these molds.

## 13.4 Evaluation of seaweed digestibility

Seaweed digestibility is mostly poorly documented. Moreover, the influence of seaweed participation on food or feed

product digestibility has been investigated rather than direct seaweed digestibility.

Table 13.1 gives data on seaweed protein digestibility provided by different authors and different assay methods.

In general, red seaweed shows higher digestibility than brown seaweed. This fact could be explained by higher content of dietary fiber in brown seaweed with a greater content of insoluble fiber in comparison to red seaweed, whose dietary fiber is mostly formed by soluble fiber. Good agreement of digestibility values of 69% and 70.2% were observed in red seaweed *Porphyra tenera* determined by the same enzyme system but in different time intervals by Goñi *et al.* (2000) and by Mišurcová *et al.* (2010), respectively. Further, the highest digestibility was established in red seaweed *Hypnea japonica* at 88.9% and *H. charoides* at 88.7% by Wong and Cheung (2001) and in *Palmaria palmata* at 87.3% by Mišurcová *et al.* (2010). A comparable high digestibility was also found in green seaweed *Ulva lactuca* where the value was 85.7% (Wong and Cheung, 2001).

On the other side, a great difference of 31% was determined in digestibility value of *Palmaria palmata* by the dialysis cell method (Galland-Irmouli *et al.*, 1999) and the gravimetric method (Mišurcová *et al.*, 2010) conducted under different analytical conditions, such as different time intervals.

In spite of red seaweed digestibility, brown seaweed showed a great difference of digestibility values within the same seaweed species *Undaria pinnatifida* of 32.1% according to Goñi *et al.* (2000) and Mišurcová *et al.* (2010). Digestibility of *Laminaria japonica* and *Laminaria digitata* varied by 55.1%. Their digestibility was analyzed under the same conditions of enzyme hydrolysis but using different time intervals.

Concerning *in vitro* digestibility determination there are many different objectives which may more or less influence the final digestibility value. To validate *in vitro* methods for seaweed digestibility, accurate conditions of all used methods should be set to increase reproducibility and to allow comparison of results obtained in different laboratories. For the evaluation of general conclusions about seaweed digestibility, many more results must be compared than have been obtained at present.

### 13.5 Contribution of seaweed to food and feed digestibility

Seaweed could be used as diet ingredients to improve digestibility of other food or feed products. Mostly it is used as a supplement to increase the dietary fiber portion in food products or feed.

The influence of added various seaweed on the protein apparent digestibility in growing male rat diet and apparent digestibility of some minerals were observed (Bocanegra *et al.*, 2003; Urbano and Goñi, 2002). From the results of both studies it is obvious that values of protein apparent digestibility decreased after the addition of seaweed ingredients to rat diets.

Nevertheless, in the case of 7% addition of both frozen dried brown seaweed *Laminaria digitata* and red seaweed *Porphyra tenera* significant differences of the values of protein apparent digestibility were not observed according to Bocanegra *et al.* (2003). The value of protein apparent digestibility decreased by 0.84% and 0.61% after the addition of *Laminaria digitata* and *Porphyra tenera*, respectively, compared to the control diet without seaweed. On the other hand, Urbano and Goñi (2002) reported that values of protein apparent digestibility in a greater extent after the addition of 14.7% both brown seaweed *Undaria pinnatifida* and red seaweed *Porphyra tenera* decreased respectively by 6.4% and 6.5% compared with the control diet with 5% addition of cellulose. However, the difference of protein apparent digestibility value after the addition of either brown or red seaweed was not found to be significant.

Further, in the papers mentioned above, there were also established effects of the addition of brown and red seaweed on the change of apparent digestibility of some minerals. Investigated seaweed samples are good sources of minerals. Unfortunately, their availability could be different, which was proved in rats. Bocanegra *et al.* (2003), in agreement with Urbano and Goñi (2002), reported that values of mineral apparent digestibility decreased more by the addition of brown seaweed than in the case of red seaweed. In contrary to protein apparent digestibility, addition of diverse seaweed had different influences on apparent digestibility of minerals. According to Urbano and Goñi (2002) all investigated minerals – calcium, magnesium, zinc, sodium, and potassium – had better availability by rats after the addition of red seaweed *Porphyra tenera* than of brown seaweed *Undaria pinnatifida*. Only iron was the exception, as its content was higher in *Undaria pinnatifida* than in *Porphyra tenera*.

So, the results of mineral availabilities were similar in the cases of the addition of red seaweed *Porphyra tenera* and brown seaweed *Laminaria digitata*, as reported by Bocanegra *et al.* (2003).

Generally, different mineral apparent digestibility could be explained by various distribution of soluble and insoluble parts of fiber. Insoluble fiber is higher in brown seaweed *Laminaria digitata* and *Undaria pinnatifida*, which caused higher retention of minerals after the addition of brown seaweed *Undaria pinnatifida*. Thus, higher contents of minerals were determined in fecal excretion by using brown seaweed, which resulted in greater mineral retention.

The significant results were observed in a survey conducted with the contribution of nori to postprandial glycemic response in healthy female volunteers (Goñi *et al.*, 2000). Nori is red seaweed from genus *Porphyra* and is high in soluble dietary fiber, having the ability to form a viscous solution with the capability to capture some nutrients such as carbohydrates. This could cause their lower accessibility to  $\alpha$ -amylase. Different *in vitro* kinetics of starch digestion of white bread with nori from the digestion of white bread itself was detected. The glycemic index of humans decreased from 100 to 68% by intake of nori-white bread (Goñi *et al.*, 2000).

### 13.6 Conclusion

Digestibility is the significant factor for evaluation of the availability of different kind of seaweed as food. Digestibility of diverse food products of vegetable or meat origin, especially protein digestibility, has been determined by many different methods over many years, but the evaluation of seaweed or seaweed products digestibility has been done poorly.

The assignment of seaweed digestibility is necessary because of changeable chemical composition within various seaweed species and the presence of many different compounds, which may influence the digestibility value positively or negatively.

Finally, it would be suitable to develop a reference method to standardize the results of seaweed digestibility and make them comparable.

### References

- Adesogan, A.T. (2005) Effect of bag type on the apparent digestibility of feeds in ANKOM Daisy<sup>II</sup> incubators. *Animal Feed Sci. Technol.*, **119**, 333–344.
- Aguilera-Morales, M., Casas-Valdez, M., Carrillo-Dominquez, S., González-Acosta, B. and Pérez-Gil, F. (2005) Chemical composition and microbiological assays of marine algae *Enteromorpha* ssp. as potential food source. *J. Food Comp. Anal.*, **18**, 79–88.
- Au, A.P. and Reddy, M.B. (2000) Caco-2 cells used to assess human iron bioavailability from a semipurified meal. *J. Nutr.*, **130**, 1329–1334.
- Bocanegra, A., Nieto, A., Blas, B. and Sánchez-Muniz, F.J. (2003) Diets containing a high percentage of Nori or Konbu algae are well-accepted and efficiently utilised by growing rats but induce different degrees of histological changes in the liver and bowel. *Food Chem. Toxicol.*, **41**, 1473–1480.
- Bodwell, C.E. (1977) Application of animal data to human protein nutrition: a review. *Cereal Chemistry*, **54**, 958–983.
- Boisen, S. and Eggum, B.O. (1991) Critical evaluation of *in vitro* methods for estimating digestibility in simple-stomach animals. *Nutr. Res. Rev.*, **4**, 141–162.
- Boisen, S. and Moughan, P.J. (1996) Different expressions of dietary protein and amino acid digestibility in pig feeds and their application in protein evaluation: a theoretical approach. *Acta Agric. Scand., Section A – Animal Science*, **46**, 165–172.
- Boza, J.J., Moënnoz, D., Vuichoud, J. *et al.* (1999) Food deprivation and refeeding influence growth, nutrient retention and functional recovery of rats. *J. Nutr.*, **129**, 1340–1346.
- Burtin, P. (2003) Nutritional value of seaweeds. *Electron. J. Env., Agric. Food Chem.*, **2**, 498–503.
- Carvalho, L.P.F., Melo, D.S.P., Pereira, C.R.M., Rodrigues, M.A.M., Cabrita, A.R.J. and Fonseca, A.J.M. (2005) Chemical composition, *in vivo* digestibility, N degradability and enzymatic intestinal digestibility of five protein supplements. *Animal Feed Sci. Technol.*, **119**, 171–178.
- Cheryan, M. (1980) Phytic acid interactions in food systems. *Crit. Rev. Food Sci. Nutr.*, **13**, 297–335.
- Cho, M.L., Han, J.H. and You, A.G. (2010) Inhibitory effects of fucan sulphates on enzymatic hydrolysis of starch. *LWT – Food Sci. Technol.*, **44**, 1164–1171.
- Coleman, M.E., Dreesen, D.W. and Wiegert, R.G. (1996) A simulation of microbial competition in the human colonic ecosystem. *Appl. Env. Microbiol.*, **62**, 3632–3639.
- Coles, L.T., Moughan, P.J. and Darragh, A.J. (2005) *In vitro* digestion and fermentation methods, including gas production techniques, as applied to nutritive evaluation of foods in the hindgut of humans and other simple-stomached animals. *Animal Feed Sci. Technol.*, **123–124**, 421–444.
- Cooper, D.A., Berry, D.A., Spendel, V.A., Kiorpes, A.L. and Peters, J.C. (1997) The domestic pig as a model for evaluating Olestra's nutritional effects. *J. Nutr.*, **127**, 1555S–1565S.
- Crespi, Ch. L., Penman, B.W. and Hu, M. (1996) Development of Caco-2 Cells expressing high levels of cDNA-derived cytochrome P4503A4. *Pharm. Res.*, **13**, 1635–1641.
- Cummings, J.H. and Macfarlane, G.T. (1991) The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol.*, **70**, 443–459.
- Darragh, A.J. and Hodgkinson, S.M. (2000) Quantifying the digestibility of dietary protein. *J. Nutr.*, **130**, 1850S–1856S.

- Davies, N.T. and Reid, H. (1979) An evaluation of the phytate, zinc, copper, iron, and manganese contents of, and Zn availability from, soya-based textured-vegetable-protein meat-substitutes or meat-extenders. *Br. J. Nutr.*, **41**, 579–589.
- Deglaire, A., Moughan, P.J., Rutherford, S.M., Bos, C. and Tomé, D. (2007) Feeding dietary peptides to growing rats enhances gut endogenous protein flows compared with feeding protein-free or free amino acid-based diets. *J. Nutr.*, **137**, 2431–2436.
- Deglaire, A., Moughan, P.J., Bos, C., Petzke, K. and Rutherford, S.M. (2008) A casein hydrolysate does not enhance gut endogenous protein flows compared with intact casein when fed to growing rats. *J. Nutr.*, **138**, 556–561.
- De Oliveira, M.N., Freitas, A.L.P., Carvalho, A.F.U. *et al.* (2009) Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. *Food Chem.*, **115**, 254–259.
- Dierick, N., Oryn, A. and De Smet, S. (2010) *In vitro* assessment of the effect of intact marine brown macro-algae *Ascophyllum nodosum* on the gut flora of piglets. *Livestock Science*, **133**, 154–156.
- Eid, A.E. and Matty, A.J. (1989) A simple *in vitro* method for measuring protein digestibility. *Aquaculture*, **79**, 111–119.
- Eggum, B.O., Hansen, I. and Larsen, T. (1989) Protein quality and digestible energy of selected foods determined in balance trials with rats. *Plant Foods for Human Nutrition*, **39**, 13–21.
- El, S.N. and Kavas, A. (1996) Determination of protein quality of rainbow trout (*Salmo irideus*) by *in vitro* protein digestibility-corrected amino acid score (PDCAAS). *Food Chem.*, **55**, 221–223.
- Elango, R., Humayun, M.A., Ball, R.O. and Pencharz, P.B. (2007) Lysine requirement of healthy school-age children determined by the indicator amino acid oxidation method. *Am. J. Clin. Nutr.*, **86**, 360–365.
- Elango, R., Ball, R.O. and Pencharz, P.B. (2008) Indicator amino acid oxidation: concept and application. *J. Nutr.*, **138**, 243–246.
- Elango, R., Ball, R.O. and Pencharz, P.B. (2009) Amino acid requirements in humans: with a special emphasis on the metabolic availability of amino acids. *Amino Acids*, **37**, 19–27.
- Fleurence, J. (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.*, **10**, 25–28.
- Friedman, M. (1996) Nutritional value of proteins from different food sources. A review. *J. Agric. Food Chem.*, **44**, 6–29.
- Galland-Irmouli, A.V., Fleurence, J., Lamghari, R. and *et al.* (1999) Nutritional value of proteins from edible seaweed *Palmaria palmata* (Dulse). *J. Nutr. Biochem.*, **10**, 353–359.
- Geurden, I., Jutfelt, F., Olsen, R.E. and Sundell, K.S. (2009) A vegetable oil feeding history affects digestibility and intestinal fatty acid uptake in juvenile rainbow trout *Oncorhynchus mykiss*. *Comp. Biochem. Physiol., Part A*, **152**, 552–559.
- Glahn, R.P., Lee, O.A., Yeung, A., Goldman, M.I. and Miller, D.D. (1998) Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an *in vitro* digestion/Caco-2 cell culture model. *J. Nutr.*, **128**, 1555–1561.
- Glahn, R.P. and Wortley, G.M. (2002) Inhibition of iron uptake by phytic acid, tannic acid, and ZnCl<sub>2</sub>: studies using an *in vitro* digestion/Caco-2 cell model. *J. Agric. Food Chem.*, **50**, 390–395.
- Golding, M. and Wooster, T.J. (2010) The influence of emulsion structure and stability on lipid digestion. *Curr. Opin. Colloid Interf. Sci.*, **15**, 90–101.
- Goñi, I., Valdivieso, L. and Garcia-Alonso, A. (2000) *Nori* seaweed consumption modifies glycemic response in healthy volunteers. *Nutr. Res.*, **20**, 1367–1375.
- Goñi, I., Urbano, M.G. and Saura-Calixto, F. (2002) *In vitro* determination of digestible and unavailable protein in edible seaweeds. *J. Sci. Food Agric.*, **82**, 1850–1854.
- Gosselink, J.M.J., Dulphy, J.P., Poncet, C., Jailler, M., Tamminga, S. and Cone, J.W. (2004) Prediction of forage digestibility in ruminants using *in situ* and *in vitro* techniques. *Animal Feed Science and Technology*, **115**, 227–246.
- Hamilton, P.B. and Archibald, R.M. (1944) A dialysis cell for rapid quantitative analytical determination of diffusible components in blood plasma. *Industrial and Engineering Chemistry Analytical Edition*, **16**, 136–137.
- Hay, M.E. (1996) Marine chemical ecology: what's known and what's next? *J. Exp. Mar. Biol. Ecol.*, **200**, 103–134.
- Hesseltine, C.W. and Wang, H.L. (1980) The importance of traditional fermented foods. *BioScience*, **30**, 402–404.
- Hodgkinson, S.M. (2006) Evaluation of the quality of protein sources for inclusion in diets for monogastric animals. *Ciencia e Investigación Agraria*, **33**, 83–90.
- Horie, Y., Sugase, K. and Horie, K. (1995) Physiological differences of soluble and insoluble dietary fibre fractions of brown algae and mushrooms in pepsin activity *in vitro* and protein digestibility. *Asia Pacific J. Clin. Nutr.*, **4**, 251–255.
- Hsu, H.W., Vavak, D.L., Satterlee, L.D. and Miller, G.A. (1977) A multienzyme technique for estimating protein digestibility. *J. Food Sci.*, **42**, 1269–1273.
- Hu, M., Li, Y., Davitt, Ch. M. *et al.* (1999) Transport and metabolic characterization of Caco-2 cells expressing

- CYP3A4 and CYP3A4 plus oxidoreductase. *Pharm. Res.*, **16**, 1352–1359.
- Humayun, M.A., Elango, R., Moehn, S. and Ball, R.O. (2007) Application of the indicator amino acid oxidation technique for the determination of metabolic availability of sulphur amino acids from casein versus soy protein isolate in adult men. *J. Nutr.*, **137**, 1874–1879.
- Hur, S.J., Lim, B.O., Decker, E.A. and McClements, D.J. (2011) *In vitro* human digestion model for food applications. *Food Chem.*, **125**, 1–12.
- Jiménez-Escrig, A. and Sánchez-Muniz, F.J. (2000) Dietary fibre from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res.*, **20**, 585–598.
- Kannan, S., Nielsen, S.S. and Mason, A.C. (2001) Protein digestibility-corrected amino acid scores for bean and bean-rice infant weaning food products. *J. Agric. Food Chem.*, **49**, 5070–5074.
- Lahaye, M. and Vigouroux, J. (1992) Liquefaction of dulse (*Palmaria palmata* (L.) Kuntze) by a commercial enzyme preparation and a purified endo- $\beta$ -1,4-D-xylanase. *J. Appl. Phycol.*, **4**, 329–337.
- Lathia, D., Hoch, G. and Kievernagel, Y. (1987) Influence of phytate on *in vitro* digestibility of casein under physiological conditions. *Plant Foods for Human Nutrition*, **37**, 229–235.
- Lemos, D., Lawrence, A.L. and Siccardi, A.J. (2009) Prediction of apparent protein digestibility of ingredients and diets by *in vitro* pH-stat degree of protein hydrolysis with species-specific enzymes for juvenile Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, **295**, 89–98.
- Levesque, C.L., Moehn, S., Pencharz, P.B. and Ball, R.O. (2010) Review of advances in metabolic bioavailability of amino acids. *Livestock Science*, **133**, 4–9.
- Liener, I.E. (1979) The nutritional significance of plant protease inhibitors. *Proc. Nutr. Soc.*, **38**, 109–113.
- Likuski, H.J.A. and Forbes, R.M. (1964) Effect of phytic acids on the availability of zinc in amino acid and casein diets fed to chicks. *J. Nutr.*, **84**, 145–148.
- Mackie, A. and Macierzanka, A. (2010) Colloidal aspects of protein digestion. *Curr. Opin. Colloid Interf. Sci.*, **15**, 102–108.
- Mager, D.R., Wykes, L.J., Ball, R.O. and Pencharz, P.B. (2003) Branched-chain amino acid requirements in school-aged children determined by indicator amino acid oxidation (IAAO). *J. Nutr.*, **133**, 3540–3545.
- Mager, D.R., Wykes, L.J., Roberts, E.A., Ball, R.O. and Pencharz, P.B. (2006) Branched-chain amino acid needs in children with mild-to-moderate chronic cholestatic liver disease. *J. Nutr.*, **136**, 133–139.
- Mahler, G.J., Shuler, M.L. and Glahn, R.P. (2009) Characterization of Caco-2 and HT29-MTX cocultures in an *in vitro* digestion/cell culture model used to predict iron bioavailability. *J. Nutr. Biochem.*, **20**, 494–502.
- Marrion, O., Schwartz, A., Fleurence, J., Guéant, J.L. and Villaume, Ch. (2003) Improvement of the digestibility of the proteins of the red alga *Palmaria palmata* by physical processes and fermentation. *Nahrung/Food*, **47**, 339–344.
- Mariotti, F., Mahé, S., Luengo, C., Benamouzig, R. and Tomé, D. (2000) Postprandial modulation of dietary and whole-body nitrogen utilization by carbohydrates in humans. *Am. J. Clin. Nutr.*, **72**, 954–962.
- Martínez, T.F. and Moyano, F.J. (2003) Effect of tannic acid on *in vitro* enzymatic hydrolysis of some protein sources. *J. Sci. Food Agric.*, **83**, 456–464.
- Miller, E.R. and Ullrey, D.E. (1987) The pig as a model for human nutrition. *Annu. Rev. Nutr.*, **7**, 361–382.
- Millward, D.J., Fereday, A., Gibson, N.R. and Pacy, P.J. (1996) Post-prandial protein metabolism. *Baillière's Clin. Endocrinol. Metab.*, **10**, 533–549.
- Millward, D.J., Layman, D.K., Tomé, D. and Schaafsma, G. (2008) Protein quality assessment: impact of expanding understanding of protein and amino acids needs for optimal health. *Am. J. Clin. Nutr.*, **87**, 1567S–1581S.
- Minekus, M., Smeets-Peters, M., Bernalier, A. *et al.* (1999) A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. *Appl. Microbiol. Biotechnol.*, **53**, 108–114.
- Mišurcová, L., Kráčmar, S., Klejdus, B. and Vacek, J. (2010) Nitrogen content, dietary fiber, and digestibility in algal food products. *Czech J. Food Sci.*, **28**, 27–35.
- Moehn, S., Bertolo, R.F.P., Pencharz, P.B. and Ball, R.O. (2004) Indicator amino acid oxidation responds rapidly to changes in lysine or protein intake in growing and adult pigs. *J. Nutr.*, **134**, 836–841.
- Moehn, S., Bertolo, R.F.P., Pencharz, P.B. and Ball, R.O. (2005) Development of the indicator amino acid oxidation technique to determine the availability of amino acids from dietary protein in pigs. *J. Nutr.*, **135**, 2866–2870.
- Moughan, P.J. (2003) Amino acid availability: aspects of chemical analysis and bioassay methodology. *Nutr. Res. Rev.*, **16**, 127–141.
- Moure, A., Cruz, J.M., Franco, D. *et al.* (2001) Natural antioxidants from residual sources. *Food Chem.*, **72**, 145–171.
- Navarro, P., Aspe, T. and Seiquer, I. (2000) Zinc transport in Caco-2 Cells and zinc balance in rats: influence of the heat treatment of a casein-glucose-fructose mixture. *J. Agric. Food Chem.*, **48**, 3589–3596.
- Negesse, T., Makkar, H.P.S. and Becker, K. (2009) Nutritive value of some non-conventional feed resources of Ethiopia determined by chemical analyses and an

- in vitro* gas method. *Anim. Feed Sci. Technol.*, **154**, 204–217.
- Oshodi, A.A., Beames, R.M. and Nakai, S. (1997) *In vitro* protein digestibility, amino acid profile and available iron of infant-weaning food prepared from maize flour and bovine blood. *Food Res. Int.*, **30**, 193–197.
- Ragan, M.A., Smidsrød, O. and Larsen, B. (1979) Chelation of divalent metal ions by brown algal polyphenols. *Mar. Chem.*, **7**, 265–271.
- Reeds, P.J. and Garlick, P.J. (2003) Protein and amino acid requirements and the composition of complementary foods. *J. Nutr.*, **133**, 2953S–2961S.
- Reilly, P., O'Doherty, J.V., Pierce, K.M., Callan, J.J., O'Sullivan, J.T. and Sweeney, T. (2008) The effects of seaweed extract inclusion on gut morphology, selected intestinal microbiota, nutrient digestibility, volatile fatty acid concentrations and the immune status of the weaned pig. *Animal*, **2**, 1465–1473.
- Rehman, Z. and Salariya, A.M. (2005) The effects of hydrothermal processing on antinutrients, protein and starch digestibility of food legumes. *Int. J. Food Sci. Technol.*, **40**, 695–700.
- Rehman, Z. and Shah, W.H. (2005) Thermal heat processing effects on antinutrients, proteins and starch digestibility of food legumes. *Food Chem.*, **91**, 327–331.
- Rodriguez, N.R. (2005) Optimal quantity and composition of protein for growing children. *J. Am. Coll. Nutr.*, **24**, 150S–154S.
- Rowan, A.M., Moughan, P.J. and Wilson, M.N. (1992) Effect of hydrolysis time on the determination of the amino acid composition of diet, ileal digesta, and feces samples and on the determination of dietary amino acid digestibility coefficients. *J. Agric. Food Chem.*, **40**, 981–985.
- Rowan, A.M., Moughan, P.J., Wilson, M.N., Maher, K. and Tasman-Jones, C. (1994) Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. *Br. J. Nutr.*, **71**, 29–42.
- Rozan, P., Lamghari, R., Linder, M. *et al.* (1997) *In vivo* and *in vitro* digestibility of soybean, lupine, and rapeseed meal proteins after various technological processes. *J. Agric. Food Chem.*, **45**, 1762–1769.
- Rupérez, P. and Saura-Calixto, F. (2001) Dietary fibre and physicochemical properties of edible Spanish seaweed. *Eur. Food Res. Technol.*, **212**, 349–354.
- Santoso, J., Gunji, S., Yosire-Stark, Y. and Suzuki, T. (2006) Mineral contents of Indonesian seaweeds and mineral solubility affected by basic cooking. *Food Sci. Technol. Res.*, **12**, 59–66.
- Sarwar, G. (1997) The protein digestibility-corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. *J. Nutr.*, **127**, 758–764.
- Sarwar, G. and Peace, R.W. (1986) Comparison between true digestibility of total nitrogen and limiting amino acids in vegetable proteins fed to rats. *J. Nutr.*, **116**, 1172–1184.
- Sarwar, G. and Peace, R.W. (1994) The protein quality of some enteral products is inferior to that of casein as assessed by rat growth methods and digestibility-corrected amino acid scores. *J. Nutr.*, **124**, 2223–2232.
- Sarwar, G., Peace, R.W., Botting, H.G. and Brullé, D. (1989) Digestibility of protein and amino acids in selected foods as determined by a rat balance method. *Plant Foods for Human Nutrition*, **39**, 23–32.
- Satterlee, L.D., Marshall, H.F. and Tennyson, J.M. (1979) Measuring Protein Quality. *J. Am. Oil Chem. Soc.*, **56**, 103–109.
- Savoie, L. and Gauthier, S.F. (1986) Dialysis cell for the *in vitro* measurement of protein digestibility. *J. Food Sci.*, **51**, 494–498.
- Savoie, L. and Charbonneau, R. (1990) Specific role of endopeptidases in modulating the nature of protein digestion products. *Plant Foods for Human Nutrition*, **40**, 233–242.
- Seligson, F.H. and Mackey, L.N. (1984) Variable predictions of protein quality by chemical score due to amino acid analysis and reference pattern. *J. Nutr.*, **114**, 682–691.
- Shan, B.E., Yoshida, Y., Kuroda, E. and Yamashita, U. (1999) Immunomodulating activity of seaweed extract on human lymphocytes *in vitro*. *Int. J. Immunopharmacol.*, **21**, 59–70.
- Schaafsma, G. (2000) The protein digestibility-corrected amino acid score. *J. Nutr.*, **130**, 1865S–1867S.
- Sultana, Z., Ahmed, S., Iqbal, S. and Chisty, A.H. (2010) Determination of *in vitro* protein digestibility of different feed ingredients for Nilotica (*Oreochromis nilotica*). *Bangladesh Research Publications Journal*, **4**, 87–94.
- Tagle, M.A. and Donoso, G. (1965) Net protein utilization determined in short- and long-term experiments with rats. *J. Nutr.*, **87**, 173–178.
- Targett, N.M., Boettcher, A.A., Targett, T.E. and Vrolijk, N.H. (1995) Tropical marine herbivore assimilation of phenolic-rich plants. *Oecologia*, **103**, 170–179.
- Thompson, L.U. (1993) Potential health benefits and problems associated with antinutrients in foods. *Food Res. Int.*, **26**, 131–149.
- Třinácý, J., Homolka, P., Zeman, L. and Richter, M. (2003) Whole tract and post ruminal digestibility determined by *in situ* ruminal, intestinal mobile nylon bag and whole tract nylon capsule methods. *Anim. Feed Sci. Technol.*, **106**, 56–67.

- Třináctý, J., Richter, M., Homolka, P., Rabišková, M. and Doležal, P. (2005) Comparison of apparent and true digestibility of nutrients determined in dairy cows either by the nylon capsule or *in vivo* method. *Czech J. Animal Sci.*, **50**, 402–410.
- Tuan, Y.H., Phillips, R.D. and Dove, C.R. (1999) Predicting integrated protein nutritional quality, Part I: Amino acid availability corrected amino acid score and nitrogen balance data fitted to linear and non-linear models for test proteins. *Nutr. Res.*, **19**, 1791–1805.
- Turner, J.M., Humayun, M.A., Elango, R. *et al.* (2006) Total sulphur amino acid requirements on healthy school-age children as determined by indicator amino acid oxidation technique. *Am. J. Clin. Nutr.*, **83**, 619–623.
- Urbano, M.G. and Goñi, I. (2002) Bioavailability of nutrients in rats fed on edible seaweeds Nori (*Porphyra tenera*) and Wakame (*Undaria pinnatifida*) as a source of dietary fibre. *Food Chem.*, **76**, 281–286.
- Wang, T., Jónsdóttir, R. and Ólafsdóttir, G. (2009) Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem.*, **116**, 240–248.
- Wang, W.W., Qiao, S.Y. and Li, D.F. (2009) Amino acids and gut function. *Amino Acids*, **37**, 105–110.
- WHO (2002) *Report of a Joint WHO/FAO/UNU Expert Consultation, WHO Technical Report Series 935. Protein and Amino Acid Requirements in Human Nutrition*. WHO Press, Geneva.
- Wolzak, A., Bressani, R. and Brenes, R.G. (1981) A comparison of *in vivo* and *in vitro* estimates of protein digestibility of native and thermally processed vegetable proteins. *Plant Foods for Human Nutrition*, **31**, 31–43.
- Wolzak, A., Elías, L.G. and Bressani, R. (1981) Protein quality of vegetable proteins as determined by traditional biological methods and rapid chemical assay. *J. Agric. Food Chem.*, **29**, 1063–1068.
- Wong, K.H. and Cheung, C.K. (2001) Nutritional evaluation of some subtropical red and green seaweeds Part II. *In vitro* protein digestibility and amino acid profiles of protein concentrates. *Food Chem.*, **72**, 11–17.
- Wortley, G., Leusner, S., Good, C., Gugger, E. and Glahn, R. (2005) Iron availability of a fortified processed wheat cereal: a comparison of fourteen iron forms using an *in vitro* digestion/human colonic adenocarcinoma (CaCo-2) cell model. *Br. J. Nutr.*, **93**, 65–71.
- Yang, Ch., Chung, D. and You, S.G. (2008) Determination of physicochemical properties of sulphated fucans from sporophyll of *Undaria pinnatifida* using light scattering technique. *Food Chem.*, **111**, 503–507.
- Yoshie, Y., Suzuki, T., Pandolf, T. and Clydesdale, F.M. (1999) Solubility of Iron and Zinc in Selected Seafoods under Simulated Gastrointestinal Conditions. *Food Sci. Technol. Res.*, **5**, 140–144.
- You, S.G., Yang, Ch., Lee, H.Y. and Lee, B.Y. (2010) Molecular characteristics of partially hydrolyzed fucoidans from sporophyll of *Undaria pinnatifida* and their *in vitro* anti-cancer activity. *Food Chem.*, **119**, 554–559.
- Young, V.R. and Pellett, P.L. (1991) Protein evaluation, amino acid scoring and the Food and Drug Administration's proposed food labeling regulations. *J. Nutr.*, **121**, 145–150.

# 14

## Metallation of Seaweed *Fucus vesiculosus* Metallothionein: $\text{As}^{3+}$ and $\text{Cd}^{2+}$ binding

Thanh T. Ngu<sup>1</sup> and Martin J. Stillman<sup>2</sup>

<sup>1</sup>Department of Chemistry, The University of Toronto, Toronto, Ontario, Canada

<sup>2</sup>Department of Chemistry, University of Western Ontario, London, Ontario, Canada

### 14.1 Introduction

The brown seaweed *Fucus vesiculosus* grows in and is resistant to toxic metals in polluted water. The gene for the protein metallothionein (MT) in *Fucus vesiculosus* has been reported (Morris *et al.*, 1999). MTs are rich in sulfur, with up to 30% of the amino acids being cysteine, an amino acid that, together with histidine, is well known to bind soft metals readily. The most studied metallation reactions have been with  $\text{Cu}^+$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$ ; recently detailed studies have been reported for  $\text{As}^{3+}$  binding. Metallation studies of recombinantly prepared *Fucus vesiculosus* metallothionein (rfMT) with cadmium and arsenic have been reported in detail and are described in this chapter.

Both  $\text{Cd}^{2+}$  and  $\text{As}^{3+}$  exhibit toxicities that have detrimentally affected large numbers of people in the past and at present. In particular, arsenic is currently a worldwide threat to humans through contamination of drinking water and is reported to currently affect millions of people, especially in Bangladesh (Hug *et al.*, 2000; Mead, 2005). Recombinant MT from *Fucus vesiculosus* has been reported to bind 6  $\text{Cd}^{2+}$  ions (Merrifield *et al.*, 2006) or 5  $\text{As}^{3+}$  ions (Merrifield *et al.*, 2004) to the thiols of the 16 cysteines in the sequence.

In this chapter we describe both equilibrium metallation experiments that establish these stoichiometric values and time- and temperature-resolved data that provided the first step-wise reaction kinetic and thermodynamic information for an algal MT.

MT is a metalloprotein, which was first characterized in 1957 (Margoshes and Vallee, 1957) from horse kidneys. Now, however, it has been found to be ubiquitous across nearly all organisms and its metal-binding properties have been described from mammals, plants, invertebrates, yeast, bacteria, and many other species (Stillman *et al.*, 1992; Suzuki *et al.*, 1993). The MT proteins exhibit remarkable metal binding properties for a very wide range of metals (Stillman *et al.*, 1992; Suzuki *et al.*, 1993). Metal coordination takes place through the large number of cysteine sulfurs present in the protein forming two metal-binding domains in mammalian, crustacean, plant, and algal MTs (Morris *et al.*, 1999; Robinson *et al.*, 1993; Stillman *et al.*, 1992; Suzuki *et al.*, 1993). A key property of the primary structure of almost all MTs is that there are no or very few aromatic amino acids, unlike most proteins, thus allowing the UV region between 230 and 300 nm to be free of

major protein absorbance. This property was invaluable in the establishment of the metal-binding properties of most MTs because absorbance from aromatic amino acids would normally mask the ligand to metal charge transfer (LMCT) absorption present when metals bind to the thiols of the cysteine residues in MT. It is particularly important to note that these LMCT bands exhibit metal-dependent band maxima meaning that the bound metal's identity can be determined quite accurately from the absorption spectrum, and even more readily, from the circular dichroism (CD) spectrum.

Each of the monovalent Group 11 and the divalent Group 12 metals bind to the protein and based on X-ray diffraction, nuclear magnetic resonance, X-ray absorption near edge structure, and X-ray absorption spectroscopy studies it has been proposed that all of these metals bind in metal-thiolate clusters in the two domains (Boulanger *et al.*, 1982; Otvos and Armitage, 1980). It has been shown that seven divalent metals and up to 12 Cu(I) and Ag(I) bind to the mammalian in a 9-cysteine  $\beta$  and an 11-cysteine  $\alpha$  domain, where the domains are connected by a short linker region (Stillman *et al.*, 1992; Suzuki *et al.*, 1993). The plant MTs (Type 1–3) generally consist of two cysteine-rich regions separated by a long interdomain linker that may be up to approximately 40 amino acids in length (Cobbett and Goldsbrough, 2002; Robinson, 1989; Robinson *et al.*, 1993). The structure for these plants MTs have been previously proposed to consist of either a hairpin motif (Domenech *et al.*, 2006) with distinct interaction between the metal binding domains, or the structure could adopt simply two separate and non-interacting metal-thiolate binding domains (Bilecen *et al.*, 2005; Loebus *et al.*, 2011; Peroza and Freisinger, 2007).

We now turn to the algal metallothionein from *Fucus vesiculosus*: rfMT has a 67 amino acid sequence within which are two regions high in cysteines; a seven-cysteine region (named  $\gamma$ ) and a nine-cysteine region (named  $\beta$ ). These two regions are separated by a 14 amino acid linker. The rfMT has a much longer linker than mammalian MT which only has 2–3 amino acids separating or linking the two domains in the native mammalian proteins. This long linker was expected to give rfMT a much more flexible structure (Rhee *et al.*, 1990). Similar to mammalian MT, it has been proposed from studies of  $\text{Cd}^{2+}$  binding to rfMT (the data are shown below), that the metals bind in two domains: 3  $\text{Cd}^{2+}$  to the 7-cysteine  $\gamma$  and 3  $\text{Cd}^{2+}$  to the 9-cysteine  $\beta$  domain (Merrifield *et al.*, 2004, 2006). These two domains were named following the convention of 11 cysteines in the mammalian  $\alpha$  domain, 9 cysteines in the mammalian  $\beta$  domain, and now 7 cysteines in a new  $\gamma$  domain.

The utility of the LMCT bands for studying metal-binding reactions of MT were mentioned earlier; however, there are limitations when using optical spectroscopy to

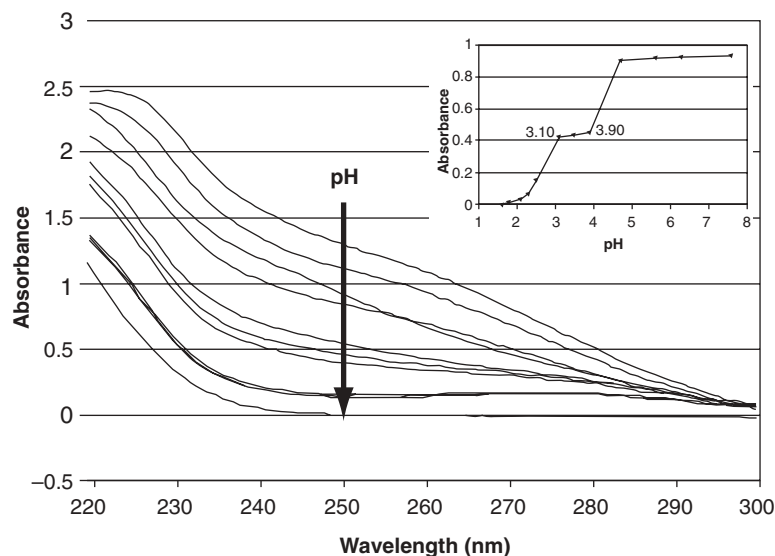
study MT. UV-visible absorption and CD spectroscopies are excellent probes to use to initially study metallation of MTs. Both techniques are dependent on the identity of the metal coordinated to MT, because the metal itself influences the wavelength of the LMCT bands. The CD spectrum of MT arises from the chiral wrapping of the peptide around the metal-thiolate cluster and is measured at the LMCT wavelengths. The CD spectrum of metal-containing MT is further dependent on the number of metals bound and coordination number (2, 3, or 4) of the metal; where the ligands are always cysteine sulfurs. The major limitation of these optical spectroscopy techniques is the inability to clearly identify intermediates because of a lack of discrimination or resolution in the absorption spectrum between intermediate species as each metal binds. However, the CD spectrum does exhibit changes that clearly show that the binding site geometry changes as a function of metal loading.

In recent years, the study of proteins has been greatly advanced with the maturity of electrospray ionization mass spectrometry (ESI-MS). Using ESI-MS, it is now possible to monitor the full metallation reaction of any metalloprotein in real-time and identify intermediate species if they have different masses and at the same time determine structural changes that occur to the protein itself that are induced by the metallation. Studying kinetic reactions using ESI-MS is a blossoming field that has the potential to revolutionize the study of protein kinetics. There have already been a number of reports of studies on protein kinetic reactions using ESI-MS (Attwood and Geeves, 2004; Bothner *et al.*, 2000; Daneshfar *et al.*, 2004; Li *et al.*, 2003; Miranker *et al.*, 1996; Shoemaker *et al.*, 2007; Wilson and Konermann, 2004; Zaia *et al.*, 1998) where the researchers have made use of these advantages.

Recent reports of metallation reactions with a wide range of metals (Blindauer *et al.*, 2003; Ejnik *et al.*, 2002; Krezel and Maret, 2007; Pattanaik *et al.*, 1992; Rigby Duncan and Stillman, 2007; Salgado and Stillman, 2004) have provided the first details of the primary steps in the metallation reactions for a few MTs. Currently, useful information can be obtained from steady-state equilibrium, time- and temperature-dependent ESI-MS. Examples from our laboratory illustrate the power of this technique.

## 14.2 Characterization of the rfMT

The rfMT protein used for all the studies described in this chapter was based on the 67-residue sequence: MAGTG CKIWE DCKCG AACSC GDSC CGTVK KGTT S RAGAG CPCGP KCKCT GQGSC NCVKD DCCGC GK. There are 16 cysteine residues present and no disulfide bonds. The  $\gamma$  domain is formed from the first 7 cysteine residues starting



**Figure 14.1** UV absorption spectra recorded during the pH titration of  $Cd_6$ -rfMT. Inset: The absorbance of  $Cd_6$ -rfMT at 250 nm as a function of pH showing the two-phase reduction in absorbance. Absorbance at 250 nm is assigned to cysteine-thiolate to cadmium charge transfer (LMCT). (Reproduced with permission from Merrifield *et al.*, 2006. Copyright American Chemical Society.)

at the N-terminus and the  $\beta$  domain is formed from the remaining 9 cysteine residues following a 14 residue linker. In addition to the sequence from the rfMT, the expression system includes the amino acid residues of the stabilizing S-peptide tag (MKETAAAKFERQHMDSPDLGTLVPRGS) on the N-terminus of the fragment (Sturzenbaum *et al.*, 1998, 2001). We have shown in previous studies that the S-tag does not interfere significantly with the reaction mechanism for binding metals in MTs (Chan *et al.*, 2007; Merrifield *et al.*, 2006; Ngu *et al.*, 2008). rfMT isoform 2 was expressed and purified as previously reported (Merrifield *et al.*, 2004; Ngu and Stillman, 2006).

### 14.3 Equilibrium metallation studies of rfMT studied using ESI-MS and UV-visible absorption techniques

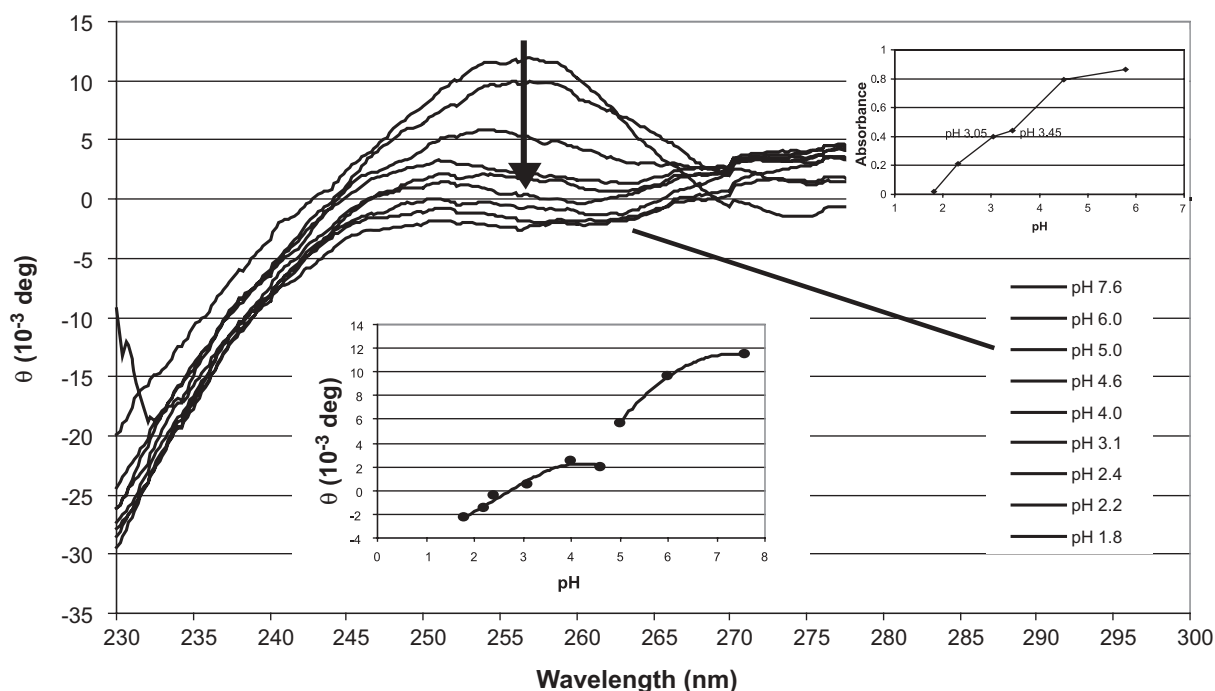
#### 14.3.1 Equilibrium data for cadmium binding

Studies by Merrifield *et al.* found that the overall metal-to-sulfur ratios of rfMT bound to  $Cd^{2+}$  and  $Zn^{2+}$  were  $Cd_6S_{16}$  and  $Zn_6S_{16}$ , respectively (Merrifield *et al.*, 2006). Mixed Cd/Zn species were also formed when  $Cd^{2+}$  was added to the Zn-containing *Fucus* MT. Analysis of the UV-

absorption, CD and ESI-MS spectral data recorded during step-wise, acid-induced demetallation supported a two-domain structure for the protein, with two separate regions that each contains three metal-binding sites. Further, the data suggested that one of the domains is significantly less stable than the other. The  $M_3S_7$  described by Merrifield *et al.* introduced a new cluster motif for MTs.

Confirmation that the cadmium atoms are bound to cysteinyl sulfurs was provided by the UV absorption (Figure 14.1) and circular dichroism spectroscopic data (Figure 14.2) at 250 nm; which is the sulfur to cadmium charge transfer band region that is prominent for cadmium-containing compounds with sulfur as the coordinating ligand (Kagi, 1993). The trends in both UV and CD absorption data recorded for  $Cd_6$ -rfMT, Figures 14.1 and 14.2 show how the absorbance at 250 nm decreases upon lowering the pH. A decrease in absorbance at 250 nm occurs when cysteinyl sulfurs bound to cadmium are protonated, thereby releasing the metal ions (Kagi, 1993). This is a characteristic property of cadmium-MTs (Stillman *et al.*, 1987; Stillman and Zelazowski, 1988) and shows that, because the absorbance is directly related to the number of S-Cd bonds, as the Scys are protonated, the bound cadmiums are displaced in a step-wise fashion.

The plateau region near pH 4 in both UV absorption (Figure 14.1) and CD (Figure 14.2) spectra clearly indicated that demetallation occurred in two distinct steps with loss



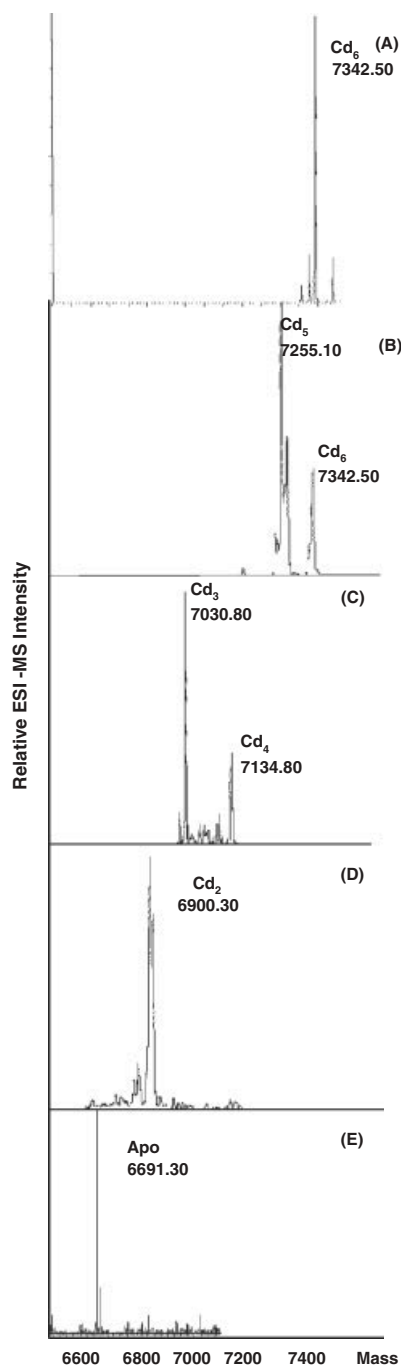
**Figure 14.2** CD spectra recorded during the pH titration of Cd<sub>6</sub>-rfMT. In this series of spectra, the Cd<sub>6</sub>-rfMT was titrated using aliquots of 1% formic acid solution starting at pH 7.6. Inset A: CD intensities of Cd<sub>6</sub>-rfMT(s) at 255 nm as a function of pH. Inset B: The absorbance of Cd<sub>6</sub>-rfMT at 250 nm as a function of pH. (Reproduced with permission from Merrifield *et al.*, 2006. Copyright American Chemical Society.)

of about 50% of the absorption in the first step, indicating loss of approximately 50% metal content from the protein.

The pH titration followed by absorption and CD spectroscopy was also monitored by ESI-mass spectrometry. The data for the pH titration of rfMT by ESI-MS are shown in Figure 14.3. The ESI-mass spectral data illustrate the sequential demetallation of the rfMT. At pH 7.60, Figure 14.3 (top; A), the molecular species has a mass of 7356.0 Da corresponding to Cd<sub>6</sub>-rfMT. A single Cd<sup>2+</sup> ion was lost by pH 6.70 (Figure 14.3B), and three Cd<sup>2+</sup> ions are lost by pH 4.30 (Figure 14.3C), where the predominant species is the Cd<sub>3</sub>-rfMT with a mass of 7030.80 Da. At pH 3.70 (Figure 14.3D), the predominant species is the Cd<sub>2</sub>-rfMT. The metal-free apo rfMT is the only species below pH 2.70 (Figure 14.3E). We can rearrange the data to connect both experiments by plotting the pH ranges that each metallated species is observed for. Figure 14.4 provides a clear picture of the pH properties, and in particular, the stability of the last three Cd<sup>2+</sup> ions. Under the ESI-MS conditions used, rfMT with a single Cd<sup>2+</sup> bound was not detected. It is possible that the conditions used in the mass spectrometer were too harsh and the final Cd<sup>2+</sup> simply was lost due to these conditions.

### 14.3.2 Equilibrium data for arsenic binding

The recombinant fMT binds up to five As<sup>3+</sup> ions. Equilibrium studies demonstrate that binding is non-cooperative and takes place in single steps. We show, in Figures 14.5 and 14.6, both the measured charge state data for a titration of apo-rfMT with As<sup>3+</sup> and the deconvoluted data that gives the mass of the parent molecular ion. It is important to note that the significance of the charge state data that clearly indicate changes that take place to the protein conformation as a result of metallation. The initial apo-rfMT exhibits a dominant +12 charge state (Figure 14.5A), but this changes to +10 when just two As<sup>3+</sup> bind, indicating that the protein folds under the influence of As<sup>3+</sup> binding. Interestingly, the data show that the folding is complete by three As<sup>3+</sup> added. The complexity in the mass spectral data between two and four As<sup>3+</sup> added arises from the overlap of the charge states from a mixture of metallated species. The spectrum is much simpler once five As<sup>3+</sup> have bound (Figure 14.5F), where the dominant charge state is +10 arising from As<sub>5</sub>-rfMT with mass of 9877.80. The deconvoluted data show clearly the build up of As<sup>3+</sup> to a maximum of five (Figure 14.6),



**Figure 14.3** Deconvoluted ESI-MS data recorded during a pH titration of  $\text{Cd}_6$ -rfMT. The pH of the solution was lowered in steps using 1% formic acid, and the ESI-TOF-MS data recorded. The maxEnt-1 deconvolution software of Micromass was used to calculate the parent ions from the measured charge states. pH of the solutions: (A) 7.6; (B) 6.7; (C) 4.3; (D) 3.7; (E) 2.7. (Reproduced with permission from Merrifield *et al.*, 2006. Copyright American Chemical Society.)

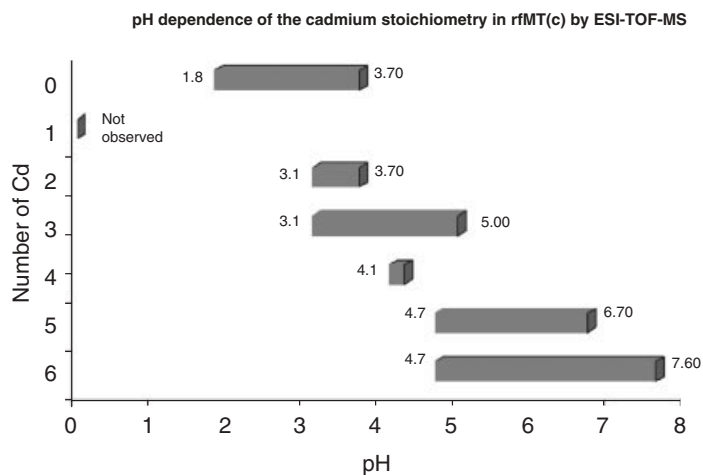
in single steps. This shows that the metallation process is non-cooperative.

## 14.4 Dynamic metallation studies of rfMT studied using ESI-MS techniques

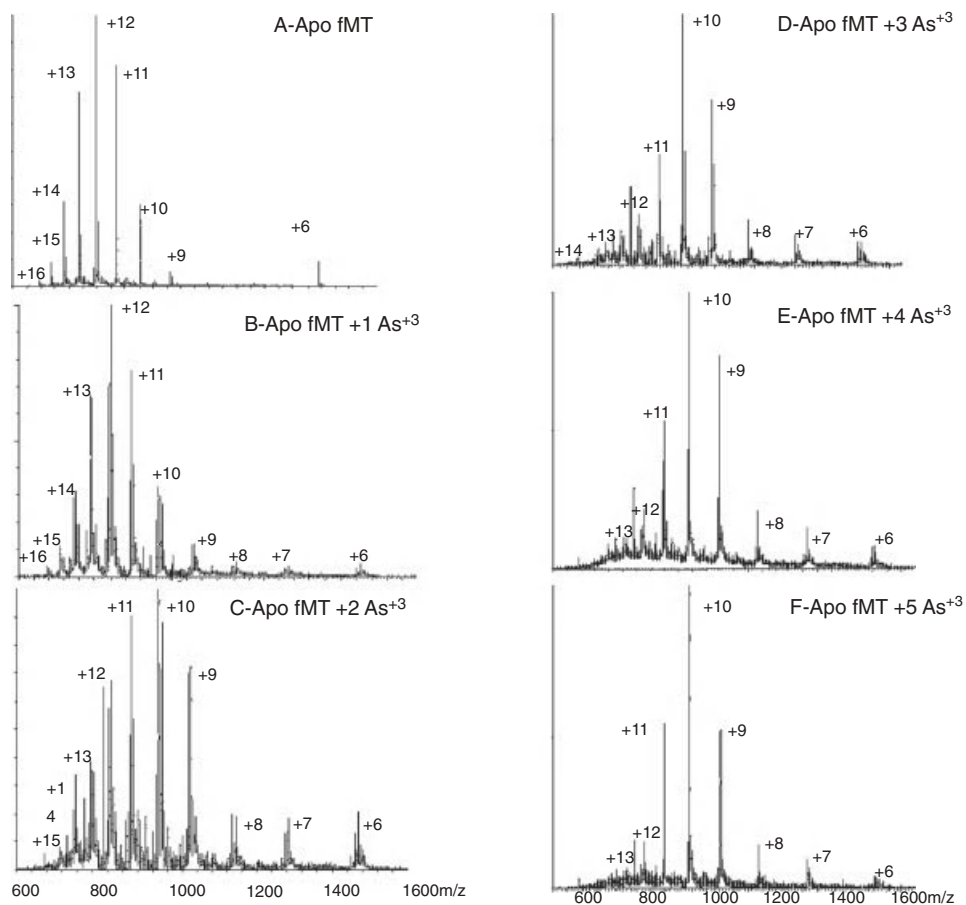
In studies with mammalian MTs, we reported that the arsenic-metallation reaction of MT was slow enough to the isolated recombinant human  $\alpha$  and  $\beta$  domains that we could determine the rate constants for the individual metallation steps for 1–3  $\text{As}^{3+}$  binding to either the  $\alpha$  and  $\beta$  domains (Ngu and Stillman, 2006). Despite the clarity afforded by the use of the isolated domains, for many years there have been questions concerning the functional significance of the two domains, and particularly, whether metallation was domain specific and/or a cooperative reaction involving both domains.

We describe in this section the step-wise, arsenic metallation of the two-domain, 16-cysteine, recombinant *Fucus vesiculosus* metallothionein using temperature- (Ngu and Stillman, 2006; Ngu *et al.*, 2008) and time-resolved ESI MS (Ngu and Stillman, 2006; Ngu *et al.*, 2009). Use of ESI-MS data for kinetic measurements has been reported by others for different proteins (Daneshfar *et al.*, 2004; Shoemaker *et al.*, 2007; Wilson and Konermann, 2004; Yu *et al.*, 1993; Zaia *et al.*, 1998; Zechel *et al.*, 1998). The time-resolved ESI-MS data showed the complete progress of the reaction from one  $\text{As}^{3+}$  bound to five  $\text{As}^{3+}$  bound (Scheme 14.1 and Figures 14.7 and 14.8). The data allowed determination of all five individual rate constants and the construction of a simulation that showed the progress of the metallation for the five  $\text{As}^{3+}$  ions and the time-resolved individual occupancies of the two domains. The data reported here clearly show that a long interdomain linker allow the domains to bind metals independently and slower, while from our previous studies we show that a short interdomain linker allowed the domains to behave as a “single domain” and bind metals faster (Ngu *et al.*, 2009).

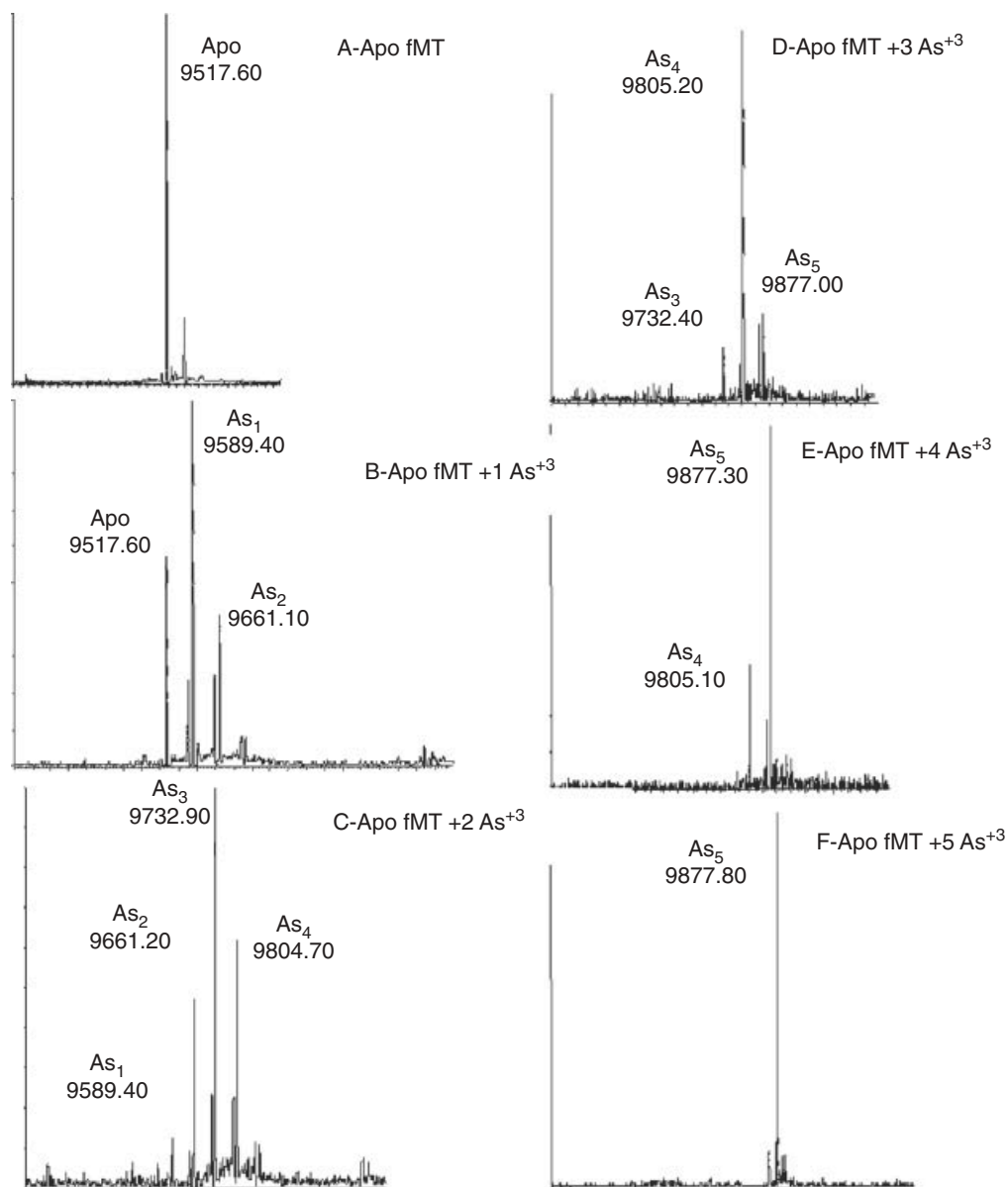
It is useful to summarize the method used in both the measurement and analysis of the data. The measured charge state data or the deconvoluted mass spectral data were used for the kinetic analyses. In the time-resolved experiments, the relative abundances were plotted against time and fitted to calculate the  $k_n$   $\beta$  or  $\gamma$  ( $n = 1$ –3 for each step in the complete metallation reaction). In the temperature-resolved experiments, the normalized relative abundances in each spectrum, following a specific reaction mixing time, were then plotted versus  $1/T$  ( $\text{K}^{-1}$ ). As kinetic data are more readily analyzed from concentration data as a function of



**Figure 14.4** pH dependence of cadmium speciation in rfMT. Cadmium stoichiometry in rfMT measured as a function of pH. The bars represent the range of pH values that the species can be detected in the mass spectrum. The relative abundances change according to the pH. (Reproduced with permission from Merrifield *et al.*, 2006. Copyright American Chemical Society.)



**Figure 14.5** ESI mass spectra from the analysis of solutions containing the apo-rfMT and increasing molar equivalents of As<sup>3+</sup> (A 0 As<sup>3+</sup>, B 1 As<sup>3+</sup>, C 2 As<sup>3+</sup>, D 3 As<sup>3+</sup>, E 4 As<sup>3+</sup>, F 5 As<sup>3+</sup>). The numbers on the peaks are the observed charged species for the reconstructed parent ions. Note that the dominant charge state decreases from +12 for the apo-protein, to +10 for the 3, 4 and 5-bound rf-MT. (Reproduced with permission from Merrifield *et al.*, 2004. Copyright Elsevier.)

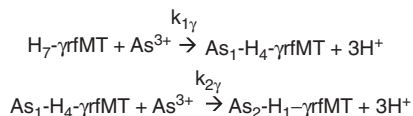
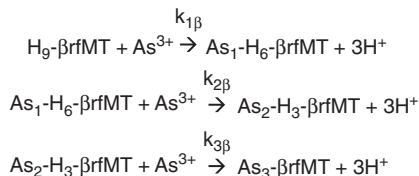


**Figure 14.6** Reconstructed spectra from the observed charge states of the spectra obtained from the addition of increasing molar equivalents of  $\text{As}^{\text{III}}$  (A-0  $\text{As}^{\text{III}}$ , B-1  $\text{As}^{\text{III}}$ , C-2  $\text{As}^{\text{III}}$ , D-3  $\text{As}^{\text{III}}$ , E-4  $\text{As}^{\text{III}}$ , F-5  $\text{As}^{\text{III}}$ ). The spectra were reconstructed using the Max Ent I program from Micromass Ltd. (Reproduced with permission from Merrifield *et al.*, 2004. Copyright Elsevier.)

time at a constant temperature, our kinetic data were rearranged into this format. This serves two purposes, first the analysis follows traditional procedures and second, the data may be directly compared to kinetic data obtained as a function of time at a fixed temperature. In addition, the data used in the subsequent analyses are averages of many different and independent datasets, which greatly improve the confidence in the calculated results. The full method has

been described in detail in previous papers by Ngu *et al.* for example (Ngu and Stillman, 2006).

It is important to recognize the complexity of the formation of  $\text{As}_5$ -rfMT. If two metal binding domains exist then a series of metallation pathways is possible, as shown in Figure 14.7. What we see in Figure 14.7 is the pathway to filling two isolated domains. In other words, the metallation in these two pathways continues independently even though

Scheme A –  $\gamma$ rfMTScheme B –  $\beta$ rfMT

**Scheme 14.1** The series of bimolecular reactions that form the sequential binding mechanism proposed for  $\text{As}^{3+}$  binding to the  $\gamma$  (A) and  $\beta$  (B) domains of rfMT. The rate constants for each step are indicated by  $k_{1,2,3}$  ( $\gamma$  or  $\beta$ ) which are for the rate of adding a single  $\text{As}^{3+}$  to the metallothionein domain to form a product with  $n$  (1, 2 or 3)  $\text{As}^{3+}$  bound. This same nomenclature is used in the text where the experimental values are reported. (Reproduced with permission from Ngu *et al.*, 2009. Copyright American Chemical Society.)

the two domains are linked together. We direct the reader to the detailed discussion of the metallation of multiple domains when short linkers are present in other recent papers by Ngu *et al.* (2008) and Ngu *et al.* (2010). To analyze the kinetic data, we used a minimization calculation that took into account the occupation of each binding site, based on the statistical probability of each species' presence. For example, the relative abundance of  $\text{As}_1\text{-rfMT}$  as measured in the mass spectrum is actually composed of two  $\text{As}_1\text{-rfMT}$  species: the first species contains one  $\text{As}^{3+}$  bound in the  $\beta$  domain and zero  $\text{As}^{3+}$  bound in the  $\gamma$  domain (shown as  $1\beta 0\gamma$  in Figure 14.7) while the second species contains zero  $\text{As}^{3+}$  bound in the  $\beta$  domain and one  $\text{As}^{3+}$  bound in the  $\gamma$  domain (shown as  $0\beta 1\gamma$  in Figure 14.7). The fractional occupation of these two domains ( $1\beta 0\gamma$  and  $0\beta 1\gamma$ ) was determined from the ratio of the individual rate constants,  $k_{1\gamma}$  and  $k_{1\beta}$ . This process was continued for each rate constant to fill the  $\beta$  and  $\gamma$  domains with  $\text{As}^{3+}$ . This is a complicated procedure made possible by the information contained in the ESI-MS data.

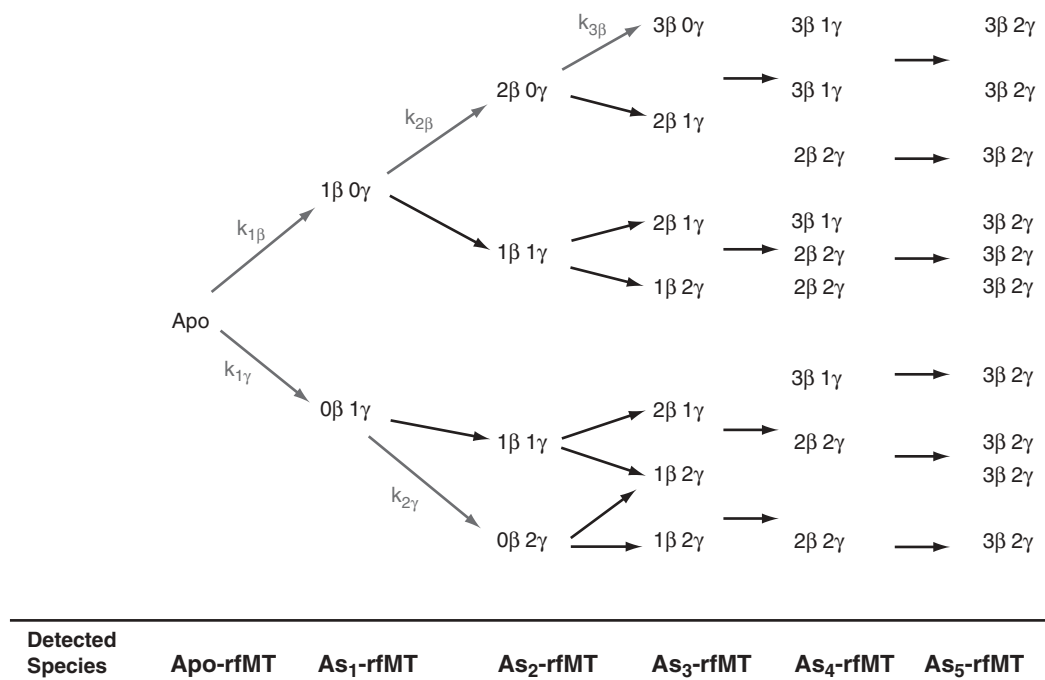
In this way the complexities of the individual time-dependent occupation of the domains by  $\text{As}^{3+}$ , as shown in Figure 14.7, were reduced to the time-dependent relative abundances of a series of five  $\text{As}^{3+}$ -containing protein species (Scheme 14.1), which were the same as measured experimentally (Figures 14.8 and 14.9). This method was more difficult and more complex than our previous studies (Ngu and Stillman, 2006; Ngu *et al.*, 2008).

Since the use of the ESI-MS technique to monitor metal binding when more than one metal is involved is currently rare, we have included a series of data sets to illustrate the several levels of information possible from this technique.

The first figure outlining mass spectral data recorded during the reaction of  $\text{As}^{3+}$  with apo-rfMT is shown in Figure 14.8 and depicts the progress of the metallation reaction at five fixed time points, namely, almost at the start, 2 min, then at 7, 20 and 80 min elapsed times. The ESI-MS charge state data were measured at mass units related to the mass/charge of the ion. An increase in the envelope charge state maximum is considered to be a result of increased exposure of the protonatable basic side chains in the protein due to an increase in volume of the protein. At the 2 minute point, the +10 and +11 charge states are maximal, but all the charge states from +6 to +12 arise from the apo-rfMT, which has a mass of 9512.1 Da. Figure 14.8 shows that there is a shift in the dominant charge states from +10/+11 for apo-rfMT to +8/+9 for  $\text{As}_5\text{-rfMT}$ , which suggests a less solvent accessible structure for the fully As-metallated species. It is clear from the time-dependent MS data in Figure 14.8 that all six species – that is apo-rfMT to  $\text{As}_5\text{-rfMT}$  – coexist and that over time the  $\text{As}_5\text{-rfMT}$  species grows in intensity.

The data were recorded in fine time steps with continuous infusion at the stated temperatures and analyzed using a reaction mechanism that required a series of consecutive bimolecular reactions leading to either the 7-cysteine gamma-domain or the 9-cysteine  $\beta$ -domain being filled with  $\text{As}^{3+}$  (Scheme 14.1 and Figure 14.7). The lower temperature experiment was chosen to expand the early stages of the reaction allowing easier analysis as the first  $\text{As}^{3+}$  bound relatively quickly. The two-domain pathway outlined in Scheme 14.1 and Figure 14.7 meant that the analysis of the ESI-MS data for the two-domains of the rfMT was much more complicated than in previous work, because the data represent the summation of all metallation products for both domains. Prior to all five sites being filled, the MS data simply show the total mass of the  $\text{As}^{3+}$  summed over both domains with no indication of the individual location of the incoming  $\text{As}^{3+}$ . However, it is clear that the distribution into each domain (the 9-cysteine  $\beta$  and the 7-cysteine  $\gamma$  domain) is controlled by the individual rate constants for the bimolecular reactions as shown in Scheme 14.1. Each symbol in Figure 14.9 represents the abundance of a single mass that is associated with a specific metallated species, as shown in the legend.

The lines in Figure 14.9 show the results of the fit but do not at this point show the occupancy of the individual domain, rather the lines in Figure 14.9 fit the experimentally determined data and, therefore, simulate the total number of  $\text{As}^{3+}$  ions that have bound to the protein, from zero (for apo-rfMT) to five for the saturated  $\text{As}_5\text{-rfMT}$ .



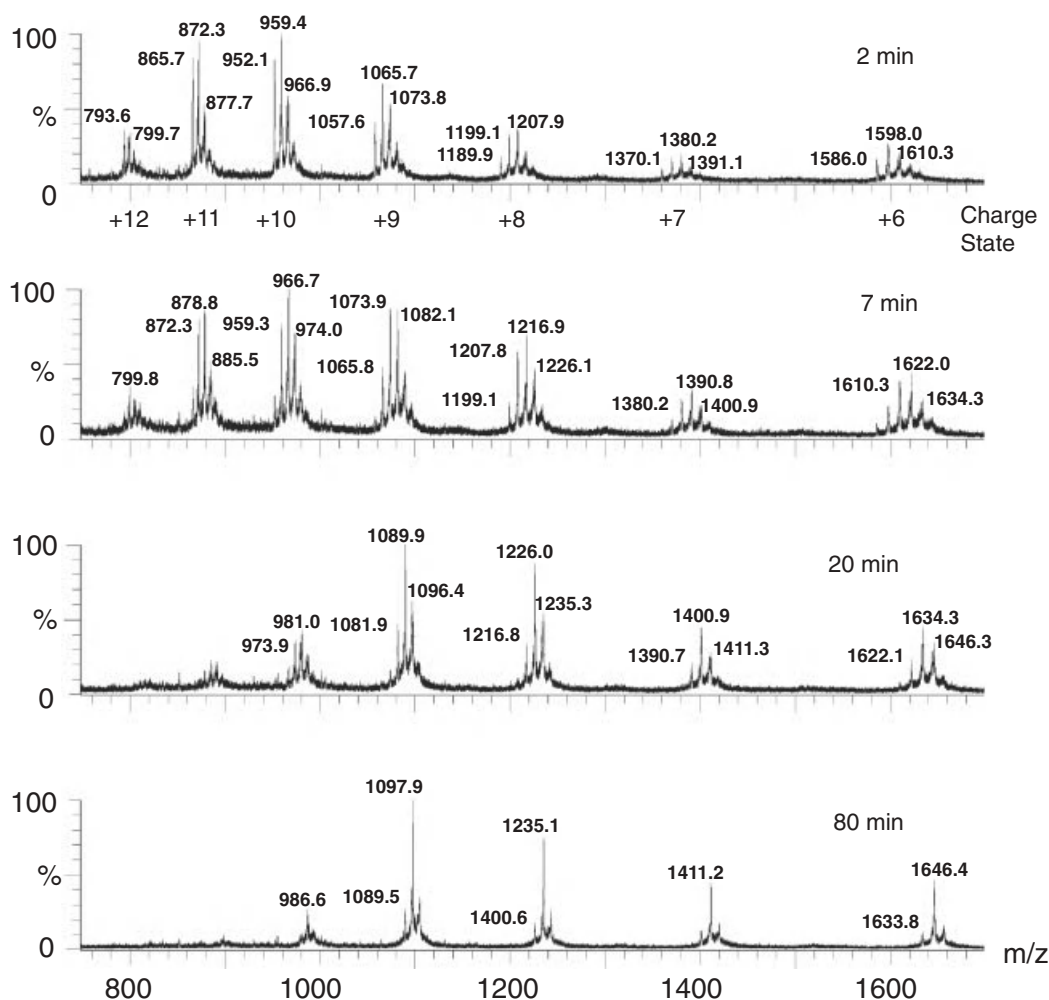
**Figure 14.7** Tree diagram illustrating the different metallation pathways for the reaction of  $\text{As}^{3+}$  with apo-rfMT, which result in various  $\text{As}_n$ -rfMT species if the binding mechanism shown in Figure 14.6 is followed. The reaction pathways and their associated rate constants for each domain are highlighted in red. Together these individual steps represent the complete model in fitting the experimental data, however, the detected species shown at the bottom of the figure are observed in the ESI mass spectra. The black lines show the complexities introduced by the simultaneous metallation of both domains into the mass spectral data, hence at each step, binding to the  $\beta$  or  $\gamma$  domain is possible. The relative distribution between the two domains is directly related to the rate constants. (Reproduced with permission from Ngu *et al.* 2009. Copyright American Chemical Society.)

The kinetic parameters, which were determined following minimization of the mechanism in Scheme 14.1 with five different rate constants provided all the parameters necessary to determine the individual domain distributions of  $\text{As}^{3+}$  during the metallation reaction, as is shown in Figure 14.10. In addition, Figure 14.10 provides a simulation of the metallation of the individual  $\gamma$  and  $\beta$  domains taking place simultaneously in the two-domain protein. The experimental data cannot discriminate between the domain occupied and cannot distinguish which domain has filled first. But the significant difference in rate constant value allows the calculated distribution to be tested against the experimental data. Combining the relative abundance of each species in Figure 14.10 and taking into account the probability of the distribution of  $\text{As}^{3+}$  between the two domains, gives the observed ESI MS data in Figure 14.9. So the fitted lines in Figure 14.9 represent the “goodness of fit” but do not indicate the partial occupancy of the domains. The rate constants ( $\text{M}^{-1}\text{s}^{-1}$ ) at 298 K calculated from the fits for the  $\gamma$  domain are:  $k_{1\gamma}$ , 19.8, and  $k_{2\gamma}$ , 1.4, and for the  $\beta$  domain:  $k_{1\beta}$ , 16.3,  $k_{2\beta}$ , 9.1 and  $k_{3\beta}$ , 2.2 (Figure 14.9).

The rate constants ( $\text{M}^{-1}\text{s}^{-1}$ ) at 286 K calculated from the fits for the  $\gamma$  domain are:  $k_{1\gamma}$ , 11.3, and  $k_{2\gamma}$ , 0.6, and for the  $\beta$  domain:  $k_{1\beta}$ , 9.7,  $k_{2\beta}$ , 4.0 and  $k_{3\beta}$ , 1.1 (Figure 14.10).

The results of the analysis shown in Figure 14.10 indicate that each domain of rfMT binds the  $\text{As}^{3+}$  ions independently in a series of two (for  $\gamma$ ) and three (for  $\beta$ ) sequential bimolecular reactions. There is no indication of cooperativity in the metallation reactions of each individual domain, that is, each of the step-wise species form in sequence rather than predominantly forming the final product,  $\text{As}_3$ - $\beta$ -domain or  $\text{As}_2$ - $\gamma$ -domain at the expense of the partially metallated species.

We have in other studies of human MT that  $\text{As}^{3+}$ -metallation of human MT is temperature- and time-dependent (Ngu *et al.*, 2006, 2008) Further, using the ESI mass spectrometer and a thermostatted mixing tee, we have shown that it was possible to measure the time-, temperature- and concentration-dependence of the reaction of  $\text{As}^{3+}$  with human MTs and extract the activation energies and Arrhenius factors for each step (Ngu *et al.*, 2006, 2008) In this current chapter, we



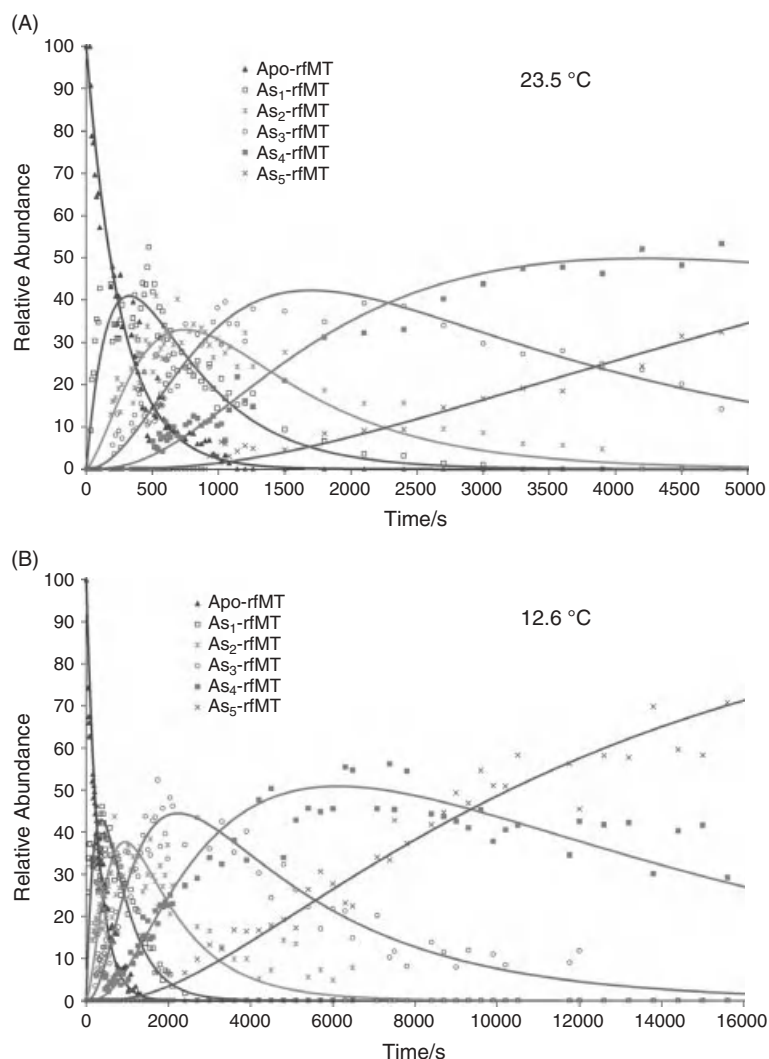
**Figure 14.8** Time-resolved ESI MS data recorded over 80 min for a solution of apo rfMT (14  $\mu\text{M}$ ) following mixing with an  $\text{As}^{3+}$  solution (261  $\mu\text{M}$ ) at 298 K. The charge states are shown underneath the top spectrum. Each spectrum corresponds to a different reaction time (2, 7, 20, and 80 min). Of special note is the change in dominant charge state from +10 at 2 min to +9 at 80 min, coupled with the complete loss of charge states for +12 and +11. (Reproduced with permission from Ngu *et al.* 2009. Copyright American Chemical Society.)

describe kinetic data that were determined for the  $\text{As}^{3+}$ -metallation of the more complex, two-domain rfMT protein for a series of fixed reaction times over a wide range of temperatures.

Figure 14.11 shows the 3D representation of the relative concentrations of each As-species in the metallation of apo-rfMT with  $\text{As}^{3+}$  from  $\text{As}_1$ -H<sub>13</sub>-rfMT to  $\text{As}_5$ -H<sub>1</sub>-rfMT as a function of time and temperature using the temperature-resolved ESI MS data. Contour plots are also included in Figure 14.11. Multiple datasets of the reaction concentrations during a set time course were extracted at a range of reaction temperatures from the 3D plots and analyzed using the reaction mechanism in Scheme 14.1 and

Figure 14.7. The data shown in Figure 14.11 are the raw mass spectral relative abundances of each individual species extracted from the spectral data that was recorded over the completed reaction time and for a range of temperatures. This allows the “life” of each species to be visualized. We should comment that in this specific case, each species although a discrete mass representing the protein bound to 0–5  $\text{As}^{3+}$  – this figure does not indicate in which domain the  $\text{As}^{3+}$  is bound.

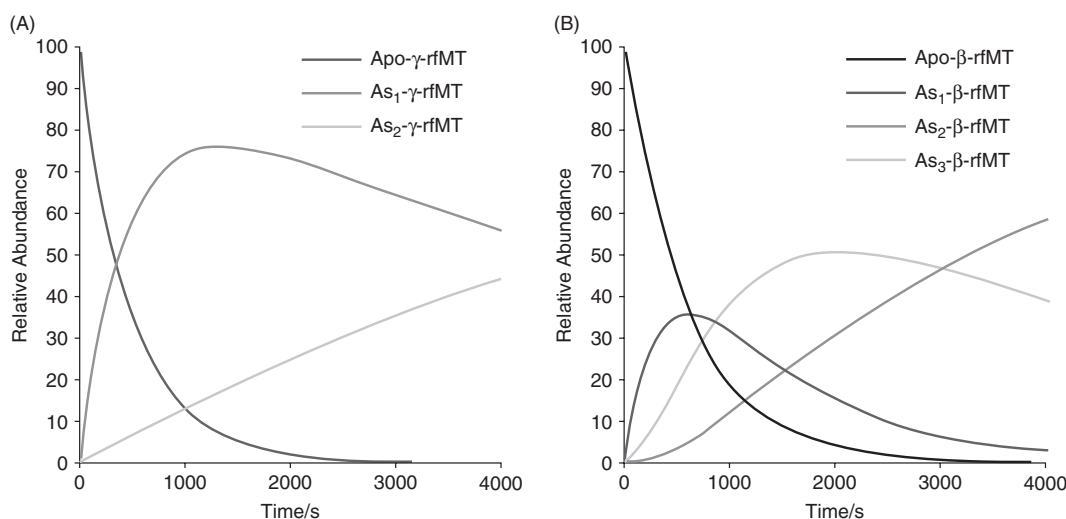
It is clear that the results of these new methods that employ a mixture of experimental and sophisticated analyses tools must be verified. We achieved this in each of our studies by testing the validity of our methods and the accuracy of



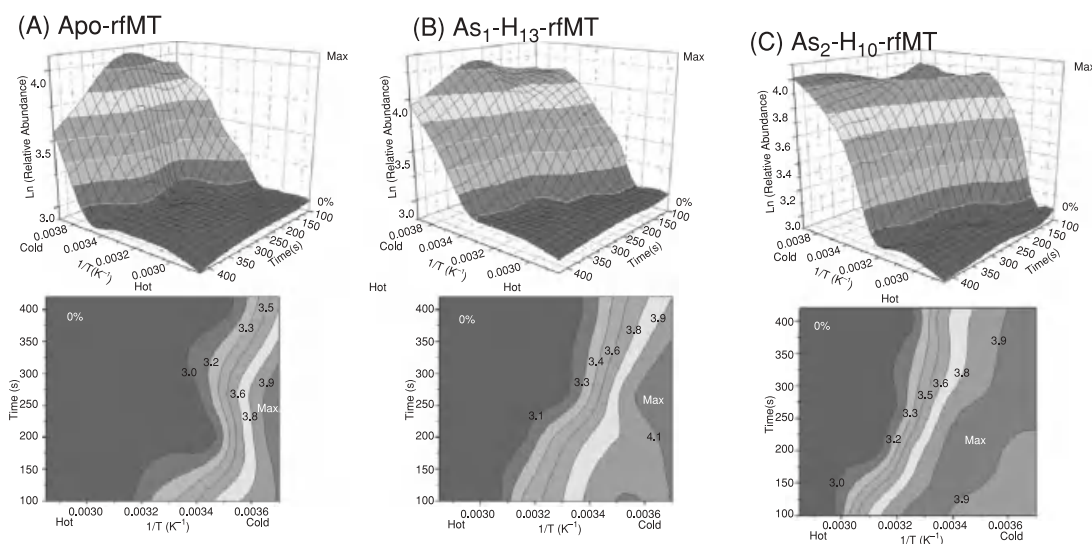
**Figure 14.9** Time-resolved, experimental and theoretical relative abundances for apo rfMT and  $\text{As}_n$ -rfMT ( $n = 1-5$ ) following reaction of  $9 \mu\text{M}$  apo rfMT with  $1081 \mu\text{M}$   $\text{As}^{3+}$  at  $23.5^\circ\text{C}$  and  $12.6^\circ\text{C}$  over  $16\,000\text{ s}$  reaction time. The legend identifies the time-course of the abundances for each mass (symbols). The lines were calculated based on minimization of the parameters in the complete analysis of the kinetic data for the relative abundance at each of the specified times using the series of sequential bimolecular reactions shown in Scheme 14.1. (Reproduced with permission from Ngu *et al.* 2009. Copyright American Chemical Society.)

the parameters calculated by simulating the relative abundance for each  $\text{As}_n$ -rfMT species ( $n = 0-5$ ) for every data point measured during the temperature-resolved experiments (Figure 14.12). The smooth lines in Figure 14.12 are simulations of the five bimolecular reactions occurring over the range of reaction times and temperatures shown. The simulations are reliant on the accuracy of the calculation used to determine each temperature-dependent rate constant that was based on analysis of the data and subsequent estimation of the step-wise activation energies and Arrhe-

nus constants,  $A$ , shown in Table 14.1. In view of the inherent difficulties involved in these temperature- and time-dependent experiments, we believe that the fits of the data are remarkably good. In Figure 14.12A, it is clear that at the  $102\text{ s}$  reaction time and at cool temperatures, the dominant species are apo- and  $\text{As}_{1-2}$ -rfMT, however at the same reaction time but at higher temperatures, the dominant species are  $\text{As}_{4-5}$ -rfMT, clearly showing that the reaction progresses more rapidly at higher temperatures. The same trend is observed for all other reaction times (Figure 14.12 B–D).



**Figure 14.10** Simulation of the sequential binding mechanism shown in Scheme 14.1 for  $\text{As}^{3+}$  binding to the  $\gamma$ -rfMT (a) and  $\beta$ -rfMT (b) domains over 4000 s. Time dependence of the fractional concentrations are plotted as the relative abundances of the protein species that form following mixing of  $9 \mu\text{M}$  apo rfMT with  $108 \mu\text{M}$   $\text{As}^{3+}$  at  $23.5^\circ\text{C}$  using the rate constants reported in the text. Model based on an  $\text{As}^{3+}$ :rfMT ratio of 12:1. (Reproduced with permission from Ngu *et al.* 2009. Copyright American Chemical Society.)



**Figure 14.11** The trends in concentrations of the individual  $\text{As}_n$ -rfMT ( $n = 0-5$ ) species as a function of temperature,  $1/T$  ( $\text{K}^{-1}$ ) and time following mixing apo-rfMT with  $\text{As}^{3+}$ . The diagrams were constructed from a series of ESI-MS traces recorded between 273 K and 346 K, and for reaction times between 102 and 402 s, from different solutions each with an  $\text{As}^{3+}$ : rfMT stoichiometric ratio of 131:1 with solutions containing  $7.6 \mu\text{M}$  apo-rfMT and  $2.5 \text{ mM}$   $\text{As}^{3+}$ . (Top) 3D visualization of  $\ln(\text{Relative Abundance})$  versus  $1/T$ , and reaction time (on the z-axis) for a single component species extracted from the total reaction. (Bottom) Contour diagrams calculated from the 3D plots. It is important to note that to aid visualization and clarity of the trend in concentration as a function of time and temperature, each 3D plot has been orientated so that the 0% relative abundance of the specific species is at the front. For example, for apo-rfMT (A) and  $\text{As}_{1-3}$ -rfMT (B-D), the conditions for 0% relative abundances are high temperatures and long reaction times and the 3D plots are orientated such that high temperatures and long reaction times are at the front. The contour diagrams provide more detail of the reaction profiles. (Reproduced with permission from Ngu *et al.* 2009. Copyright American Chemical Society.)

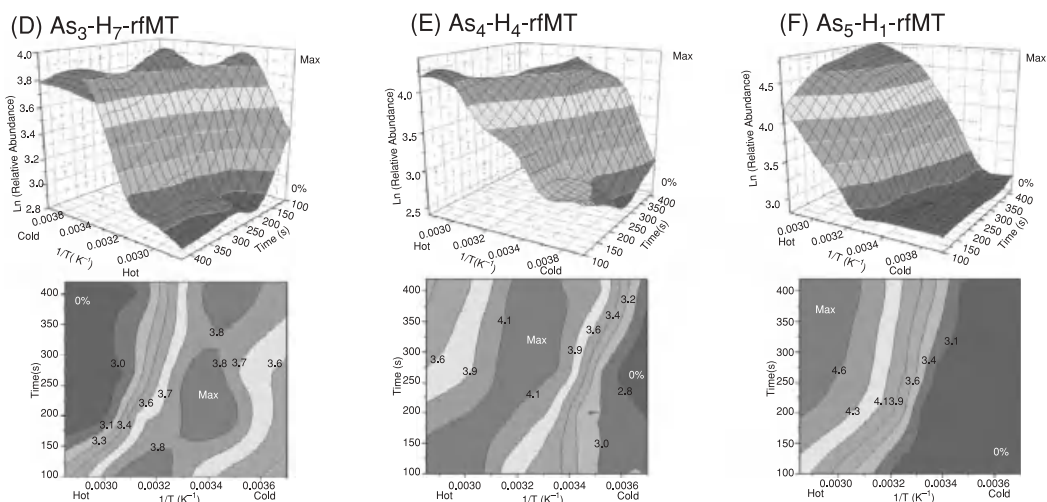
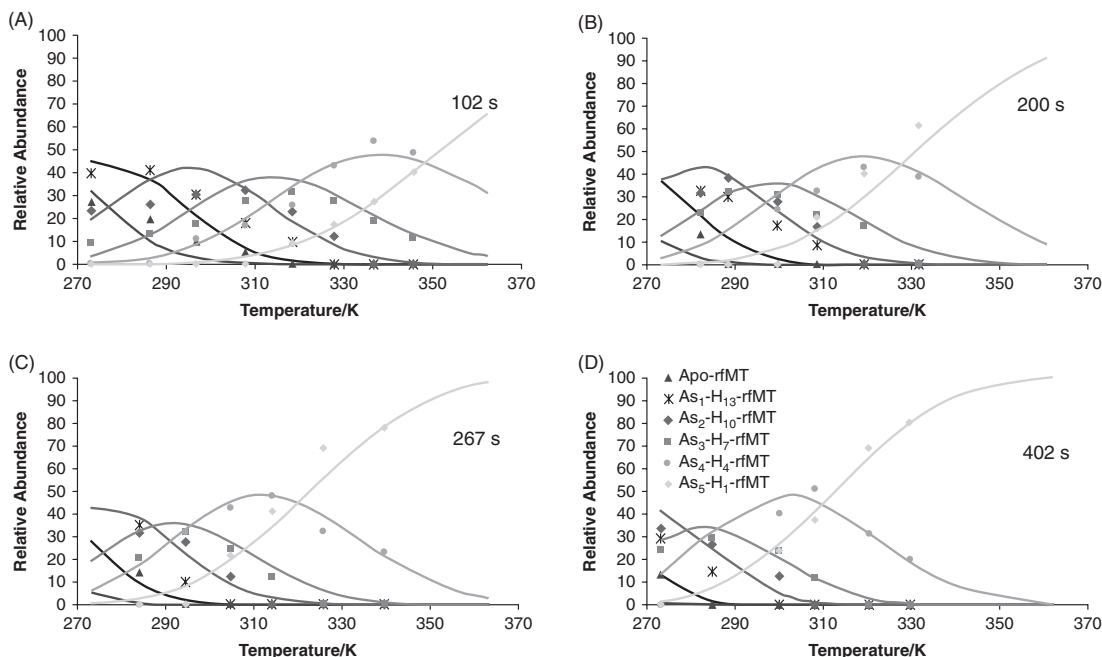


Figure 14.11 (Continued)



**Figure 14.12** Tests of the analytical methods in reproducing the measured kinetic data. Temperature-resolved relative abundances of apo-rfMT and  $\text{As}_n$ -rfMT ( $n = 1-5$ ) following reaction of apo-rfMT with  $\text{As}^{3+}$  at fixed reaction times. ESI MS data were obtained for apo-rfMT at a series of fixed reaction times with increasing temperatures (273–346 K) in the presence of excess  $\text{As}^{3+}$ . The fixed reaction times were 102 s (A), 200 s (B), 267 s (C), and 402 s (D). The reaction was carried out with an  $\text{As}^{3+}$ : rfMT stoichiometric ratio of 131:1 with solutions containing 7.6  $\mu\text{M}$  apo-rfMT and 2.5 mM  $\text{As}^{3+}$ . The smooth lines were calculated based on the complete analysis of the kinetic data for the relative abundance of each component species for every set temperature at the specified times. The lines are connected by a series of five sequential reactions shown in Scheme 14.1 and described in the text. The data points that comprise the theoretical lines were calculated from analysis of data sets measured at all reaction times and temperatures to obtain the kinetic parameters:  $k_{\text{temp}}$ ,  $A$ ,  $E_A$ , which were used with the experimental concentrations of the protein and the  $\text{As}^{3+}$  to predict the concentration of each species at the specified temperature and time. There will always be considerable uncertainty in any simulation that uses parameters extracted from the entire temperature-time-relative abundance data set that comprise many different experiments. (Reproduced with permission from Ngu *et al.* 2009. Copyright American Chemical Society.)

**Table 14.1** Rate constants, activation energies ( $E_A$ ), Arrhenius factor ( $A$ ), activation enthalpies ( $\Delta H^\ddagger$ ), activation entropies ( $\Delta S^\ddagger$ ), and activation free energies ( $\Delta G^\ddagger$ ) for the  $\text{As}^{3+}$ -induced metallation of the rfMT at pH 3.5

Arsenic species formed	$n^a$	$k_n^a$ at 298 K (mol/s)	$E_A^b$ (kJ/mol)	$A$ ( $10^{-5}$ mol/s)	$\Delta H^\ddagger$ (kJ/mol)	$\Delta S^\ddagger$ (J/K/mol)	$\Delta G^\ddagger$ at 298 K (kJ/mol)
$\text{As}_1\text{-H}_4\text{-}\gamma\text{rfMT}$	$1\gamma$	19.8	34	231	32	-112	65
$\text{As}_2\text{-H}_1\text{-}\gamma\text{rfMT}$	$2\gamma$	1.4	$33 \pm 14$	$9.2 \pm 4$	$31 \pm 14$	$-138 \pm 115$	$72 \pm 69$
$\text{As}_1\text{-H}_6\text{-}\beta\text{rfMT}$	$1\beta$	16.3	32	62	29	-123	66
$\text{As}_2\text{-H}_3\text{-}\beta\text{rfMT}$	$2\beta$	9.1	$35 \pm 9$	$77 \pm 18$	$33 \pm 9$	$-121 \pm 49$	$69 \pm 34$
$\text{As}_3\text{-}\beta\text{rfMT}$	$3\beta$	2.2	$27 \pm 7$	$1.6 \pm 0.4$	$25 \pm 6$	$-153 \pm 81$	$71 \pm 42$

<sup>a</sup>For reactions as shown in Scheme 14.1, where  $k_n$  refers to the rate constant for a single step that involves addition of a single As forming a product with  $n$  As bound. Each rate constant has a percent standard deviation of  $\pm 7\%$

<sup>b</sup>A temperature range of 270–350 K and 51 data points were used to construct the 3D plots that generated these activation parameters.

Reproduced with permission from Ngu *et al.* (2009). Copyright American Chemical Society.

## 14.5 Conclusions

Our goal in this chapter was to illustrate the extraordinary power of the ESI-MS technique in determining complex metallation properties of metalloproteins, here the MT from *Fucus vesiculosus*. The detailed results described here rely heavily on the ESI-MS technique. As is clear from the many and varied studies published recently, the ESI-MS technique provides vastly more information concerning metallation studies than could be obtained previously with this precision and speed. It is the unambiguous nature of the data that is so persuasive. However, we still require the spectroscopic tools, for instance, here the absorption spectrum tells us that the  $\text{Cd}^{2+}$  is binding to the thiols of the cysteines and the CD provides folding information. The data we describe here are unusual for MTs because the seaweed *Fucus vesiculosus* MT protein has two domains connected by a long interdomain linker. This is atypical for MTs, when compared to mammalian MTs, although is common in MTs from plant and algae (Morris *et al.*, 1999; Robinson *et al.*, 1993; Suzuki *et al.*, 1993) The analysis of the stepwise metallation data was complicated requiring a statistical probability analysis based on the individual specific rate constants and metal-domain occupation within the individual domains.

A major result of the analysis of the ESI-MS data described above is that the two domains in rfMT act independently when binding  $\text{As}^{3+}$ , whereas, studies of  $\text{As}^{3+}$ -metallation of recombinant human  $\beta\alpha$ MT show that the two domains in that protein interact during metallation (Ngu *et al.*, 2008) We believe that the increase in interdomain space for rfMT compared to human MT allows the

domains in rfMT to act independently of each other when the protein metallates.

## Acknowledgments

We thank NSERC of Canada for equipment, operating and student support funding. We are grateful to the Department of Biochemistry, University of Western Ontario for use of the cell culture equipment.

## References

- Attwood, P.V. and Geeves, M.A. (2004) Kinetics of an enzyme-catalyzed reaction measured by electrospray ionization mass spectrometry using a simple rapid mixing attachment. *Anal. Biochem.*, **334**, 382–389.
- Bilecen, K., Ozturk, U.H., Duru, A.D., *et al.* (2005) *Triticum durum* metallothionein. *J. Biol. Chem.*, **280**, 13701–13711.
- Blindauer, C.A., Polfer, N.C., Keiper, S.E., *et al.* (2003) Inert site in a protein zinc cluster: isotope exchange by high resolution mass spectrometry. *J. Am. Chem. Soc.*, **125**, 3226–3227.
- Bothner, B., Chavez, R., Wei, J., *et al.* (2000) Monitoring enzyme catalysis with mass spectrometry. *J. Biol. Chem.*, **275**, 13455–13459.
- Boulanger, Y., Armitage, I.M., Miklossy, K.-A. and Winge, D.R. (1982)  $^{113}\text{Cd}$  NMR study of a metallothionein fragment: evidence for a two-domain structure. *J. Biol. Chem.*, **257**, 13717–13719.

- Chan, J., Huang, Z., Watt, I., *et al.* (2007) Characterization of the conformational changes in recombinant human metallothioneins using ESI-MS and molecular modeling. *Can. J. Chem.*, **85**, 898–912.
- Cobbett, C. and Goldsbrough, P. (2002) Phytochelators and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.*, **53**, 159–182.
- Daneshfar, R., Kitova, E.N. and Klassen, J.S. (2004) Determination of protein-ligand association thermochemistry using variable-temperature nanoelectrospray mass spectrometry. *J. Am. Chem. Soc.*, **126**, 4786–4784.
- Domenech, J., Mir, G., Huguet, G., *et al.* (2006) Plant metallothionein domains: functional insight into physiological metal binding and protein folding. *Biochimie*, **2006**, 583–593.
- Ejnik, J., Robinson, J., Zhu, J., *et al.* (2002) Folding pathway of apo-metalllothionein induced by  $Zn^{2+}$ ,  $Cd^{2+}$  and  $Co^{2+}$ . *J. Inorg. Biochem.*, **88**, 144–152.
- Hug, S., Wegelin, M., Gechter, D. and Canonica, L. (2000) Arsenic contamination of ground water: disastrous consequences in Bangladesh. *EAWAG News*, **49**, 18–20.
- Kagi, J.H.R. (1993) Evolution structure and chemical activity of class I metallothioneins: A personal perspective. In: *Metallothionein III* (eds K.T. Suzuki, N. Imura, and M. Kimura). Birkhauser Verlag, Switzerland, pp. 29–56.
- Krezel, A. and Maret, W. (2007) Dual nanomolar and picomolar Zn(II) binding properties of metallothionein. *J. Am. Chem. Soc.*, **129**, 10911–10921.
- Li, Z., Sau, A.K., Whitehouse, C., *et al.* (2003) A snapshot of enzyme catalysis using electrospray ionization mass spectrometry. *J. Am. Chem. Soc.*, **125**, 9938–9939.
- Loebus, J., Peroza, E.A., Blüthgen, N., *et al.* (2011) Protein and metal cluster structure of the wheat metallothionein domain  $\gamma$ -E(c)-1: the second part of the puzzle. *J. Biol. Inorg. Chem.*, **16**, 683–694.
- Margoshes, M. and Vallee, B.L. (1957) A cadmium protein from equine kidney cortex. *J. Am. Chem. Soc.*, **79**, 4813.
- Mead, M.N. (2005) Arsenic. In search of an antidote to a global poison. *Env. Health Persp.*, **113**, A378–A386.
- Merrifield, M.E., Chaseley, J., Kille, P. and Stillman, M.J. (2006) Determination of the Cd/S cluster stoichiometry in *Fucus vesiculosus* metallothionein. *Chem. Res. Toxicol.*, **19**, 365–375.
- Merrifield, M.E., Ngu, T. and Stillman, M.J. (2004) Arsenic binding to *Fucus vesiculosus* metallothionein. *Biochem. Biophys. Res. Commun.*, **324**, 127–132.
- Miranker, A., Robinson, C.V., Radford, S.E. and Dobson, C.M. (1996) Investigation of protein folding by mass spectrometry. *FASEB J.*, **10**, 93–101.
- Morris, C.A., Nicolaus, B., Sampson, V., *et al.* (1999) Identification and characterization of a recombinant metallothionein protein from a marine alga, *Fucus vesiculosus*. *Biochem. J.*, **338**, 553–560.
- Ngu, T.T. and Stillman, M.J. (2006) Arsenic binding to human metallothionein. *J. Am. Chem. Soc.*, **128**, 12473–12483.
- Ngu, T., Sturzenbaum, S.R. and Stillman, M.J. (2006) Cadmium binding studies to the earthworm *Lumbricus rubellus* metallothionein by electrospray mass spectrometry and circular dichroism spectroscopy. *Biochem. Biophys. Res. Commun.*, **351**, 229–233.
- Ngu, T., Easton, A. and Stillman, M. J. (2008) Kinetic analysis of arsenic-metallation of human metallothionein: Significance of the two-domain structure. *J. Am. Chem. Soc.*, **130**, 17016–17028.
- Ngu, T.T., Lee, J.A., Rushton, M.K. and Stillman, M. J. (2009) Arsenic-metallation of seaweed *Fucus vesiculosus* metallothionein: the importance of the interdomain linker in metallothionein. *Biochemistry*, **48**, 8806–8816.
- Ngu, T., Lee, J.A., Pinter, T. and Stillman, M. J. (2010) Arsenic-metallation of triple domain human metallothioneins: support for the evolutionary advantage and interdomain metalation of multiple-metal-binding domains. *J. Inorg. Biochem.*, **104**, 232–244.
- Otvos, J.D. and Armitage, I.M. (1980) Structure of the metal clusters in rabbit liver metallothionein. *Proc. Natl Acad. Sci. U.S.A.*, **77**, 7094–7098.
- Pattanaik, A., Bachowski, G., Laib, J., *et al.* (1992) Properties of the reaction of *cis*-Dichlorodiammineplatinum (II) with metallothionein. *J. Biol. Chem.*, **267**, 16121–16128.
- Peroza, E.A. and Freisinger, E. (2007) Metal ion binding properties of *Tricium asetivum* E<sub>c</sub>-1 metallothionein: evidence supporting two separate metal thiolate clusters. *J. Biol. Inorg. Chem.*, **12**, 377–391.
- Rhee, I.-K., Lee, K.S. and Huang, P.C. (1990) Metallothioneins with interdomain hinges expanded by insertion mutagenesis. *Protein Eng.*, **3**, 221–226.
- Rigby Duncan, K.E. and Stillman, M.J. (2007) Evidence for noncooperative metal binding to the  $\alpha$  domain of human metallothionein. *FEBS J.*, **274**, 2253–2261.
- Robinson, N.J. (1989) Algal metallothioneins: secondary metabolites and proteins. *J. Appl. Phycol.*, **1**, 5–18.
- Robinson, N.J., Tommey, A.M., Kuske, C. and Jackson, P. J. (1993) Plant metallothioneins. *Biochem. J.*, **295**, 1–10.
- Salgado, M.T. and Stillman, M.J. (2004)  $Cu^{+}$  distribution in metallothionein fragments. *Biochem. Biophys. Res. Commun.*, **318**, 73–80.
- Shoemaker, G.K., Kitova, E.N., Palcic, M.M. and Klassen, J.S. (2007) Equivalency of binding sites in protein-ligand complexes revealed by time-resolved tandem mass spectrometry. *J. Am. Chem. Soc.*, **129**, 8674–8675.

- Stillman, M.J. and Zelazowski, A.J. (1988) Domain specificity in metal binding to metallothionein. *J. Biol. Chem.*, **263**, 1–6.
- Stillman, M.J., Cai, W. and Zelazowski, A.J. (1987) Cadmium binding to metallothioneins: domain specificity in reactions of  $\alpha$  and  $\beta$  fragments, apometallothionein, and zinc metallothionein with  $\text{Cd}^{2+}$ . *J. Biol. Chem.*, **262**, 4538–4548.
- Stillman, M.J., Shaw, C.F., III and Suzuki, K.T. (1992) *Metallothioneins*. VCH Publishers, New York.
- Sturzenbaum, S.R., Kille, P. and Morgan, A.J. (1998) The identification, cloning and characterization of earthworm metallothionein. *FEBS Lett.*, **431**, 437–442.
- Sturzenbaum, S.R., Winters, C., Galay, M., *et al.* (2001) Metal ion trafficking in earthworms: identification of a cadmium-specific metallothionein. *J. Biol. Chem.*, **276**, 34013–34018.
- Suzuki, K.T., Imura, N. and Kimura, M. (1993) *Metallothionein III*. Birkhauser, Boston.
- Wilson, D.J. and Konermann, L. (2004) Mechanistic studies on enzymatic reactions by electrospray ionization MS using a capillary mixer with adjustable reaction chamber volume for time-resolved measurements. *Anal. Chem.*, **76**, 2537–2543.
- Yu, X., Wojciechowski, M. and Fenselau, C. (1993) Assessment of metals in reconstituted metallothioneins by electrospray mass spectrometry. *Anal. Chem.*, **65**, 1355–1359.
- Zaia, J., Fabris, D., Wei, D., *et al.* (1998) Monitoring metal ion flux in reactions of metallothionein and drug-modified metallothionein by electrospray mass spectrometry. *Protein Sci.*, **7**, 2398–2404.
- Zechel, D.L., Konermann, L., Withers, S.G. and Douglas, D.J. (1998) Pre-steady state kinetic analysis of an enzymatic reaction monitored by time-resolved electrospray ionization mass spectrometry. *Biochemistry*, **37**, 7664–7669.

# **PART III**

## **Biological Properties of Molecules Derived from Seaweeds**

# 15

## *In Vivo* and *in Vitro* Toxicity Studies of Fucoxanthin, a Marine Carotenoid

Yoshimi Niwano<sup>1</sup> and Fumiaki Beppu<sup>2</sup>

<sup>1</sup>Tohoku University Graduate School of Dentistry, Sendai, Miyagi, Japan

<sup>2</sup>Faculty of Fisheries, Hokkaido University, Minato, Hakodate, Hokkaido, Japan

### 15.1 Introduction

Various types of brown algae, such as hijiki (*Sargassum fusiforme*), kombu (*Laminaria japonica*), and wakame (*Undaria pinnatifida*), are used especially in East Asian diet. Fucoxanthin (FX) (Figure 15.1), an orange-colored xanthophyll derivative, is a major non-provitamin A carotenoid present in edible brown algae (Haugan *et al.*, 1992). It has been reported that FX shows antiobesity effect through uncoupling protein (UCP)1 expression in white adipose tissue (WAT) in KK-*A*<sup>y</sup> mice, an animal model of type 2 diabetes with obesity (Maeda *et al.*, 2005). FX, as well as its metabolite fucoxanthinol (FXOH), has been shown to reduce the expression of peroxisome proliferator-activated receptor (PPAR) $\gamma$  in 3T3-L1 preadipocytes, which in turn resulted in the inhibited differentiation to mature adipocytes (Maeda *et al.*, 2006), suggesting that dietary FX also acts as an inhibitor against adipocyte maturation in addition to as a stimulator for UCP1 expression in WAT.

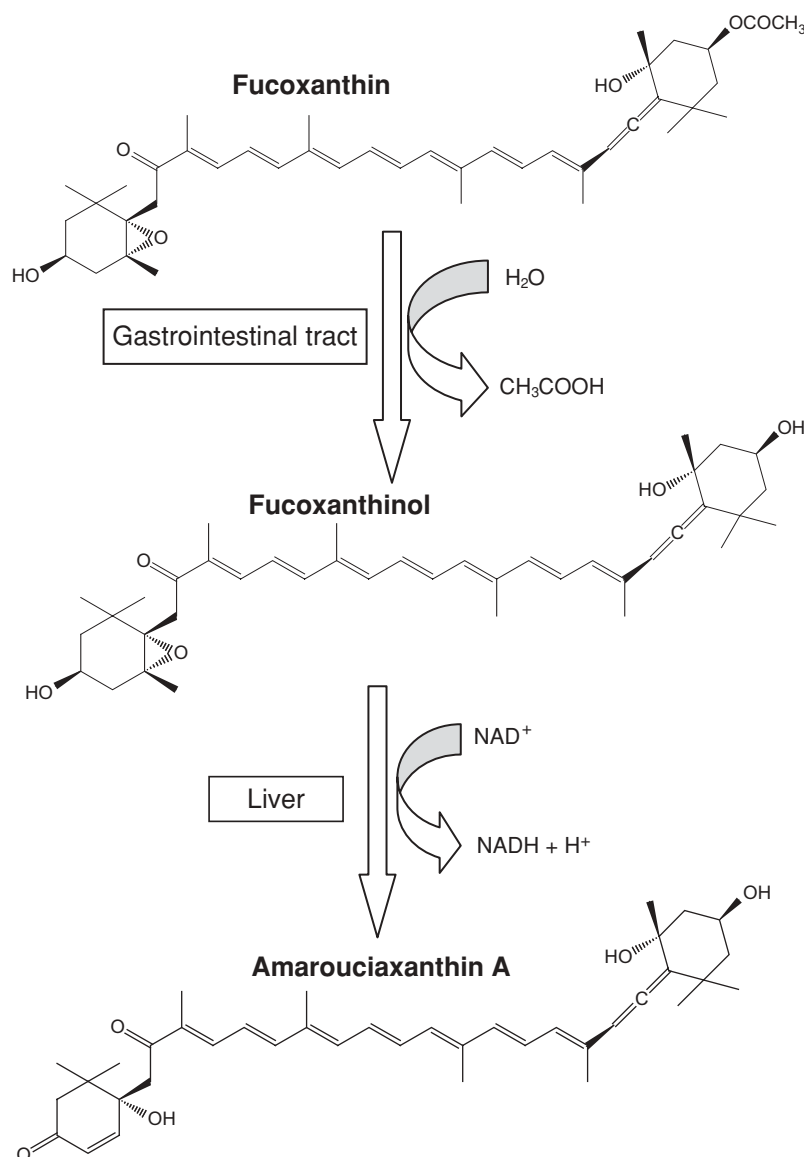
Besides the antiobesity effect, it has been reported that dietary FX or FXOH increases the amount of docosahexaenoic acid (DHA) in the liver of KK-*A*<sup>y</sup> mice (Tsukui *et al.*, 2007), and DHA is expected to induce clinically important benefits such as improvement of lipid metabolism, hypertension and Alzheimer's disease (Fischer *et al.*, 2008; Harris *et al.*, 2008; Kelley *et al.*, 2008; Lindmark and Clough, 2007; Ma *et al.*, 2007). *In vitro* and *in vivo* studies further revealed that FXOH and/or FX possesses antioxidant activity

(Sachindra *et al.*, 2007), anticancer activity against different kinds of cancer cells (Das *et al.*, 2008; Ishikawa *et al.*, 2008; Konishi *et al.*, 2006; Satomi and Nishino, 2007, 2009) and anti-inflammatory action (Shiratori *et al.*, 2005).

As described above, there are many reports on FX in terms of its effect on physiological and pharmacological implications. However, although dietary supplements containing FX have been developed in some countries, only a few studies have been conducted on the safety evaluation of purified FX. This paper reviews the toxicological aspect of FX based mainly on our studies (Beppu *et al.*, 2009a, b).

### 15.2 *In vivo* oral toxicity study

In our single oral dose study (Beppu *et al.*, 2009a), 6-week-old male and female ICR mice were orally administered with FX at doses of 1000 and 2000 mg/kg or its vehicle (0.5% (w/v) carboxymethyl cellulose-Na (CMC-Na) solution containing 0.5% (v/v) Tween 80) alone. No mortality and no abnormalities in gross appearance of the animals were observed during the experimental period. Body weight gain in any of the male and female FX groups was similar to that in the corresponding control groups, and no abnormal findings were observed in the macroscopic observation on day 14. Thus, the 50% lethal dose (LD50) of FX in mice is more than 2000 mg/kg.



**Figure 15.1** Proposed metabolic pathway of dietary fucoxanthin in mammals (Sugawara *et al.*, 2002; Asai *et al.*, 2004). FX is hydrolyzed to form FXOH in the gastrointestinal tract. Absorbed FXOH is dehydrogenated/isomerized to form amarouciaxanthin A in the liver.

Regarding repeated oral dose toxicity of FX, there have been only two studies reported so far. One is a rat study (Kadekaru *et al.*, 2009), and the other one is a mouse study (Beppu *et al.*, 2009a). In the rat study, 6-week-old male and female Crl:CD (SD) rats were orally administered with FX at doses of 10 and 50 mg/kg/day or its vehicle (corn oil) alone for consecutive 28 days. No mortality and no abnormalities in gross appearance of the animals were observed during the experimental period. In both male and female animals, body weight gain in FX 10 mg/kg and FX 50 mg/kg groups

was similar to that of the corresponding control groups, and no significant differences also found in feed intake. According to the urinary and hematologic examination conducted on the last day of the experiment, no changes with toxicological implications were found in any of the FX groups. In contrast, serum biochemical examination revealed that total cholesterol and/or high-density lipoprotein (HDL)-cholesterol levels were significantly elevated in any of the FX groups. That is, 28 days-repeated oral administration of FX at doses of 10 and 50 mg/kg to male and female

**Table 15.1** Plasma biochemical parameters reflecting liver and kidney functions in mice administered with fucoxanthin for 30 days

Sex	Male			Female		
	Fucoxanthin			Fucoxanthin		
Group	Vehicle	500 mg/kg	1000 mg/kg	Vehicle	500 mg/kg	1000 mg/kg
Total protein (g/dl)	5.26 ± 0.34	4.99 ± 0.35	5.16 ± 0.32	5.46 ± 0.32	5.20 ± 0.16	5.25 ± 0.20
Albumin (g/dl)	3.31 ± 0.24	3.00 ± 0.24*	3.08 ± 0.22	3.67 ± 0.26	3.44 ± 0.16*	3.49 ± 0.11
A/G	1.70 ± 0.12	1.51 ± 0.12	1.48 ± 0.10	2.08 ± 0.24	1.97 ± 0.17	2.00 ± 0.19
Total-bilirubin (mg/dl)	0.11 ± 0.02	0.30 ± 0.04**	0.39 ± 0.06**	0.03 ± 0.03	0.28 ± 0.04**	0.31 ± 0.04**
AST(U/l)	59 ± 19	65 ± 22	62 ± 16	95 ± 29	78 ± 24	65 ± 18
ALT (U/l)	25 ± 8	27 ± 6	29 ± 8	25 ± 7	23 ± 6	23 ± 6
ALP(U/l)	196 ± 47	204 ± 37	226 ± 70	335 ± 72	257 ± 54	259 ± 50
γ-GTP(U/l)	<LOQ	<LOQ	<LOQ	0.9 ± 1.2	0.4 ± 1.1	0.9 ± 1.8
Urea nitrogen (mg/dl)	21.6 ± 2.8	23.7 ± 3.6	23.3 ± 6.6	20.1 ± 4.9	25.5 ± 5.1*	21.4 ± 3.4
Uric acid (mg/l)	0.93 ± 0.23	0.95 ± 0.19	1.12 ± 0.14	0.95 ± 0.18	1.08 ± 0.23	0.96 ± 0.18
Creatinine (mg/dl)	0.09 ± 0.02	0.09 ± 0.02	0.11 ± 0.02	0.09 ± 0.02	0.11 ± 0.03	0.10 ± 0.02

Each value represents the mean and standard deviation of 10 mice.

Asterisks show significant differences from corresponding vehicle-treated control groups (\* $p < 0.05$ , \*\* $p < 0.01$ ) assessed by Dunnet's multiple comparison test.

Note that the Increased levels of total bilirubin in the FX groups were most likely false results, because the endpoint of bilirubin assay based on the vanadic acid oxidation method was proved to be interfered with by FXOH that is a major metabolite of FX in the circulation.

Reproduced from Beppu *et al.* (2009a) with permission.

rats resulted in significant increases in serum total cholesterol concentration to be approximately 1.5–1.7-fold and 1.9–2.2-fold, respectively. Any changes relevant to elevated cholesterol level were found in necropsy, organ weights, and histological findings.

In our mouse study where the animals were orally administered with FX at doses of 500 and 1000 mg/kg or its vehicle (CMC-Na solution containing 0.5% (v/v) Tween 80) alone, daily, for 30 days (Beppu *et al.*, 2009a), similar results to those in the rat study (Kadekaru *et al.*, 2009) were obtained. No mortality, as well as no abnormal changes or findings were observed in body weight gain, feed intake, organ weight, except for liver, as shown in Table 15.3, macroscopy, necropsy, and histological findings. However, as seen in Tables 15.1 and 15.2, where serum biochemical parameters are summarized, plasma total cholesterol and phospholipid concentrations increased significantly in any of the FX group as compared with those in the corresponding control groups. This cholesterol elevation by FX was reproduced in another repeated oral dose study (unpublished data). In the study, as is the case with the rat study (Kadekaru *et al.*, 2009), HDL-cholesterol also increased by FX. Regarding other carotenoids, it has been reported that rats fed with diets containing 0.1% of canthaxanthin and astaxanthin significantly increased plasma total cholesterol

levels to 1.8-fold and 1.3-fold, respectively (Murillo, 1992). In the case of astaxanthin, there have been three published subchronic oral toxicity studies. Ono *et al.* (1999) reported that dietary exposure of F344 rats to *Haemato-coccus* extract at the highest dietary dosage of 5% (equivalent to 0.25% astaxanthin; ~125 mg/kg/day) for 90 days did not affect body weight, feed intake, blood cell morphology, plasma biochemistry, organ weight or histology. Takahashi *et al.* (2004) also reported that daily oral (gavage) administration of an astaxanthin-rich extract of *H. pluvialis*, resulting in a daily intake of up to 50 mg astaxanthin/kg/day to Sprague–Dawley rats for 90 days, did not cause treatment-related adverse effects. Stewart *et al.* (2008) reported that dietary exposure of male and female Wistar rats to an astaxanthin-rich *H. pluvialis* biomass for 90 days, resulting in intakes of up to 500 mg astaxanthin/kg/day, did not show biologically significant adverse effect on a series of health-related parameters. However, in the study by Stewart *et al.*, total cholesterol concentration was significantly higher in animals receiving 50000 and 200000 ppm of the biomass. The authors speculated that the increase in cholesterol levels noted in high and intermediate-groups may be associated with the high fat content of the test article matrix. Besides astaxanthin-rich studies, rats fed with diets containing 0.63–5% *Dunaliella* carotene significantly increased

**Table 15.2** Plasma concentration of glucose, lipids and minerals in mice administered with fucoxanthin for 30 days

Sex	Male			Female		
	Fucoxanthin			Fucoxanthin		
Group	Vehicle	500 mg/kg	1000 mg/kg	Vehicle	500 mg/kg	1000 mg/kg
Glucose (mg/dl)	77 ± 17	86 ± 31	75 ± 8	81 ± 19	83 ± 21	81 ± 16
TG (mg/dl)	49.2 ± 19.6	87.5 ± 47.9	79.9 ± 41.8	38.9 ± 15.1	58.8 ± 42.6	62.6 ± 29.8
Total-cholesterol (mg/dl)	127 ± 25	216 ± 36**	249 ± 41**	107 ± 18	175 ± 15**	186 ± 23**
Phospholipids (mg/dl)	233 ± 28	320 ± 48**	340 ± 44**	195 ± 28	268 ± 22**	276 ± 29**
NEFA (μEq/l)	1210 ± 216	1234 ± 249	1393 ± 280	1442 ± 212	1495 ± 355	1579 ± 435
Calcium (mg/dl)	9.19 ± 0.26	9.15 ± 0.28	9.16 ± 0.30	9.65 ± 0.23	9.36 ± 0.31	9.29 ± 0.22
Inorganic phosphorus (mg/dl)	6.48 ± 0.40	7.53 ± 0.75**	7.44 ± 0.52**	7.44 ± 0.60	7.33 ± 0.97	6.54 ± 0.96
Magnesium (mg/dl)	2.06 ± 0.21	2.10 ± 0.23	2.05 ± 0.17	2.21 ± 0.17	2.14 ± 0.21	2.02 ± 0.12
Sodium (mmol l <sup>-1</sup> )	151.3 ± 1.0	151.8 ± 1.7	151.4 ± 0.9	151.9 ± 1.7	152.3 ± 1.6	149.6 ± 2.8
Potassium (mM)	6.50 ± 1.25	6.67 ± 1.37	6.89 ± 0.87	6.72 ± 1.02	6.47 ± 0.83	6.51 ± 0.95
Chloride (mM)	113.2 ± 1.8	113.7 ± 2.3	114.7 ± 2.5	115.5 ± 2.2	115.6 ± 2.2	113.2 ± 2.9

Each value represents the mean and standard deviation of 10 mice.

Asterisks show significant differences from corresponding vehicle-treated control groups (\* $p < 0.05$ , \*\* $P < 0.01$ ) assessed by Dunnet's multiple comparison test.

Reproduced from Beppu *et al.* (2009a) with permission.

serum total cholesterol levels in a dose dependent manner (Kuroiwa *et al.*, 2006), suggesting that some carotenoids may have an ability to increase circulating cholesterol level in rodents as a common feature. This point also needs to be clarified as well as the mechanisms by which FX and other xanthophylls increases circulating cholesterol. Besides the total cholesterol concentrations, plasma total bilirubin concentrations increased significantly in any of the FX groups. However, these increased levels of total bilirubin in the FX groups, as shown in Table 15.1, were likely false results, because the endpoint of bilirubin assay based on the vanadic acid oxidation method was proved to be interfered with by fucoxanthinol (FXOH), which is a major metabolite of FX in the circulation. Furthermore, since parameters for liver function other than total bilirubin, such as aspartate amino transferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP) were not influenced by FX, and histological observation for liver sections showed no abnormal changes by FX, it was suggested that the increased level of total bilirubin by FX was most likely artifact caused by substances such as FXOH existed in the plasma. Indeed, in the rat study (Kadekaru *et al.*, 2009), no increases in the bilirubin concentration were found in the FX groups.

Besides purified FX, two extracts from the brown seaweed *Fucus vesiculosus* containing 28.8% polyphenols or 18% polyphenols plus 0.0012% fucoxanthin were studied to determine their toxicity in mice and rats (Zaragoza *et al.*,

2008). The extracts were shown to lack any relevant toxic effects in an acute toxicity test in rats and mice, and in a 4 week toxicity study in rats. However, in terms of organ weight, relative liver weight increased in male rats treated with the high dose of one extract. The authors speculated that the increased liver weight stemmed from neither fat accumulation nor alcohol. Moreover, they concluded that the absence of any changes in liver functional parameters (transaminases, ALP, bilirubin, urea and creatinine) supports a lack of toxicity in the liver. In our FX study, the relative liver weight increased significantly by the administration of FX (Table 15.3). As is the case with the study on *Fucus vesiculosus* extracts (Zaragoza *et al.*, 2008), no abnormal histological findings were observed by the light microscopy, and no abnormal values were found in liver functional parameters. Thus, it is speculated that one of the possibilities is induction of cytochrome P450 in the liver as reported in the previous studies (Amacher *et al.*, 1998; Khan *et al.*, 1998; Lake *et al.*, 1984), which needs to be confirmed by biochemical analysis in the future studies.

### 15.3 *In vitro* and *in vivo* mutagenicity study

For *in vitro* toxicity studies, it is important to figure out which metabolites exist in the circulation. As shown in Figure 15.1, it is proposed that dietary FX is hydrolyzed

**Table 15.3** Relative tissues weight (g/100 g body weight) of mice administered with fucoxanthin for 30 days

Sex	Male			Female		
	Fucoxanthin			Fucoxanthin		
Group	Vehicle	500 mg/kg	1000 mg/kg	Vehicle	500 mg/kg	1000 mg/kg
Body weight (g)	34.7 ± 1.7	34.5 ± 1.3	35.4 ± 1.4	28.3 ± 1.1	28.5 ± 1.4	29.3 ± 1.6
Liver	3.72 ± 0.16	4.33 ± 0.31**	4.17 ± 0.38**	3.91 ± 0.29	4.43 ± 0.22**	4.60 ± 0.25**
Kidney	1.47 ± 0.19	1.54 ± 0.12	1.52 ± 0.27	1.23 ± 0.11	1.24 ± 0.08	1.28 ± 0.07
Spleen	0.22 ± 0.06	0.22 ± 0.03	0.21 ± 0.04	0.30 ± 0.05	0.37 ± 0.10	0.40 ± 0.07*
Testes	0.81 ± 0.07	0.79 ± 0.05	0.78 ± 0.09	—	—	—
Prostate	1.03 ± 0.16	1.22 ± 0.16*	1.08 ± 0.11	—	—	—
Ovary	—	—	—	0.10 ± 0.03	0.09 ± 0.01	0.09 ± 0.02
Uterus	—	—	—	0.51 ± 0.13	0.50 ± 0.16	0.53 ± 0.10

Each value represents the mean and standard deviation of 10 mice.

Asterisks show significant differences from corresponding vehicle-treated control groups (\* $p < 0.05$ , \*\* $p < 0.01$ ) assessed by Dunnet's multiple comparison test.

Reproduced from Beppu *et al.* (2009a) with permission.

into FXOH in the gastrointestinal tract before absorption from the intestine. Then, the absorbed FXOH is metabolized to amarouciaxanthin A in the liver (Asai *et al.*, 2004; Sugawara *et al.*, 2002). Indeed, it was also reported that FXOH is a major gastrointestinal metabolite of dietary FX in humans, indicating that the major compound in the circulation after FX intake is FXOH (Asai *et al.*, 2008). Therefore, in an *in vitro* mutagenicity assay, FXOH instead of FX should be used even though S9 mix is used to estimate the effect of metabolites formed in the liver. In our study (Beppu *et al.*, 2009b), mutagenicity of FXOH *in vitro* was evaluated by the Ames test, which is the bacterial reverse mutation test developed by Ames *et al.* (1972 and 1973), and mutagenicity of FX *in vivo* was evaluated by the micronucleus test, which has been a commonly used assay for the screening of potential genotoxicants (MacGregor *et al.*, 1987).

In Ames test, in any of the FXOH-treated tester strains regardless of the presence or absence of S9, a liver subcellular fraction that contains drug-metabolizing enzymes including the cytochromes P450, flavin monooxygenases, and uridine diphosphate, the number of colonies was less than twice the number of colonies in the corresponding negative control. Table 15.4 shows the result of assay with S9 (data not shown for without S9). Since sedimentation of FXOH was observed at doses of 78.1 µg/plate or more, effective concentrations might be reduced by the sedimentation in a range of high doses of FXOH. Under these conditions, the results indicate that the mutagenicity of FXOH, a mother compound in the circulation after dietary FX intake, was found to be negative in all test strains.

In the micronucleus assay, preliminary study was conducted to determine the sampling time. In one study, animals were orally administered with FX at a dose of 2000 mg/kg, and the bone marrow cells were taken 24, 48, and 72 h after the administration. In the other study, FX at dose of 2000 mg/kg was orally administered to animals twice or three times at 24 h intervals. No clear differences were found in the incidences of micronucleus cells among the experimental groups. In addition, it is indicated that growth of bone marrow cells was not inhibited in any of the experimental groups, since no differences were found in the PCE (polychromatic erythrocytes): NCE (normochromatic erythrocytes) ratio among the groups. Based on the result of the preliminary study, the bone marrow specimens were taken 24 h after the single administration of FX. The doses of FX in the final study were 500, 1000 and 2000 mg/kg, and CPA (cyclophosphamide) at 50 mg/g was used as a positive control. The results are summarized in Table 15.5. The incidence of micronucleus cells in the CPA treated group was significantly higher than that of the negative control group. In the FX-treated groups, no such differences of the incidence of micronucleus cells were found as compared with that of the negative control group. The estimated PCE:NCE ratio in the bone marrow preparations of FX-treated groups also showed no significant differences from that in the negative control group, indicating no cytotoxic effects. Hence, these results indicate that FX has no genotoxic/mutagenic effects on the bone marrow cells of mice *in vivo*. In the aforementioned oral dose study, FX at 2000 mg/kg as the maximal dose was orally administered to mice, and neither mortality nor abnormal findings were observed. In

**Table 15.4** Analysis of the mutagenicity of fucoxanthinol in four strains of *Salmonella typhimurium* and one strain of *Escherichia coli* with S9 mix

			The number of revertant colonies (colonies/plate)				
			Base-pair substitution type			Frameshift type	
						TA98	TA1537
Concentration (μg/plate)			TA100	TA1535	WP2 <i>uvrA</i>		
Control	DMSO		102	11	27	39	33
Fucoxanthinol		39.1	139				
		78.1 +	141				
		156 +	138	12	27	39	33
		313 +	166	13	25	38	30
		625 +	127	10	21	32	18
		1250 +	150	17	21	32	16
		2500 +	140	13	27	37	14
		5000 +	149	7	27	28	17
Positive control	2-AA	0.5				534	
		1	975				
		2		498			169
		10			930		

Each data represents the mean of duplicate cultures.

2-AA: 2-Aminoanthracene

+: Sediment of fucoxanthinol was observed.

Reproduced from Beppu *et al.* (2009b) with permission.

**Table 15.5** Analysis of mutagenicity of fucoxanthin (Fx) in the micronucleus test in the bone marrow cells of mice

Group	Number of MNPCE per animal					Total (%)	
	1	2	3	4	5	MNPCE/PCE	PCE/Erythrocytes
Negative control	7	3	12	7	9	$0.38 \pm 0.16$	$53.0 \pm 0.70$
Fx 500 mg/kg	6	8	5	4	8	$0.31 \pm 0.08$	$51.5 \pm 5.45$
Fx 1000 mg/kg	10	8	1	4	10	$0.33 \pm 0.18$	$50.8 \pm 4.92$
Fx 2000 mg/kg	5	8	3	7	5	$0.28 \pm 0.09$	$53.6 \pm 3.78$
CPA 50 mg/kg	23	19	17	30	25	$1.14 \pm 0.23^{**}$	$46.5 \pm 6.50$

The number of micronucleated polychromatic erythrocytes (MNPCE) was evaluated in the bone marrow cells of mice 24 hr after the single administration.

Total (%) is expressed as the mean  $\pm$  standard deviation.

PCE: Polychromatic erythrocytes

CPA: Cyclophosphamide used as a positive control

Asterisk shows significant differences from the negative control ( $p < 0.01$ ) assessed by Dunnet's multiple comparison test

Reproduced from Beppu *et al.* (2009b) with permission.

this study, no significant difference in the incidence of micronucleus cells was induced by FX even at the maximal dose (2000 mg/kg).

Regarding astaxanthin, two mutagenicity studies have been reported so far (Takahashi *et al.*, 2004, 2010). In their studies, no mutagenic activity of astaxanthin was detected when assayed by *in vitro* Ames test, *in vitro* chromosomal aberration test, and *in vivo* micronucleus test.

## 15.4 Conclusion

Our study with mice clearly showed that consecutive oral administration of FX at a high dose induces hypercholesterolemia (Beppu *et al.*, 2009a). In addition, the rat study also clearly showed the ability of FX, even at a dose as low as 10 mg/kg, to increase blood cholesterol level (Kadekaru *et al.*, 2009). It is well known that animal models have limitations. One of the major limitations is related to cholesterol and lipoprotein metabolism since these lipid metabolic pathways significantly vary in animals as compared with those in humans (Green and Moghadasian, 2004; Groot *et al.*, 2005; Dietschy and Turley, 2002). For instance, unlike humans, rabbits and mice lack hepatic lipase and cholesteryl ester transfer protein, respectively. Mice and rats have poor cholesterol absorption, while rabbits absorb cholesterol more efficiently, making this species closer to humans. Therefore, it is not necessarily the case that FX induces hypercholesterolemia in humans, even though it increases blood cholesterol level significantly in rodents. Nevertheless, attention should be paid to lipid metabolism when dietary supplements containing FX are developed. Regarding mutagenicity of dietary FX, the data in our study permit us to presume that orally administered FX is a safe compound under the experimental conditions employed here.

## References

- Amacher, D.E., Schomaker, S.J. and Burkhardt, J.E. (1998) The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies. *Food Chem. Toxicol.*, **36**, 831–839.
- Ames, B.N., Durston, W.E., Yamasaki, E. and Lee, F.D. (1973) Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. *Proc. Natl Acad. Sci. USA*, **70**, 2281–2285.
- Ames, B.N., Gurney, E.G., Miller, J.A. and Bartsch, H. (1972) Carcinogens as frameshift mutagens: Metabolites and derivatives of 2-acetylaminofluorene and other aromatic amine carcinogens. *Proc. Natl Acad. Sci. USA*, **69**, 3128–3132.
- Asai, A., Sugawara, T., Ono, H. and Nagao, A. (2004) Bio-transformation of fucoxanthinol into amarouciaxanthin A in mice and HepG2 cells: formation and cytotoxicity of fucoxanthin metabolites. *Drug Metab. Disp.* **32**, 205–211.
- Asai, A., Yonekura, L. and Nagao, A. (2008) Low bioavailability of dietary epoxyxanthophylls in humans. *Br. J. Nutr.*, **100**, 273–277.
- Beppu, F., Niwano, Y., Tsukui, T., *et al.* (2009a) Single and repeated oral dose toxicity study of fucoxanthin, a marine carotenoid, in mice. *J. Toxicol. Sci.*, **34**, 501–510.
- Beppu, F., Niwano, Y., Sato, E., *et al.* (2009b) *In vitro* and *in vivo* evaluation of mutagenicity of fucoxanthin and Its metabolite fucoxanthinol. *J. Toxicol. Sci.*, **34**, 693–698, 2009.
- Das, S.K., Hashimoto, T. and Kanazawa, K. (2008) Growth inhibition of human hepatic carcinoma HepG2 cells by fucoxanthin is associated with down-regulation of cyclin D. *Biochim. Biophys. Acta*, **1780**, 743–749.
- Dietschy, J.M. and Turley, S.D. (2002) Control of cholesterol turnover in the mouse. *J. Biol. Chem.*, **277**, 3801–3804.
- Fischer, R., Dechend, R., Qadri, F., *et al.* (2008): Dietary n-3 polyunsaturated fatty acids and direct rennin inhibition improve electrical remodeling in a model of high human renin hypertension. *Hypertension*, **51**, 540–546.
- Green, T.J. and Moghadasian, M.H. (2004) Species-related variations in lipoprotein metabolism: the impact of FFER(HDL) on susceptibility to atherogenesis. *Life Sci.*, **74**, 2441–2449.
- Groot, P.H., Pearce, N.J., Yates, J.W., *et al.* (2005) Synthetic LXR agonists increase LDL in CETP species. *J. Lipid Res.*, **46**, 2182–2191.
- Harris, W.S., Miller, M., Tighe, A.P., *et al.* (2008) Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. *Atherosclerosis*, **197**, 12–24.
- Haugan, J.A., Aakermann, T., and Liaaen-Jensen, S. (1992) Isolation of fucoxanthin and peridinin. *Methods Enzymol.*, **213**, 231–245.
- Ishikawa, C., Tafuku, S., Kadekaru, T., *et al.* (2008) Anti-adult T-cell leukemia effects of brown algae fucoxanthin and its deacetylated product, fucoxanthinol. *Int. J. Cancer*, **123**, 2702–2712.
- Kadekaru, T., Toyama, H. and Yasumoto, T. (2008) Safety evaluation of fucoxanthin purified from *Undaria pinnatifida*. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **55**, 304–308 (in Japanese).
- Kelley, D.S., Siegel, D., Vemuri, M., *et al.* (2008): Docosahexaenoic acid supplementation decreases remnant-like particle-cholesterol and increases the (n-3) index in hypertriglyceridemic men. *J. Nutr.*, **138**, 30–35.
- Khan MA, Jovanovich LV, Martin LT, *et al.* (1998) Effects of photoisomers of cyclodiene insecticides on liver and

- microsomal cytochrome P450 in rats. *Arch. Toxicol.*, **72**, 74–83.
- Konishi, I., Hosokawa, M., Sashima, T., *et al.* (2006) Halocynthiaxanthin and fucoxanthinol isolated from *Halocynthia roretzi* induce apoptosis in human leukemia, breast and colon cancer cells. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.*, **142**, 53–59.
- Kuroiwa, Y., Nishikawa, A., Imazawa, T., *et al.* (2006) A subchronic toxicity study of dunaliella carotene in F344 rats. *Food Chem. Toxicol.* **44**, 138–145.
- Lake, B.G., Rijcken, W.R., Gray, T.J., *et al.* (1984) Comparative studies of the hepatic effects of di- and mono-n-octyl phthalates, di-(2-ethylhexyl) phthalate and clofibrate in the rat. *Acta Pharmacol. Toxicol. (Copenh)*, **54**, 167–176.
- Lindmark, L. and Clough, P. (2007) A 5-month open study with long-chain polyunsaturated fatty acids in dyslexia. *J. Med. Food*, **10**, 662–666.
- Ma, Q.L., Teter, B., Ubeda, O.J., *et al.* (2007) Omega-3 fatty acid docosahexaenoic acid increases SorLA/LR11, a sorting protein with reduced expression in sporadic Alzheimer's disease (AD): relevance to AD prevention. *J. Neurosci.* **27**, 14299–14307.
- MacGregor, J.T., Heddle, J.A., Hite, M., *et al.* (1987) Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. *Mutat. Res.* **189**, 103–112.
- Maeda, H., Hosokawa, M., Sashima, T., *et al.* (2005) Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commun.*, **332**, 392–397.
- Maeda, H., Hosokawa, M., Sashima, T., *et al.* (2006) Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *Int. J. Molec. Med.*, **18**, 147–152.
- Murillo, E. (1992) Hypercholesterolemic effect of canthaxanthin and astaxanthin in rats. *Archivos Latinoamericanos de Nutrición* **42**, 409–413 (in Spanish).
- Ono, A., Sekita, k., Saitoh, M., *et al.* (1999) A 13-week subchronic oral toxicity study of *Haematococcus color*. *Kokuritsu Iyakuhiin Shokuhin Eisei Kenkyusho Hokoku (Bulletin of National Institute of Health Sciences)* **117**, 91–98 (in Japanese).
- Takahashi, J., Tsukahara, H. and Minota, S. (2004) Toxicological studies of astaxanthin from *Haematococcus pluvialis* – Ames test, oral single dose and 90-days subchronic toxicity studies in rats. *J. Clin. Ther. Med.*, **20**, 867–881 (in Japanese).
- Takahashi, J., Tsukahara, H. and Hotta, Y. (2010) Toxicological studies of astaxanthin from *Haematococcus pluvialis*: II: *In Vivo* micronucleus test in mice and *in vitro* chromosome aberration test using mammalian cultured cells of AstaREAL Oil 50F. *J. Clin. Ther. Med.*, **26**, 287–295.
- Tsukui, T., Konno, K., Hosokawa, M., *et al.* (2007) Fucoxanthin and fucoxanthinol enhance the amount of docosahexaenoic acid in the liver of KKAY obese/diabetic mice. *J. Agric. Food Chem.*, **55**, 5025–5029.
- Sachindra, N.M., Sato, E., Maeda, H., *et al.* (2007) Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *J. Agric. Food Chem.*, **55**, 8516–8522.
- Satomi, Y. and Nishino, H. (2007) Fucoxanthin, a natural carotenoid, induces G1 arrest and GADD45 gene expression in human cancer cells. *In Vivo*, **21**, 305–309.
- Satomi, Y. and Nishino, H. (2009) Implication of mitogen-activated protein kinase in the induction of G1 cell cycle arrest and gadd45 expression by the carotenoid fucoxanthin in human cancer cells. *Biochim. Biophys. Acta*, **1790**, 260–266.
- Shiratori, K., Ohgami, K., Ilieva, I., *et al.* (2005) Effects of fucoxanthin on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. *Exp. Eye Res.*, **81**, 422–428.
- Stewart, J.S., Lignell, A., Pettersson, A., *et al.* (2008) Safety assessment of astaxanthin-rich microalgae biomass: Acute and subchronic toxicity studies in rats. *Food Chem. Toxicol.*, **46**, 3030–3036.
- Sugawara, T., Baskaran, V., Tsuzuki, W. and Nagao, A. (2002) Brown algae fucoxanthin is hydrolyzed to fucoxanthinol during absorption by Caco-2 human intestinal cells and mice. *J. Nutr.*, **132**, 946–951.
- Zaragoza, M.C., López, D., P. Sáiz, M., *et al.* (2008) Toxicity and antioxidant activity *in vitro* and *in vivo* of two *Fucus vesiculosus* extracts. *J. Agric. Food Chem.*, **56**, 7773–7780.

# 16

## Brown Seaweed Lipids as Potential Source of Omega-3 PUFA in Biological Systems

Kazuo Miyashita<sup>1</sup>, Bhaskar Narayan<sup>2</sup>, Takayuki Tsukui<sup>1</sup>, Hiroyuki Kamogawa<sup>1</sup>, Masayuki Abe<sup>1,3</sup> and Masashi Hosokawa<sup>1</sup>

<sup>1</sup>*Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan*

<sup>2</sup>*Department of Meat, Fish & Poultry Technology, CFTRI, Mysore, India*

<sup>3</sup>*Kaneka Co., Kita-ku, Osaka, Japan*

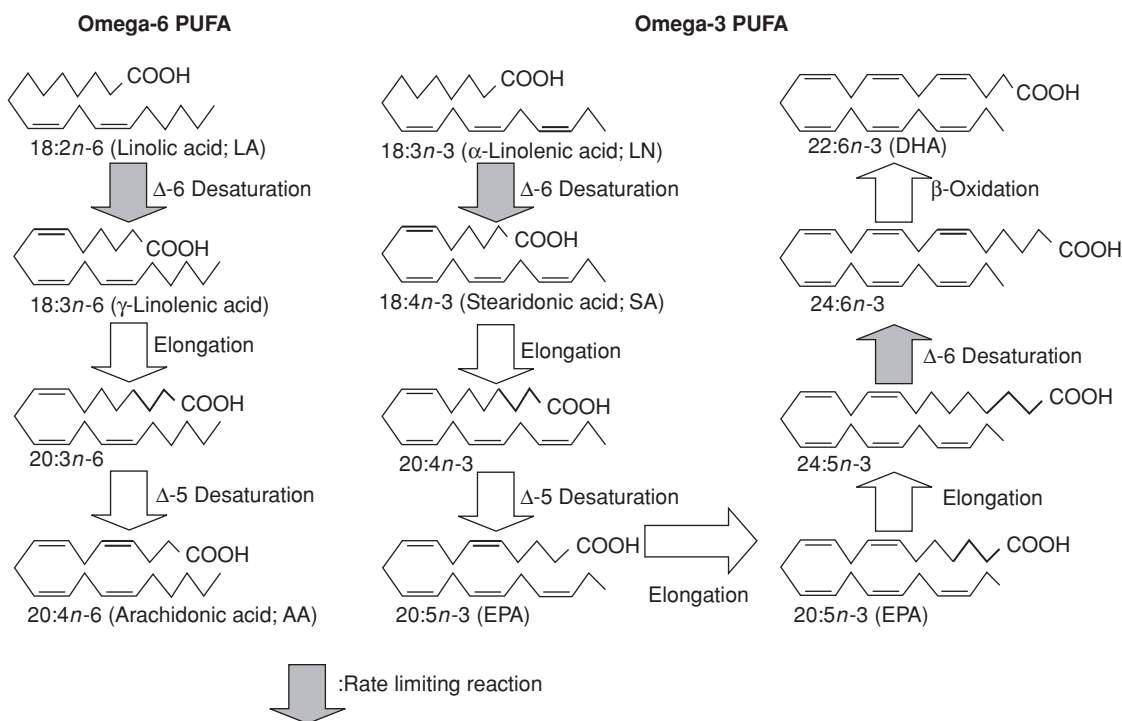
### 16.1 Introduction

The occurrence of polyunsaturated fatty acids (PUFA), mainly omega-3 fatty acids, that have been found to have significant health effects, is unique feature of marine lipids. Although the marine animals have been exploited thoroughly as source of omega-3 PUFA, their plant counterparts, seaweeds, have not been exploited to the same extent. Unlike their terrestrial counterparts, seaweeds have not been looked upon as important substrates for further processing of their lipids due to the relatively small amounts of lipids present in them. In spite of their low lipid content PUFA, and other lipid-related compounds from seaweeds, have aroused considerable interest among researchers for their nutritional impact on human health.

The fatty acid composition of algae is connected with environmental factors (Kaneniwa *et al.*, 1987) that in turn influence the comparisons between algae from different parts of the world (Stefanov *et al.*, 1988). Added to this, other researchers have found that distribution of fatty acids in seaweeds to be closely linked with the taxonomic position (Kayama *et al.*, 1989; Narayan *et al.*, 2008). Studies on differ-

ent lipid classes and their fatty acid composition would be helpful in locating the sources of PUFA that have profound physiological benefits and will also provide substantial information for chemotaxonomic inferences (Narayan *et al.*, 2008).

Docosahexaenoic acid (22:6 $n$ -3, DHA) and arachidonic acid (20:4 $n$ -6, AA) are important PUFA for human health. Both PUFA are derived from essential  $\alpha$ -linolenic acid (18:3 $n$ -3, LN) and linoleic acid (18:2 $n$ -6, LA), respectively. The same enzymatic systems are involved in the biosynthesis of DHA and AA. We have found that the brown seaweed carotenoid, fucoxanthin, increased DHA content in mouse liver and that this effect would be attributed to the up-regulation of related enzymes (Tsukui *et al.*, 2007, 2009). Brown seaweeds, especially harvested in cold waters, contain high levels of omega-3 PUFA such as stearidonic (18:4 $n$ -3, SA) and eicosapentaenoic acid (20:5 $n$ -3, EPA) (Li *et al.*, 2002; Narayan *et al.*, 2004; Terasaki *et al.*, 2009). These omega-3 PUFA can be more easily convert to DHA in biological systems as compared with LN mainly originated from vegetable oils, suggesting that brown seaweed lipids will be a good source of DHA.



**Figure 16.1** Molecular pathway of omega-6 and omega-3 PUFA metabolism.

Interest in functionality of marine nutraceuticals continues to grow year by year, due to the fact that prevention of disease through marine dietary means has been better understood and recognized by the public at large. Seaweed lipids, especially brown seaweed lipids, have drawn increased interest due to several health benefits they afford. The major interest has been paid to omega-3 PUFA and fucoxanthin, a characteristic carotenoid found in brown seaweeds (Dembitsky and Maoka, 2007; Marquardt and Hanelt, 2004; Matsuno, 2001; Schubert *et al.*, 2006). This chapter focuses mainly on the brown seaweed lipids as a good source of omega-3 PUFA along with special emphasis on the DHA-increasing effect of fucoxanthin in biological systems.

## 16.2 Omega-3 and omega-6 PUFA

Traditionally, LA (omega-6) and LN (omega-3) are considered the only essential fatty acids. Essential fatty acids play a key role in many metabolic processes, and cannot be synthesized by mammals. Since animals lack a  $\Delta$ -15 or  $\Delta$ -12 desaturase, they are unable to form omega-3 or omega-6 PUFA *de novo* and must be acquired through dietary sources (Innis 2003; Jump 2004; Sampath and Ntambi, 2004). From two 18-carbon essential PUFA, LA and LN, all

downstream fatty acids can be synthesized (Figure 16.1). Among these downstream products are the highly physiologically relevant AA, DHA, and EPA. These are considered critical metabolites as they are important precursors to both eicosanoids and prostanoids, mediating numerous physiological and biochemical processes. AA, EPA, and DHA are sometimes considered conditional essential fatty acids because their production may be inadequate in certain conditions such as prematurity and periods of growth, thus requiring exogenous supplementation.

EPA and DHA are omega-3 PUFA found in marine lipids. Both long-chain PUFA have been shown to cause significant biochemical and physiological changes in the body (Lands, 2005; Li *et al.*, 2003; Narayan *et al.*, 2006; Shahidi and Miraliakbari, 2006; Sinclair *et al.*, 2005) that most of the times result in positive influence on human nutrition and health. On the other hand, omega-6 PUFA and their derivatives, mainly AA, play an important role in biological system such as in the immune response, in thrombosis, and in brains (Hoffman *et al.*, 2009; Le *et al.*, 2009).

Simopoulos (2008) demonstrated that today in Western societies the omega-6/omega-3 ratio is about 16/1 due to the high intake of vegetable oils such as soybean, corn, sunflower, and safflower oils, which are high in LA. Indeed, the ratio of omega-6/omega-3 PUFA in the food chain in Europe (Sanders, 2000) and United States (Kris-Etherton

*et al.*, 2000) is still much higher than that recommended by FAO or WHO. Although it is difficult to establish the favorable ratio of omega-6 and omega-3 in dietary lipids, it is apparent that the adequate intake of omega-3 PUFA is higher than that found in Western diet (Russo, 2010). Therefore, marine oils and several vegetable oils such as canola oil should be regarded as healthy oil because of its relatively higher level of omega-3 PUFA.

## 16.3 Importance of omega-3 PUFA on human health

Substantial epidemiological and case-control study data demonstrate that the risk of coronary heart disease (CHD) is lowest among those with the highest long chain omega-3 PUFA such as EPA and DHA intake (Calder, 2004; Kris-Etherton *et al.*, 2002; Leaf *et al.*, 2008; Wang *et al.*, 2006). The important cardioprotective effect of omega-3 PUFA has been also demonstrated by clinical implications (Russo, 2010) and by genetic and nutrigenetic approach (Allayee *et al.*, 2009). Thus, American and European heart associations recommend the intake of 1 g/day of EPA and DHA for prevention of sudden cardiac death and other cardiovascular dysfunctions (De Backer *et al.*, 2003; Smith *et al.*, 2006). It is apparent that EPA and DHA consumption is of benefit in reducing the risk of CHD. Cardioprotection has been extensively reviewed elsewhere and is thought to occur through various mechanisms, including the reduction of serum triacylglycerol (TG) levels, anti-arrhythmic effects, decreasing platelet aggregation, plaque stabilization, and/or reduction of blood pressure (Allayee *et al.*, 2009; Givens and Gibbs, 2008; Harris *et al.*, 2008; Leaf *et al.*, 2008; Russo, 2010; Tziomalos *et al.*, 2008).

High TG levels have been shown to be an independent risk factor for CHD in a meta-analysis of 17 large, population-based studies ( $N > 56,000$ ) (Hokanson and Austin, 1996). EPA and DHA can alter the serum lipid profile. The most consistent action is a reduction in TG levels. EPA and DHA also increase high-density lipoprotein cholesterol levels in many studies. However, DHA and EPA supplementation does not affect total cholesterol (TC) levels, and it also reduces the proportion of small dense low-density lipoprotein (LDL) particles (Kelley *et al.*, 2007; Satoh *et al.*, 2007), which are potentially more atherogenic (Gazi *et al.*, 2007). The TG-lowering effect of EPA and DHA are not completely understood, but several potential mechanisms are as follows; reduction of hepatic very low-density lipoprotein (VLDL)-TG synthesis and secretion, enhancement of TG clearance from chylomicrons and VLDL particles (Davidson, 2006; Harris and Bulchandani, 2006).

EPA and DHA have been expected to prevent atherosclerosis (Leaf *et al.*, 2008) through the reduction of serum lipid levels and blood pressure. Atherosclerosis is one of the main conditions affecting the coronary arteries. In the development of atherosclerosis gradual uptake of oxidized LDL by the endothelium occurs and results in an inflammatory response leading to deposition of plaques in the arterial walls. Oxidized LDL also stimulates endothelial cells to produce chemokines and other factors that have direct chemotactic activity for monocytes to adhere to the endothelium. In addition, oxidized LDL is preferentially taken up by macrophage cells via scavenger receptors, and they consequently become loaded with lipids and convert into "foam cells". These foam cells tend to accumulate in fatty streaks, by which the inside diameter of the vein is reduced. Thus, blood flow is restricted, which can aggravate or produce hypertension and eventually cause irreparable damage to the heart.

Anti-atherosclerotic effect of EPA and DHA will be also related to regulation of eicosanoid productions (Calder, 2006; Mori and Beilin, 2004; Vila, 2004). Importantly, the effects of eicosanoids can be driven, in large part, by competition between AA and EPA as substrates for enzymes that catalyze release of the fatty acids from cell membranes or their conversion into a variety of metabolites. The activities of cyclo-oxygenases (COX) and lipoxygenases (LOX) on AA leads to the synthesis of 2-series prostanoids and 4-series leukotrienes, while EPA are converted by the same enzymes to 3-series prostanoids and 5-series leukotrienes, respectively. In most cases EPA is a poor substrate for COX and LOX enzymes than AA, suggesting that the biological activities of COX- and LOX-derived EPA eicosanoids are strictly depend upon their intracellular availability, with respect to AA-derived compounds present at higher concentrations (Jump, 2002).

The 2-series prostaglandins and the 4-series leukotrienes exert a more potent proinflammatory effect compared to 3-series prostaglandins and the 5-series leukotrienes. Thus, the lower concentration of AA in favor of EPA induces the less production of specific cell types such as monocytes, neutrophils, and eosinophils, that cause atherosclerosis. Furthermore, increasing EPA intake and, consequently, decreasing the lipid AA/EPA ratio, result in the reduction of the pro-thrombotic agent thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and the production of the anti-inflammatory agent prostaglandin E<sub>3</sub> (PGE<sub>3</sub>) and thromboxanes with reduced pro-aggregatory and vasoconstrictive properties (TXA<sub>3</sub>).

Marine omega-3 PUFA such as EPA and DHA reduce vascular risk not only by preventing the development of atherosclerosis and atherothrombosis, but also through their anti-arrhythmic properties. Several epidemiologic studies showed that increased dietary intake of EPA

and DHA reduces the risk of sudden cardiac death (Albert *et al.*, 2002; Daviglus *et al.*, 1997; Mozaffarian *et al.*, 2003). The molecular explanation for the anti-arrhythmic effects of omega-3 PUFA has not been fully elucidated. Human data are strongly supported by observational and interventional studies, but lack a mechanistic demonstration from a molecular point of view. Data obtained on animal models and cultured cardiomyocytes suggest the involvement of the activity of myocyte sarcolemma ion channels (sodium and L-type calcium). Harris *et al.* (2008) demonstrated that EPA and DHA are incorporated into myocardial cell membranes and can modulate electrophysiological properties of cardiac myocytes, potentially altering both eicosanoid production and ion-channel function.

Another important role of DHA is an essential constituent of mammalian central nervous system such as membrane lipids of brain gray matter and the visual elements of the retina (Belkind-Gerson *et al.*, 2008; Hoffman *et al.*, 2009; Innis, 2008). DHA is the major omega-3 PUFA esterified in the glycerophospholipids that form the structural matrix of brain gray matter and retinal membranes (Belkind-Gerson *et al.*, 2008; Hoffman *et al.*, 2009; Guisto *et al.*, 2002). Humans and other animals can take in DHA directly from marine products or obtain it after bioconversion of DHA precursors, LN, or intermediate PUFA between LN and DHA. DHA accumulation in the brain and retina, as in other organs, depends on the amount and types of omega-3 PUFA in the diet, and on dietary intake of omega-6 PUFA, which interacts and competes with omega-3 PUFA in the fatty acid metabolic pathway. Thus, the current high intakes of omega-6 PUFA, or low intakes of omega-3 PUFA contribute to poor infant neural development and function, and cause several kinds of biological disorders.

## 16.4 Brown seaweed lipids

The lipid content of seaweed varies with species, geographical location, season, temperature, salinity, light intensity and type of species and/or combination of these factors. Sanchez-Machado *et al.* (2004a,b) reported that tropical species have significantly less lipid than cold water species. The quantitative lipid analysis of a major brown seaweed family, Sargassaceae, in subarctic zone (Terasaki *et al.*, 2009) showed a relatively higher quantity of total lipids as compared with those in tropical zone (Bhaskar *et al.*, 2004a,b; Bhaskar and Miyashita, 2005). Terasaki *et al.* (2009) also reported a seasonal variation of total lipid content of brown seaweeds. In all the species, the highest lipid contents were observed in a growth period between winter and spring and total lipid of several brown seaweed

species reached to near 10% per dry weight. Main lipid class of brown seaweed lipids is glycolipids consisting of monogalactosyl-diacylglycerols (MGDG), digalactosyl-diacylglycerol (DGDG), and sulfoquinovosyl-diacylglycerol (SQDG) (Bhaskar *et al.*, 2004a,b,c; Bhaskar and Miyashita, 2005; Kamenarska *et al.*, 2002; Vaskovsky *et al.*, 1996). The glycolipid level is usually more than half the total lipid extract. Other lipid classes are phospholipids and triacylglycerols.

Seaweeds can be source of essential fatty acids including both omega-3 and omega-6 PUFA (Arao and Yamada, 1989; Bhaskar *et al.*, 2004a,b,c; Bhaskar and Miyashita, 2005; Kamenarska *et al.*, 2002; Li *et al.*, 2002; Terasaki *et al.*, 2009; Vaskovsky *et al.*, 1996). The red and brown algae are particularly rich in omega-3 PUFA such as SA and EPA. Both seaweed lipids also contain high level of AA as omega-6 PUFA. The ratio of omega-6 to omega-3 PUFA is very important as a nutraceutical for human intake as both of these compete for the same enzyme to synthesize prostaglandins derived from both omega-6 and omega-3 families. We have reported the compositions of major fatty acids of total lipids from six brown seaweeds (Terasaki *et al.*, 2009). Major PUFA from these algae were LA (18:2*n*-6; 3.8–11.2%), LN (18:3*n*-3; 4.3–10.1%), SA (18:4*n*-3; 5.6–11.8%), AA (20:4*n*-6; 6.1–15.2%), and EPA (20:5*n*-3; 9.7–13.2%). Palmitoleic acid (16:1*n*-7; 2.0–6.6%) was the major monoenic fatty acid apart from oleic acid (18:1*n*-9; 5.6–14.8%), which is in contrast to higher plants (Harwood and Jones, 1989). AA is a precursor of eicosanoids with pharmacological significance, while SA is a precursor for EPA and DHA. The importance of EPA and DHA in human health promotion has been confirmed through research. Several studies demonstrated that recommend a ratio of 1:1.5 to 1:2 between omega-3 and omega-6 PUFA (Hamazaki and Okuyama, 2003; Gebauer *et al.*, 2004). Although the ratio recommended by them may be too high, it is very clear that consumption of omega-3 PUFA, especially EPA and DHA, in modern food chain is less than their adequate intake levels. Thus, application of brown seaweed lipids to the food products will be expected as they have a nutritionally desirable ratio of omega-3 to omega-6 PUFA.

Seaweeds belonging to the same genus from different parts of the world generally have similar fatty acid profiles, although the actual content of PUFA are influenced by the site of collection. The difference in the fatty acid composition of algae depends on environmental factors. For instance, it is well established that algae accumulate PUFA when there is decrease in the environmental temperature (Kayama *et al.*, 1985; Khotimchenko, 1991). This in turn influences the comparisons between algae from different parts of the world (Bhaskar *et al.*, 2004b). Attempts to

use fatty acid composition as an aid in taxonomical conclusions of higher plants have been reviewed thoroughly. Some researchers have also found that distribution of fatty acids in marine plants to be closely linked with taxonomic position (Khotimchenko and Svetashev, 1987; Kayama *et al.*, 1989; Bhaskar *et al.*, 2004a). For instance, a C20 non-methylene interrupted fatty acid has been shown to be characteristic feature of the genus *Sargassum* (Bhaskar *et al.*, 2004b) while conjugated fatty acids have been shown to be present in a specific group of red seaweeds, namely Ceramiales (Burgess *et al.*, 1991; Wise *et al.*, 1994; Bhaskar *et al.*, 2004a).

## 16.5 Bioconversion of LN to DHA

Although plant-derived omega-3 counterpart of EPA and DHA, LN, is the principal dietary omega-3 PUFA consumed in the typical Western diet (Barre, 2007; Kris-Etherton *et al.*, 2002), it may be true that humans have a poor ability to form DHA from LN (Figure 16.1). Tracer studies have shown that the proportion of the conversion of LN to DHA in infants is very low, less than 1% (Goyens *et al.*, 2005; Hussein *et al.*, 2005; Innis, 2009). On the other hand, studies in normal healthy adults consuming western diets, which are rich in LA, show that supplemental LN raises EPA and DPA status in the blood and in breast milk. Addition of LN to the diets of formula-fed infants does raise DHA, but no level of LN tested raises DHA to levels achievable with preformed DHA at intakes similar to typical human milk DHA supply (Brenna *et al.*, 2009). Males and females differ in their ability to synthesize DHA from LN and that this disparity is associated with gender differences in the circulating concentrations of DHA, which is higher in females (Childs *et al.*, 2008).

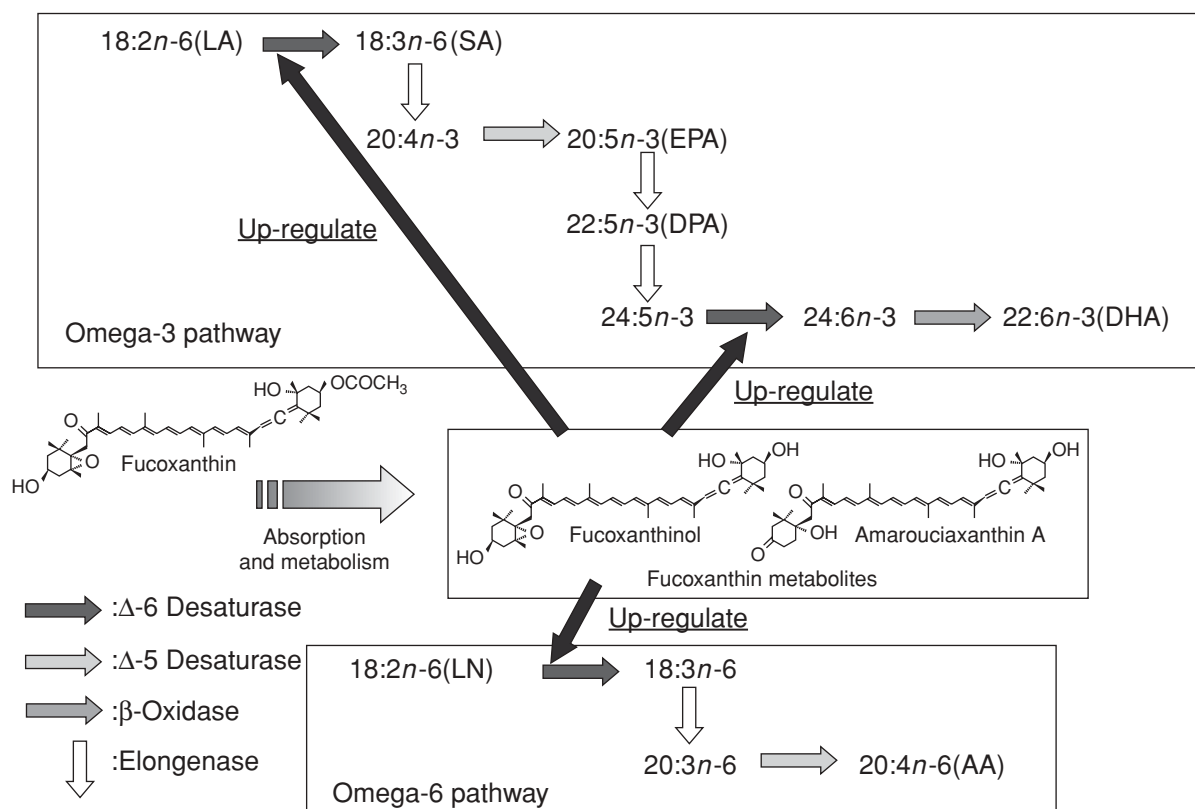
The tissue composition of omega-3 PUFA depends on both dietary intake of these PUFA and metabolism controlled by genetic polymorphisms, resulting that at the same dietary intake of LN, its respective health effects in each tissue may differ due to genetic differences in metabolism (Simopoulos, 2010). Therefore, it is important to understand the extent of their metabolism from LN in the tissue of various mammals. In addition, the metabolism and functions of the omega-3 and omega-6 PUFA interrelate on many levels. High intake of LA inhibits the conversion of LN to DHA. DHA in liver and brain decreased at intake of LA above 3% energy (Novak *et al.*, 2008; Innis 2008). Modern human diets contain in excess of 3% energy LA (Innis and Jacobson, 2007; Simopoulos, 1999), leading to the argument that omega-6 PUFA intakes are now so high as to flood the fatty acid metabolic pathway and suppress metabolism of the omega-3 PUFA (Figure 16.1).

The ability of mammals, and humans in particular, to metabolize LN to DHA is an important nutritional interest since there is evidence that enhanced DHA status is important for optimal health. Although more work is required to make clear the ability to form DHA from LN in humans, the need of LN is ever more apparent, given that LN is by far the predominant form of omega-3 PUFA consumed and intakes of EPA and DHA are typically very low in the Western diet (Anderson and Ma, 2009). Brown seaweed lipids contain high levels of omega-3 PUFA such as LN, SA, and EPA; they are potential sources of DHA in biological systems. Moreover, our recent studies showed that fucoxanthin, a typical brown seaweed carotenoid, can enhance DHA synthesis from LN in rodent (Tsukui *et al.*, 2007, 2009) (Figure 16.2). Therefore, brown seaweed lipids will increase DHA levels in biological system, giving an opportunity to develop a new type of DHA supplement.

## 16.6 Hepatic DHA enhancement in mice by fucoxanthin

Fucoxanthin is a major carotenoid present in chloroplasts of brown seaweeds. It is the most abundant of all carotenoids accounting for >10% of estimated total natural production of carotenoids (Matsuno, 2001). Fucoxanthin has a unique structure including an unusual allenic bond and 5,6-monoepoxide in its molecule (Figure 16.2). Of approximately 700 naturally occurring carotenoids, about 40 carotenoids contain the allenic bond. The principal allenic carotenoids are fucoxanthin in brown seaweeds, peridinin in microalgae, and neoxanthin in higher plants (Dembitsky and Maoka, 2007). Fucoxanthin has several physiological activities as found in other carotenoids. They show antioxidant (Conn *et al.*, 1991; Miller *et al.*, 1996; Nishino, 1998; Nomura *et al.*, 1997; Sachindra *et al.*, 2007; Yan *et al.*, 1999), anti-carcinogenic (Das *et al.*, 2010; Hosokawa *et al.*, 2009; Kim *et al.*, 2010a; Liu *et al.*, 2009; Nishino *et al.*, 2009; Satomi and Nishino, 2009) and anti-inflammatory (Heo *et al.*, 2010; Kim *et al.*, 2010b; Shiratori *et al.*, 2005) activities.

More attention has been paid to some characteristic physiological effects of fucoxanthin: antiobesity and antidiabetic effects. We have found that fucoxanthin feeding significantly reduced abdominal WAT weight of obese model mice or normal mice fed a high-calorie diet, while no effect was found on normal mice fed normal diet (Hosokawa *et al.*, 2010; Maeda *et al.*, 2005, 2007a,b, 2009; Miyashita, 2009; Miyashita and Hosokawa, 2009; Miyashita *et al.*, 2010), suggesting that suppressive effect of fucoxanthin on the WAT weight gain is specific for adiposity in the development of



**Figure 16.2** Possible molecular mechanism for enhancement of hepatic DHA and AA in mice fed fucoxanthin.

obesity in mice. This effect of fucoxanthin is partly due to the induction of uncoupling protein 1 (UCP1) in abdominal WAT (Maeda *et al.*, 2005, 2007b). UCP1 induction by fucoxanthin metabolites accumulated in the WAT has been highly interested because UCP1 is usually expressed only in brown adipose tissue (BAT) not in WAT. In addition, Fucoxanthin improves insulin resistance and decreases blood glucose level through the regulation of adipocytokines secreted from WAT and of GLUT 4 expressions in muscle (Maeda *et al.*, 2007b, 2009; Miyashita *et al.*, 2010). The details of the antiobesity and antidiabetic effects of fucoxanthin can be seen in other chapters of this book.

Another interesting characteristic activity of fucoxanthin is to enhance DHA content of mouse liver (Tsukui *et al.*, 2007, 2009). Although the molecular mechanism has been still unclear, the effect may be important to understand the physiological effects of fucoxanthin in biological systems. DHA can be biosynthesized through desaturation and elongation reaction steps beginning with LN in the liver (Brenna *et al.*, 2009; Innis, 2009) (Figure 16.1). AA is also synthesized from LA through the same step (Le *et al.*, 2009). Several kinds of desaturase and elongenase involve this en-

zymatic reaction and  $\Delta$ -6 desaturase is the rate-limiting enzyme in these PUFA biosynthetic pathways (Cho *et al.*, 1999; Sprecher 1981) (Figure 16.1). Enhancement of desaturase and elongenase activity, especially of  $\Delta$ -6 desaturase activity, will increase DHA and AA biosynthesis.

Initial experiments on DHA increase by fucoxanthin intake have been done using crude fucoxanthin prepared from *Undaria pinnatifida*, the most common edible brown seaweed in Japan. Crude fucoxanthin contained 78% fucoxanthin. Although there was little difference in the fatty acid composition of dietary lipids, 0.18 and 0.36% fucoxanthin containing diets significantly increased the proportion of DHA in the fatty acids of liver lipids of obese/diabetes model mice (KK-A<sup>y</sup> mice) (Tsukui *et al.*, 2007). The proportion of DHA in mice fed a diet containing 0.36% fucoxanthin was found to be more than twice that of the control group. In addition, increases in the proportion of stearic acid and AA were also observed in the group receiving diets supplemented with crude fucoxanthin. In contrast, the proportions of oleic acid (18:1n-9) and LN in the fatty acid composition of liver lipids were reduced. This effect of fucoxanthin has been confirmed using purified

fucoxanthin and fucoxanthinol, a main metabolite of fucoxanthin (Tsukui *et al.*, 2007, 2009) (Figure 16.2), where a quantitative assessment of DHA by using an internal standard was carried out.

In KK-*A<sup>y</sup>* mice fed 0.1 and 0.2% purified fucoxanthin, and 0.2% purified fucoxanthinol, the amount of hepatic DHA was 1.7, 1.9, and 1.8 times higher than that of the control group, respectively (Tsukui *et al.*, 2007). There was a trend for the amount of hepatic 20:4*n*-6 to increase with fucoxanthin diets, although this increase was not significant. An increase in hepatic DHA and AA was also observed in C57BL/6J normal mice even at 0.05% fucoxanthin intake in the diet (Tsukui *et al.*, 2009). On the other hand, in the small intestine of KK-*A<sup>y</sup>* mice fed 0.2% purified fucoxanthin or fucoxanthinol, an increase in DHA or AA was not observed. Since the liver has been considered to be the primary tissue for desaturation and elongation of unsaturated fatty acids, the increase in DHA and AA seen with fucoxanthin- and fucoxanthinol-supplemented diets was thought to result from a modification in the biosynthesis and degradation of PUFA in liver. However, an increase in hepatic 20:4*n*-6 was less compared to DHA, especially in mice fed fucoxanthin diets. It is therefore proposed that DHA and AA may be synthesized by independent pathways involving omega-3 and omega-6 specific enzymes (Infante and Huszagh, 1998; Rodriguez *et al.*, 1999) and that the degradation of 20:4*n*-6 is faster than that of DHA (Figure 16.2).

Previous studies showed that synthetic peroxisome proliferators, fenofibrate (Blond *et al.*, 1989), and clofibrate (Kawashima *et al.*, 1990) increased the hepatic proportion of 20:4*n*-6 in obese Zucker rat and normal Wistar rats, but not DHA. This effect of proliferators is based on the activation of  $\Delta$ -6 desaturase in normal and obese rats. Another study reported the increase in DHA and EPA in adipose tissue but not in the liver of type 2 diabetes model mouse given a PPARR ligand, rosiglitazone (Watkins *et al.*, 2002). In addition, vitamin A deficiency has also been reported to enhance the proportion of DHA in the liver of rats fed LN (Zhou *et al.*, 2006). However, there are only a few studies regarding the enhancement of DHA biosynthesis through the regulation of *de novo* synthesis. Our studies (Tsukui *et al.*, 2007, 2009) clearly indicated that dietary fucoxanthin and fucoxanthinol enhance the amount of DHA in the liver of mice. Because purified fucoxanthin and fucoxanthinol diets contained only LN as the precursor fatty acid of DHA, increased DHA is considered to be converted from LN through elongation and desaturation steps. Therefore, the results obtained in our study suggest that dietary fucoxanthin and fucoxanthinol may modify the biosynthesis and metabolic pathways of omega-3 and omega-6 PUFA.

## 16.7 Conclusion

Most characteristic feature of brown seaweed lipids is high contents of omega-3 PUFA such as LN (18:3*n*-3), SA (18:4*n*-3), and EPA (20:5*n*-3), and of omega-6 PUFA, AA (20:4*n*-6). The importance of these PUFA for human health has been proven through extensive scientific research in the recent past. Brown seaweed lipids contain many kinds of bioactives other than omega-3 and omega-6 PUFA; these are polyphenols, sterols, and fucoxanthin. Among them much attention has been paid to fucoxanthin relating to its ability to enhance DHA biosynthesis from other kinds of omega-3 PUFA such as LN, SA, and EPA. Animal experiments revealed the significant increase in hepatic DHA level by fucoxanthin intake, showing that brown seaweed lipids are potential source of DHA even though they contain no DHA.

Major American and European heart associations recommend the intake of 1 g/day of EPA and DHA for prevention of sudden cardiac death and other cardiovascular dysfunctions (De Backer *et al.*, 2003; Smith *et al.*, 2006). However, it may be difficult for consumers to have this level from fish oil, because of its undesirable flavor and low stability. On the other hand, we have found that a powder from *Undaria pinnatifida* (wakame), the most common edible brown seaweed in Japan, can be a pasta ingredient (Prabhasankar *et al.*, 2009). Pasta with 10% wakame was acceptable sensorially. The sensorily acceptable pasta had a mild flavor with taste similar to control pasta, as assessed by panelists. The ratio of omega-3 to omega-6 fatty acid in seaweed incorporated pasta was 1:3.4 as compared to 1:15.2 in the control, showing the drastic increase in the omega-3 content to recommended level by mixing brown seaweed powder to flour without any adverse changes. Heat processing involved in pasta preparation and cooking did not destroy fucoxanthin, whose level was enough to show the enhancement of hepatic DHA. This opens up a new line of research and development for effective and safe DHA supplementation to humans.

## References

- Albert, C.M., Campos, H., Stampfer, M.J., *et al.* (2002) Blood levels of long-chain *n*-3 fatty acids and the risk of sudden death. *N. Engl. J. Med.*, **346**, 1113–1118.
- Allayee, H., Roth, N. and Hodis, H.N. (2009) Polyunsaturated fatty acids and cardiovascular disease: implications for nutrigenetics. *J. Nutrigenet. Nutrigenomics*, **2**, 140–148.
- Anderson, B.M. and Ma, D.W.L. (2009) Are all *n*-3 polyunsaturated fatty acids created equal? *Lipids Health Dis.*, **8**, 33.

- Arao, T. and Yamada M. (1989) Positional distribution of fatty acids in galactolipids of algae. *Phytochemistry*, **28**, 805–810.
- Barre D.E. (2007) The role of consumption of alpha-linolenic, eicosapentaenoic and docosahexaenoic acids in human metabolic syndrome and type 2 diabetes – a mini-review. *J. Oleo Sci.*, **56**, 319–325.
- Belkind-Gerson, J., Carreón-Rodríguez, A., Contreras-Ochoa, C.O., Estrada-Mondaca, S. and Parra-Cabrera M.S. (2008) Fatty acids and neurodevelopment *J. Pediatr. Gastroenterol., Nutr.* **47**, S7–S9.
- Bhaskar, N., Tomohisa, K., Miyashita, K., Park, S.-B., Endo, Y. and Fujimoto, K. (2004a) Occurrence of conjugated polyenoic fatty acids in seaweeds from the Indian Ocean. *Z. Naturforsch.*, **59C**, 310–314.
- Bhaskar, N., Hosokawa, M. and Miyashita, K. (2004b) Comparative evaluation of fatty acid composition of different *Sargassum* (Fucales, Phaeophyta) species harvested from temperate and tropical waters. *J. Aquatic Product Tech.*, **13**, 53–70.
- Bhaskar, N., Hosokawa, M. and Miyashita, K. (2004c) Growth inhibition of human pro-myelocytic leukemia (9HL-60) cells by lipid extracts of marine alga *Sargassum marginatum* (Fucales, Phaeophyta) harvested off Goa (west coast of India) with special reference to fatty acid composition. *Indian J. Marine Sci.*, **33**, 355–360.
- Bhaskar, N. and Miyashita, K. (2005) Lipid composition of *Padina tetrastomatica* (Dictyotalea, Phaeophyta), brown seaweed of the west coast of India. *Indian J. Fish.*, **52**, 263–268.
- Blond, J.P., Clouet, P., Bezard, J. and Legendre, C. (1989) Effect of fenofibrate treatment on linoleic acid desaturation in liver of obese Zucker rats. *Biochem. Pharmacol.*, **38**, 2741–2744.
- Brenna, J.T., Salem Jr., N., Sinclair, A.J. and Cunnane, S.C. (2009)  $\alpha$ -Linolenic acid supplementation and conversion to *n*-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot. Essent. Fatty Acids*, **80**, 85–91.
- Burgess, J.R., De la Rosa, R.I., Jacobs, R.S. and Butler, A. (1991) A new eicosapentanoic acid formed from Arachidonic acid in the coralline red algae *Bassiella orbigniana*. *Lipids*, **26**, 162–165.
- Calder, P.C. (2004) *n*-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin. Sci.*, **107**, 1–11.
- Calder, P.C. (2006) *N*-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am. J. Clin. Nutr.*, **83**, 1505S–1519S.
- Childs, C.E., Romeu-Nadal, M., Burdge, G.C. and Calder, P.C. (2008) Gender differences in the *n*-3 fatty acid content of tissues. *Proc. Nutr. Soc.*, **67**, 19–27.
- Cho, H.P., Nakamura, M. and Clarke, S.D. (1999) Cloning, expression, and nutritional regulation of the mammalian delta-6 desaturase. *J. Biol. Chem.*, **274**, 471–477.
- Conn, P.F., Schalch, W. and Truscott, T.G. (1991) The singlet oxygen and carotenoid interaction. *J. Photochem. Photobiol. B*, **11**, 41–47.
- Das, S.K., Ren R., Hashimoto, T. and Kanazawa, K. (2010) Fucoxanthin induces apoptosis in osteoclast-like cells differentiated from RAW 264.7 cells. *J. Agric. Food Chem.*, **58**, 6090–6095.
- Davidson, M. (2006) Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids. *Am. J. Cardiol.*, **98**, 27i–33i.
- Daviglus, M.L., Stamler, J., Orenchia, A.J., et al. (1997) Fish consumption and the 30-year risk of fatal myocardial infarction. *N. Engl. J. Med.*, **336**, 1046–1053.
- De Backer, G., Ambrosioni, E., Borch-Johnsen, K., et al. (2003) European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. *Eur. Heart J.*, **24**, 1601–1610.
- Dembitsky, V.M. and Maoka, T. (2007) Allenic and cumulenenic lipids. *Prog. Lipid Res.*, **46**, 328–375.
- Gazi, I.F., Tsimihodimos, V., Tselepis, A.D., Moses Elisaf, M. and Mikhailidis, D.P. (2007) Clinical importance and therapeutic modulation of small dense lowdensity lipoprotein particles. *Expert. Opin. Biol. Ther.*, **7**, 53–72.
- Gebauer, S., Harris, W. S., Kris-Etherton, P. M. and Etherton, T. D. (2004) Dietary *n*-6:*n*-3 fatty acid ratio and health. In: *Healthful Lipids* (eds. Akoh, C.C. and Lai, O.-M). AOCS Press, Champaign, Illinois, pp. 221–248.
- Givens, D.I. and Gibbs, R.A. (2008) Current intakes of EPA and DHA in European populations and the potential of animal-derived foods to increase them. *Proc. Nutr. Soc.*, **67**, 273–280.
- Goyens, P.L., Spilker, M.E., Zock, P.L., Katan, M.B. and Mensink, R.P. (2005) Compartmental modeling to quantify alpha-linolenic acid conversion after longer term intake of multiple tracer boluses. *J. Lipid. Res.*, **46**, 1474–1483.
- Guisto, N.M., Salvador, G.A., Castagnet, P.I., Pasquare, S.J. and Ilincheta de Bscherio, M.G. (2002) Age-associated changes in central nervous system glycerophospholipids composition and metabolism. *Neurochem. Res.*, **27**, 1513–1523.
- Hamazaki, T. and Okuyama, H. (2003) The Japan society for lipid nutrition recommends to reduce the intake of linoleic acid. A review and critique of the scientific evidence. *World Rev. Nutr. Diet.*, **92**, 109–132.

- Harris, W. and Bulchandani, D. (2006) Why do omega-3 fatty acids lower serum triglycerides? *Curr. Opin. Lipidol.*, **17**, 387–393.
- Harris, W.S., Miller, M., Tighe, A.P., Davidson, M.H. and Schaefer, E.J. (2008) Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. *Atherosclerosis*, **197**, 12–24.
- Harwood, J.L. and Jones, A.L. (1989) Lipid metabolism in algae. *Adv. Bot. Res.*, **16**, 1–53.
- Heo, S.-J., Yoon, W.-J., Kim, K.-N., *et al.* (2010) Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages. *Food Chem. Toxicol.*, **48**, 2045–2051.
- Hoffman, D.R., Boettcher, J.A. and Diersen-Schade, D.A. (2009) Toward optimizing vision and cognition in term infants by dietary docosahexaenoic and arachidonic acid supplementation: A review of randomized controlled trials. *Prostaglandins Leukotr. Essent. Fatty Acids*, **81**, 151–158.
- Hokanson, J.E. and Austin, M.A. (1996) Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J. Cardiovasc. Risk*, **3**, 213–219.
- Hosokawa, M., Miyashita, T., Nishikawa, S., *et al.* (2010) Fucoxanthin regulates adipocytokine mRNA expression in white adipose tissue of diabetic/obese KK-Ay mice. *Arch. Biochem. Biophys.*, **504**, 17–25.
- Hosokawa, M., Okada, T., Mikami, N., Konishi, I. and Miyashita, K. (2009) Bio-functions of marine carotenoids. *Food Sci. Biotech.*, **18**(1), 1–11.
- Hussein, N., Ah-Sing, E., Wilkinson, P., *et al.* (2005) Long-chain conversion of [<sup>13</sup>C] linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men. *J. Lipid Res.*, **46**, 269–280.
- Infante, J.P. and Huszagh, V.A. (1998) Analysis of the putative role of 24-carbon polyunsaturated fatty acids in the biosynthesis of docosapentaenoic (22:5n-6) and docosahexaenoic (22:6n-3) acids. *FEBS Lett.*, **431**, 1–6.
- Innis, S.M. (2003) Perinatal biochemistry and physiology of long chain polyunsaturated fatty acids. *J. Pediatr.*, **143**, S1–S8.
- Innis S.M. (2008) Dietary omega 3 fatty acids and the developing brain. *Brain Res.*, **1237**, 35–43.
- Innis, S.M. (2009) Omega-3 fatty acids and neural development to 2 years of age: do we know enough for dietary recommendations? *J. Pediatr. Gastroenterol. Nutr.*, **48**, S16–S24.
- Innis, S.M. and Jacobson, K. (2007) Dietary lipids in early development and intestinal inflammatory disease. *Nutr. Rev.*, **65**, 5188–5189.
- Jump, D.B. (2002) The biochemistry of n-3 polyunsaturated fatty acids. *J. Biol. Chem.*, **277**, 8755–8758.
- Jump, D. (2004) Fatty acid regulation of gene transcription. *Crit. Rev. Clin. Lab. Sci.*, **41**, 41–78.
- Kamenarska, Z., Funda N. Yalçın, F.D., Ersöz, T., Çalış, I., Stefanova, K. and Popova, S. (2002) Chemical composition of *Cystoseira crinita* bory from the eastern Mediterranean. *Z. Naturforsch.*, **57c**, 584–590.
- Kaneniwa, M., Itabashi, Y. and Takagai, T. (1987) Unusual 5-olefinic acids in the lipids of algae from Japanese water. *Bull. Jap. Soc. Sci. Fish.*, **53**, 861–866.
- Kayama, M., Araki, S. and Sato, S. (1989) Lipids of marine plants. In: *Marine Biogenic Lipids, Fats and Oils*, Vol. II (eds Ackman, R.G). CRC Press Inc., Boca Raton, FL, pp. 3–48.
- Kayama, M., Iijima, N., Kuwahara, M., Sado, T., Araki, S. and Sakurai, T. (1985) Effect of water temperature on the fatty acid composition of *Porphyra*. *Bull. Jpn. Soc. Sci. Fish.*, **51**, 687.
- Kawashima, Y., Musoh, K. and Kozuka, H. (1990) Peroxisome proliferators enhance linoleic acid metabolism in rat liver. Increased biosynthesis of omega 6 polyunsaturated fatty acids. *J. Biol. Chem.*, **265**, 9170–9175.
- Kelley, D.S., Siegel, D., Vemuri, M. and Mackey, B.E. (2007) Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am. J. Clin. Nutr.*, **86**, 324–333.
- Khotimchenko, S.V. (1991) Fatty acid composition of seven *Sargassum* species. *Phytochemistry*, **30**, 2639–2641.
- Khotimchenko, S.V. and Svetashev, V.I. (1987) Fatty acids of marine macrophytes. *Biol. Morya (Vladivostok)*, **6**, 3–15.
- Kim, K.-N., Heo, S.-J., Kang, S.-M., Ahn, G. and Jeon, Y.J. (2010a) Fucoxanthin induces apoptosis in human leukemia HL-60 cells through a ROS-mediated Bcl-xL pathway. *Toxicol. In Vitro*, **24**, 1648–1654.
- Kim, K.N., Heo, S.-J., Yoon, W.-J., *et al.* (2010b) Fucoxanthin inhibits the inflammatory response by suppressing the activation of NF-κB and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages. *Eur. J. Pharmacol.*, **649**, 369–375.
- Kris-Etherton, P.M., Harris, W.S. and Appel, L.J. (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, **106**, 2747–2757.
- Kris-Etherton, P.M., Taylor, D.S., Yu-Poth, S., *et al.* (2000) Polyunsaturated fatty acids in the food chain in the United States. *Am. J. Clin. Nutr.*, **71**, S179–S188.
- Lands, W.E.M. (2005) *Fish, Omega-3 and Human Health*, 2nd edn. AOCS Press, Champaign, IL, USA, pp. 3–160.
- Le, H.D., Meisel, J.A., de Meijer, V.E., Gura, K.M. and Puder, M. (2009) The essentiality of arachidonic acid and docosahexaenoic acid. *Prostaglandins Leukotr. Essent. Fatty Acids*, **81**, 165–170.

- Leaf, A., Kang, J.X. and Xiao, Y.-F. (2008) Fish oil fatty acids as cardiovascular drugs. *Curr. Vasc. Pharmacol.*, **6**, 1–12.
- Li, D. and Bode, O., Drummond, H. and Sinclair, A.J. (2003) Omega-3 (*n*-3) fatty acids. In: *Lipids for Functional Foods and Nutraceuticals* (ed. Gunstone, F.D.) The Oily Press, Bridgwater, UK, pp. 225–262.
- Li, X., Fan, X., Han, L. and Lou, Q. (2002) Fatty acids of some algae from the Bohai Sea. *Phytochemistry*, **59**, 157–161.
- Liu, C.-L. Yung-Sheng Huang, Y.-S., Hosokawa, M., Miyashita, K. and Hu, M.-L. (2009) Inhibition of proliferation of a hepatoma cell line by fucoxanthin in relation to cell cycle arrest and enhanced gap junctional intercellular communication. *Chem. Biol. Int.*, **182**, 165–172.
- Maeda, H., Hosokawa, M., Sashima, T., Funayama, K. and Miyashita, K. (2005) Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commun.*, **332**, 392–397.
- Maeda, H., Hosokawa, M., Sashima, T., Funayama, K. and Miyashita, K. (2007a) Effect of medium-chain triacylglycerols on anti-obesity effect of fucoxanthin. *J. Oleo Sci.*, **56**, 615–621.
- Maeda, H., Hosokawa, M., Sashima, T. and Miyashita, K. (2007b) Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decrease blood glucose in obese/diabetic KK-*A*<sup>y</sup> mice. *J. Agric. Food Chem.*, **55**, 7701–7706.
- Maeda, H., Hosokawa, M., Sashima, T., Murakami-Funayama, K. and Miyashita, K. (2009) Anti-obesity and anti-diabetic effects of fucoxanthin on diet-induced obesity conditions in a murine model. *Mol. Med. Rep.*, **2**, 897–902.
- Marquardt, J. and Hanelt, D. (2004). Carotenoid composition of *Delesseria lancifolia* and other marine red algae from polar and temperate habitats. *Eur. J. Phycol.*, **39**, 285–292.
- Matsuno, T. (2001) Aquatic animal carotenoids. *Fish. Sci.*, **67**, 771–783.
- Miller, N.J., Sampson, J., Candeias, L.P., Bramley, P.M. and Rice-Evans, C.A. (1996) Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.*, **384**, 240–242.
- Miyashita, K. (2009) The carotenoid fucoxanthin from brown seaweed affects obesity. *Lipid Tech.*, **21**, 186–190.
- Miyashita, K. and Hosokawa, M. (2009) Anti-obesity effect of allenic carotenoid, fucoxanthin. In: *Nutrigenomics and Proteomics in Health and Disease: Impact of Food Factors-Gene Interactions* (eds Mine, Y., Miyashita, K. and Shahidi, F.). John Wiley & Sons, Inc., Ames, IA, USA. pp. 145–160.
- Miyashita, K., Maeda, H., Okada, T., Abe, M. and Hosokawa, M. (2010) Anti-obesity and anti-diabetic effects of allenic carotenoid, fucoxanthin. *AgroFOOD*, **21**, 24–27.
- Mori, T.A. and Beilin, L.J. (2004) Omega-3 fatty acids and inflammation. *Curr. Atheroscler. Rep.*, **6**, 461–467.
- Mozaffarian, D., Lemaitre, R.N., Kuller, L.H., Burke, G.L., Tracy, R.P. and Siscovick, D.S. (2003) Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study. *Circulation*, **107**, 1372–1377.
- Narayan, B., Miyashita, K. and Hosokawa, M. (2004) Comparative evaluation of fatty acid composition of different Sargassum (Fucales, Phaeophyta) species harvested from temperate and tropical waters. *J. Aqua. Food Product Tech.*, **13**, 53–70.
- Narayan, B., Miyashita, K. and Hosokawa, M. (2006) Physiological effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)-a review. *Food Rev. Int.*, **22**, 291–307.
- Narayan, B., Kumar, C.S., Sashima, T., Maeda, H., Hosokawa, M. and Miyashita, K. (2008) Composition, functionality and potential applications of seaweed lipids. In: *Biocatalysis and Bioenergy* (ed. Ho, C.T.). John Wiley & Sons, Inc., New York, pp. 463–490.
- Nishino, H. (1998) Cancer prevention by carotenoids. *Mutat. Res.*, **402**, 159–163.
- Nishino, H., Murakoshi, M., Tokuda, H. and Satomi, Y. (2009) Cancer prevention by carotenoids. *Arch. Biochem. Biophys.*, **483**, 165–168.
- Nomura, T., Kikuchi, M., Kubodera, A. and Kawakami, Y. (1997) Proton-donative antioxidant activity of fucoxanthin with 1,1-diphenyl-2-picrylhydrazyl (DPPH). *Biochem. Mol. Biol. Int.*, **42**, 361–370.
- Novak, E., Dyer, R.A. and Innis, S.M. 2008. High dietary omega-6 fatty acids contribute to reduced docosahexaenoic acid in the developing brain and inhibit secondary neurite growth. *Brain Res.*, **1237**, 136–145.
- Prabhasankar, P., Ganesan, P., Bhaskar, N., et al. (2009) Edible Japanese seaweed, Wakame (*Undaria pinnatifida*) as an ingredient in pasta: chemical, functional and structural evaluation. *Food Chem.*, **115**, 501–508.
- Rodriguez, A., Sarda, P., Boulot, P., Leger, C.L. and Descomps, B. (1999) Differential effect of *N*-ethyl maleimide on delta 6 desaturase activity in human fetal liver toward fatty acids of the *n*-6 and *n*-3 series. *Lipids*, **34**, 23–30.
- Russo, G.L. (2010) Dietary *n*-6 and *n*-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochem. Pharmacol.*, **235**, 785–795.
- Sachindra, N.M., Sato, E., Maeda, H., et al. (2007). Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *J. Agric. Food Chem.*, **55**, 8516–8522.

- Sampath, H. and Ntambi, J. (2004) Polyunsaturated fatty acid regulation of gene expression. *Nutr. Rev.*, **62**, 333–339.
- Sanchez-Machado, D.I., Lopez-Cervantes, J., Lopez-Hernandez, J. and Paseiro-Losada, P. (2004a) Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chem.*, **85**, 439–444.
- Sanchez-Machado, D.I., Lopez-Hernandez, P., Paseiro-Losada, P. and Lopez-Cervantes, J. (2004b) An HPLC method for the quantification of sterols in edible seaweeds. *Biomed. Chromatogr.*, **18**, 183–190.
- Sanders, T.A. (2000) Polyunsaturated fatty acids in the food chain in Europe. *Am. J. Clin. Nutr.*, **71**, 176S–178S.
- Satoh, N., Shimatsu, A., Kotani, K., *et al.* (2007) Purified eicosapentaenoic acid reduces small dense LDL, remnant lipoprotein particles, and C-reactive protein in metabolic syndrome. *Diabetes Care*, **30**, 144–146.
- Satomi, Y. and Nishino, H. (2009) Implication of mitogen-activated protein kinase in the induction of G1 cell cycle arrest and *gadd45* expression by the carotenoid fucoxanthin in human cancer cells. *Biochim. Biophys. Acta*, **1790**, 260–266.
- Schubert, N., García-Mendoza, E. and Pacheco-Ruiz, I. (2006) Carotenoid composition of marine red algae. *J. Phycol.*, **42**, 1208–1216.
- Shahidi, F. and Miraliakbari, H. (2006) Marine oils: compositional characteristics and health effects. In: *Nutritional and Specialty Lipids and Their Co-Products* (ed. Shahidi, F.). CRC Press, New York, USA, pp. 227–250.
- Shiratori, K., Ohgami, K., Ilieva, I., *et al.* (2005) Effects of fucoxanthin on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. *Exp. Eye Res.*, **81**, 422–428.
- Simpoulous, A.P. (1999) Essential fatty acids in health and chronic disease. *Am. J. Clin. Nutr.*, **70**, 560–595.
- Simopoulos, A.P. (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp. Biol. Med.*, **233**, 674–688.
- Simopoulos, A.P. (2010) Genetic variants in the metabolism of omega-6 and omega-3 fatty acids: their role in the determination of nutritional requirements and chronic disease risk. *Exp. Biol. Med.*, **235**, 785–795.
- Sinclair, A., Wallace, J., Martin, M., Attar-Bashi, N., Weisinger, R. and Li, D. (2005) The effects of eicosapentaenoic acid in various clinical conditions. In: *Healthful Lipids* (eds Akoh, C.C. and Lai, O.-M.). AOCS Press, Champaign, IL, USA, pp. 361–394.
- Smith Jr, S.C., Allen, J., Blair, S.N., *et al.* (2006) AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update: endorsed by the National Heart, Lung, and Blood Institute. *Circulation*, **113**, 2363–2372.
- Sprecher, H. (1981) Biochemistry of essential fatty acids. *Prog. Lipid Res.*, **20**, 13–22.
- Stefanov, K., Konaklieva, M., Brechany, E.Y. and Christie, W.W. (1988) Fatty acid composition of some algae from the Black sea. *Phytochemistry*, **27**, 3495–3497.
- Terasaki, M., Hirose, A., Narayan, B., *et al.* (2009) Evaluation of recoverable functional lipid components with special reference to fucoxanthin and fucosterol contents of several brown seaweeds of Japan. *J. Phycol.*, **45**, 974–980.
- Tziomalos, K., Athyros, V.G., Karagiannis, A. and Mikhailidis, D.P. (2008) Omega-3 fatty acids: how can they be used in secondary prevention? *Curr. Atherosclerosis Rep.*, **10**, 510–517.
- Tsukui, T., Konno, K., Hosokawa, M., Maeda, H., Sashima, T. and Miyashita, K. (2007) Fucoxanthin and fucoxanthinol enhance the amount of docosahexaenoic acid in the liver of KKAY obese/diabetic mice. *J. Agric. Food Chem.*, **55**, 5025–5029.
- Tsukui, T., Baba, T., Hosokawa, M., Sashima, T. and Miyashita, K. (2009) Enhancement of hepatic docosahexaenoic acid and arachidonic acid contents in C57BL/6J mice by dietary fucoxanthin. *Fish. Sci.*, **75**, 261–263.
- Vaskovsky, V.E., Khotimchenko, S.V., Xia, B. and Hefang, L. (1996) polar lipids and fatty acid of some marine macrophytes from the Yellow Sea. *Phytochemistry*, **42**, 1347–1356.
- Vila, L. (2004) Cyclooxygenase and 5-lipoxygenase pathways in the vessel wall: role in atherosclerosis. *Med. Res. Rev.*, **24**, 399–424.
- Wang, C., Harris, W.S., Chung, M., *et al.* (2006) Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary prevention studies: a systematic review. *Am. J. Clin. Nutr.*, **84**, 5–17.
- Watkins, S.M., Reifsnnyder, P.R., Pan, H.J., German, J.B. and Leiter, E.H. (2002) Lipid metabolome-wide effects of the PPARgamma agonist rosiglitazone. *J. Lipid Res.*, **43**, 1809–1817.
- Wise, M.L., Hamberg, M. and Gerwick, W.H. (1994) Biosynthesis of conjugated triene containing fatty acids by novel isomerase from the red marine alga *Ptilota filicina*. *Biochemistry*, **33**, 15231–15232.
- Yan, X., Chuda, Y., Suzuki, M. and Nagata, T. (1999) Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Biosci. Biotechnol. Biochem.*, **63**, 605–607.
- Zhou, D., Ghebremeskel, K., Crawford, M.A. and Reifen, R. (2006) Vitamin A deficiency enhances docosahexaenoic and arachidonic acids in liver of rats fed an R-linolenic acid-adequate diet. *Lipids*, **41**, 213–219.

# 17

## Immune Regulatory Effects of Phlorotannins Derived From Marine Brown Algae (*Phaeophyta*)

Phuong Hong Nguyen<sup>1</sup>, il-Whan Choi<sup>2</sup>, Se-Kwon Kim<sup>3</sup> and Won-Kyo Jung<sup>1</sup>

<sup>1</sup>Department of Marine Life Science, and Marine Life Research & Education Center, Chosun University, Gwangju, Republic of Korea

<sup>2</sup>Department of Microbiology, College of Medicine and Advanced Research Center for Multiple Myeloma, Inje University, Busan, Republic of Korea

<sup>3</sup>Marine Bioprocess Research Center, Pukyong National University, Busan, Republic of Korea

### 17.1 Introduction

Population and communities of marine macroalgae have always been depending on the rate of consumption by the aquatic herbivores. In order to minimize this impact, marine macroalgae have to express the chemical defense by the action of the produced secondary metabolites (Paul, 1992). The most crucial chemicals for these defenses are phlorotannins or polyphloroglucinol phenolics, which are known to have primary roles in cell-wall construction (Altena *et al.*, 1992).

Phlorotannins are a type of tannins composed of phloroglucinol oligomers (Shibata *et al.*, 2004). Phlorotannins constitute around 15% of the dry weight of total biomass (Ragan and Glombitza, 1986a, b; Targett and Arnold, 1998). Phlorotannins are further classified as fucols, phloroethols or fuhalols (Table 17.1). Their molecular sizes have been found to range from about 126 Da–125 kDa (Ragan and Glombitza, 1986a, b), but are most commonly found in the 10 to 100 kDa range (Boettcher and Targett, 1993; McClintock and Baker, 2001). In marine algae,

phloroglucinolins have been investigated from marine brown algae and red algae that possess variety of the low, intermediate, and high molecular weight with both phenyl and phenoxy units (Singh and Bharate, 2006; Glombitza and Li, 1991).

Although the chemical structures of phlorotannins have been discovered and investigated since the 1980s (Figure 17.1), their diverse activities have been demonstrated in cell culture and animal models like mouse. As shown in Table 17.1, many investigations have verified that phlorotannins contribute to the potential pharmacological and biological activities such as anti-oxidant (Li *et al.*, 2009), anti-microbial (Nagayama *et al.*, 2002), anti-proliferative (Kong *et al.*, 2009), radioprotective (Zhang *et al.*, 2008), anti-human immunodeficiency virus (HIV) (Artan *et al.*, 2008), anti-Alzheimer's disease (Yoon *et al.*, 2009), and especially anti-inflammatory (Jung *et al.*, 2009) activities.

In this chapter, we focus on immune regulatory effects of marine algae-derived phlorotannins as potential nutraceutical, cosmeceutical, and therapeutic agents for various inflammatory and neurodegenerative diseases.

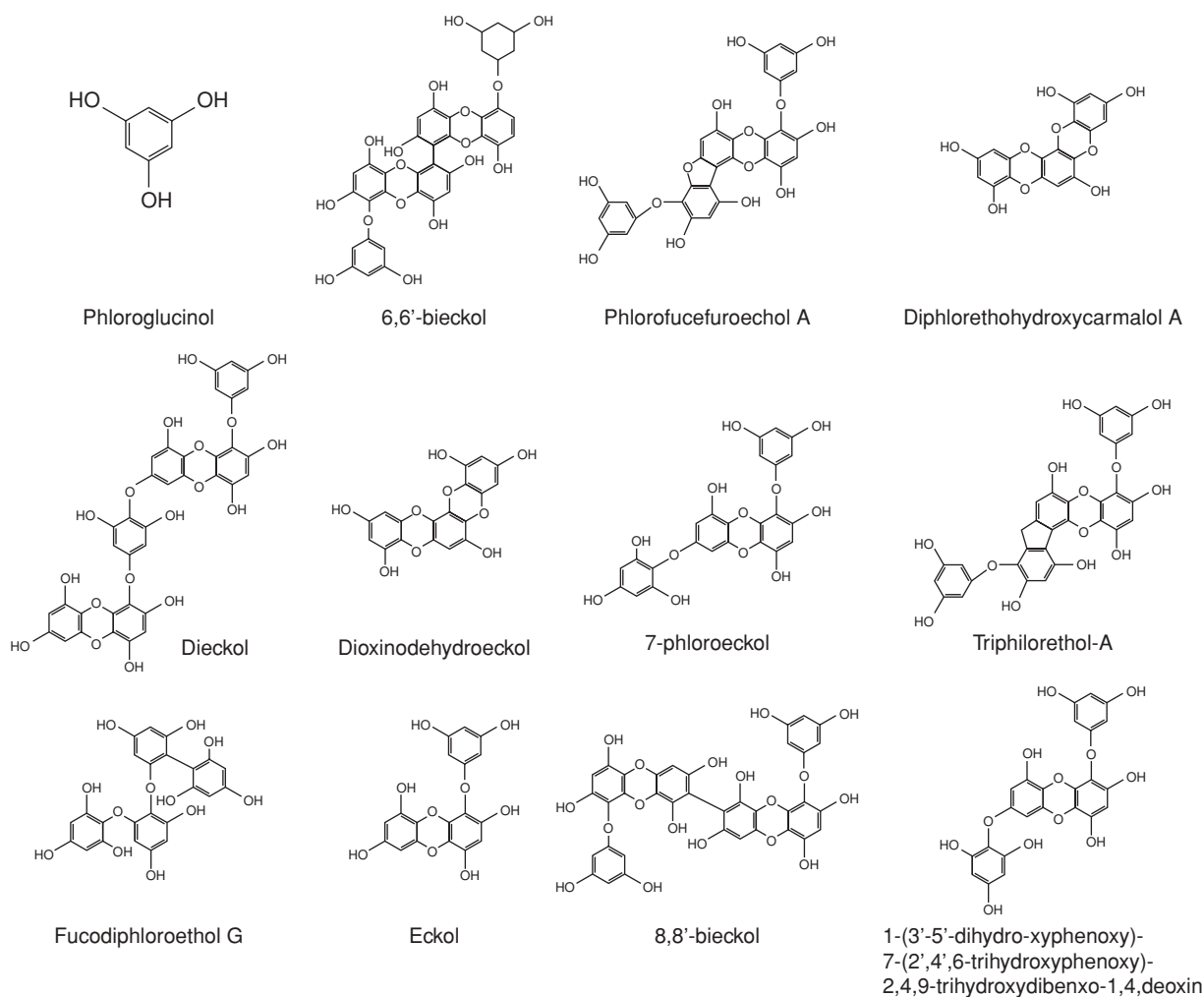
**Table 17.1** Marine macroalgae-derived phlorotannins and their immune regulatory effects

Organ	Phlorotannin derivatives	Activities	Mechanism	References
Unnamed	Phloroglucinol	Anti-inflammation	Suppression of NIK and activation of AP-1 transcription factor via MARK pathway and matrix metalloproteinase inhibition	Kim and Kim, 2010
<i>Ecklonia stolonifera</i>	Phlorofucofuroeckol A, dieckol, and dioxinodehydroeckol	Anti-inflammation	Effect of phlorofucofuroeckol A on iNO, COX-2 inhibition	Kim <i>et al.</i> , 2009
<i>Eisenia bicyclis</i>	Phloroglucinol; eckol, phlorofucofuroeckol A, dieckol, and 8,8'-bieckol	Anti-inflammation	sPLA <sub>2</sub> , COX-1 and COX-2 inhibition	Shibata <i>et al.</i> , 2003
<i>Ecklonia cava</i>	Phlorotannin extracts	Matrix metalloproteinase inhibition	Uninvestigated	Kim <i>et al.</i> , 2006
<i>Eisenia arborea</i>	Extract and three (6,6'-bieckol, 6,8'-bieckol and phlorofucofuroeckol -B)	Anti-allergy	Inhibition of beta-hexosaminidase release	Sugiura <i>et al.</i> , 2007
<i>Ecklonia cava</i>	Phloroglucinol, dieckol, 6,6'-dieckol, and 1-(3',5'-dihydroxyphenoxy)-7-(2'',4'',6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin	Anti-allergy	Inhibition of binding activity between IgE and FcεRI	Le <i>et al.</i> , 2009
<i>Laurencia undulate</i>	Extracts	Anti-asthmatis	Inhibition of TNF-α and Th <sub>2</sub>	Jung <i>et al.</i> , 2009
<i>Ecklonia cava</i>	Extracts	Anti-asthmatis	Th2 cytokine reduction	Kim <i>et al.</i> 2008

(Continued)

Table 17.1 (Continued)

Organ	Phlorotannin derivatives	Activities	Mechanism	References
<i>Eisenia bicyclis</i>	Dioxinodehydroeckol, eckol, phlorofurofueckol-A, dieckol, triphlo roethol A, 7-phloroethol	Neuroprotective effect	BACE1 ( $\beta$ -site of APP cleaving enzyme) inhibitory activity	Jung <i>et al.</i> , 2010
<i>Ecklonia cava</i>	Extract containing phenolic and compounds and phlorotannins	Neuroprotective effect	iNOS, COX-2, NF- $\kappa$ B inhibition	Jung <i>et al.</i> , 2009
<i>Ishige okamurae</i>	Phloroglucinol, 6,6'-bieckol, and diphlorethohydroxycarmalol	Neuroprotective effect	6,6'-bieckol effect on cholinesterase inhibition	Yoon <i>et al.</i> , 2009
<i>Ecklonia cava</i>	Methanolic extract, dieckol and 1-(3',5'-dihydroxyphenoxy)-7-(2',4',6'-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4,-dioxin	Anti-Rheumatoid arthritis	iNOS and COX-2 promotion, JNK and p38 MAPK suppression	Ryu <i>et al.</i> , 2009
<i>Ecklonia cava</i>	Phlorotannin LDA103 extract	Anti-rheumatoid arthritis	PGE <sub>2</sub> and interleukin-1 $\alpha$ inhibition	Shin <i>et al.</i> , 2005
<i>Ecklonia cava</i>	Phlorotannin venol	Anti-rheumatoid arthritis	PGE <sub>2</sub> and interleukin-1 $\alpha$ inhibition	Kang <i>et al.</i> , 2004
<i>Ecklonia cava</i>	Dieckol	Anti-diabetes	Uninvestigated	Lee <i>et al.</i> , 2010
<i>Ishige okamurae</i>	Diphlorethohydroxycarmalol	Anti-diabetes	Uninvestigated	Heo <i>et al.</i> , 2009
<i>Ecklonia cava</i>	Crude polysaccharide and crude polyphenolic fractions	Anti-proliferative and anti-radical activities	Superoxide anion (O <sub>2</sub> <sup>-</sup> ), hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> ) and hydroxyl radical (OH $\cdot$ )	Athukoral <i>et al.</i> , 2006
<i>Ishige okamurae</i>	Diphlorethohydroxycarmalol	Anti-oxidant	Uninvestigated	Heo and Jeon, 2009
<i>Eisenia bicyclis</i> , <i>Ecklonia cava</i> , and <i>Ecklonia kurome</i>	Phloroglucinol, eckol, phlorofucofuroeckol A, dieckol, and 8,8'-bieckol	Anti-oxidant	Uninvestigated	Shibata <i>et al.</i> , 2007



**Figure 17.1** Chemical structures of phlorotannins derived from marine macroalgae.

## 17.2 Anti-inflammatory effects of phlorotannins on RAW264.7 macrophage cells

The inhibitory effect of the inflammatory mediators such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in RAW264.7 cells stimulated by lipopolysaccharide was investigated in phloroglucinol, the monomer of phlorotannins abundant in brown algae (Kim and Kim, 2010). These results are compared with the inhibitory activities on human fibrosarcoma cell line (HT1080), where phloroglucinol diminished the expression of matrix metalloproteinase that show an important role in chronic inflammatory diseases. Phloroglucinol inactivated the inflammatory mediators via the suppression of nuclear factor (NF)- $\kappa$ B inducible

kinase (NIK) and activation of activator protein 1 (AP-1) transcription factor via the mitogen-activated protein kinase (MAPK) pathway. Phloroglucinol also regulated the reduction of AP-1 transcription factor via the inactivation of extracellular signal-regulated kinase (ERK). This result suggested that phloroglucinol could be a potential agent for the inhibition of chronic inflammation. Anti-inflammatory studies of the brown alga *Ecklonia stolonifera* showed profound inhibitory effect on nitric oxide (NO) production and PGE<sub>2</sub> in lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophage cells (Kim *et al.*, 2009). Three phlorotannins of phlorofucuroechol A, dieckol, and dioxinodehydroeckol were evaluated in this study. Among of them, phlorofucuroechol A showed significant inhibition in the production of NO and PGE<sub>2</sub> through the down-regulation of inducible nitric oxide

synthase (iNOS) and cyclo-oxygenase-2 (COX-2) protein expression. This study concluded that phlorofucofuroeckol A from *E. stolonifera* is a potential anti-inflammatory agent.

### 17.3 Neuroprotective effects of phlorotannins on BV2 microglial cells

Alzheimer's disease (AD) is a neurodegenerative disease of the brain that causes changes in brain function together with the full complexity of local peripheral inflammatory response. Jung *et al.* (2010) reported an anti-AD effect of phlorotannins from *Eisenia bicyclis* with BACE1 ( $\beta$ -site of APP cleaving enzyme) inhibitory activity. This extract had performed non-competitive inhibition against BACE1 through TYR132 and THR133 residues from the phlorotannin types of dioxinodehydroeckol, eckol, phlorofucofuroeckol-A, dieckol, triphloroethol A, 7-phloroethol. It is suggested that the extract of *E. bicyclis* can be applied for the development of preventive agents for AD, which is being considered for further studies on pharmacotherapy.

The anti-neurodegenerative effect of brown algae *Ecklonia cava* extract with high amount of phlorotannins was investigated using LPS-stimulated BV2 microglia (Jung *et al.*, 2009a). The extract exhibited the inhibition of NO, PGE<sub>2</sub> production in a concentration-dependent manner and iNOS and COX-2 in BV2 microglia without significant cytotoxicity. Further, the extract showed the suppression of

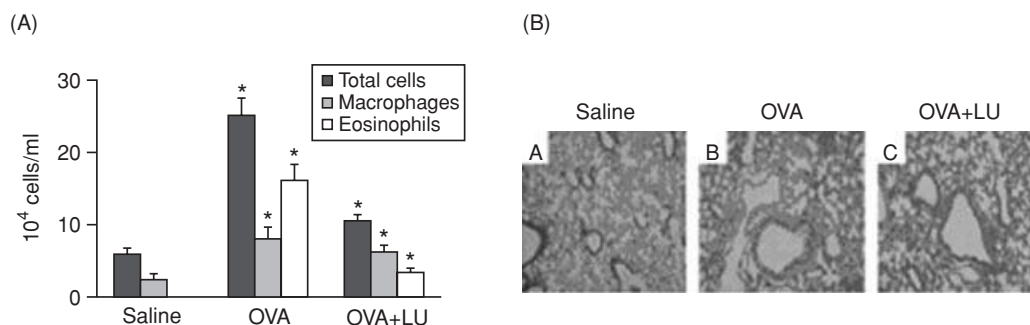
NF- $\kappa$ B translocation and DNA-binding in LPS-stimulated BV2 microglia. This finding indicates that the extract inhibited the proinflammatory cytokines via pathways blocking NF- $\kappa$ B and MAPK activation.

Yoon *et al.* (2009) investigated the inhibitory effects of algae *Ishige okamurai* phlorotannins on cholinesterase (acetylcholinesterase and butyrylcholinesterase) that are responsible for a crucial role in cholinergic transmission by hydrolyzing the neurotransmitter acetylcholine, which is implicated to a chronic and progressive neurodegenerative disorder of Alzheimer's disease. Three structures were characterized from this brown algae as phloroglucinol, 6,6'-bieckol, and diphlorethohydroxycarmalol. The 6,6'-bieckol compound showed strong inhibitory effect against acetylcholinesterase. This study suggested that the brown algae *I. okamurai* should be a potential marine source of natural products, which suppress Alzheimer's disease.

### 17.4 Anti-allergic effects of phlorotannins

#### 17.4.1 Anti-asthma

A Polyphenolic extracts derived from marine red alga *Lau-rencia undulate* (LU) showed anti-asthma effect against ovalbumin (OVA)-induced murine allergic airway reactions using *in vivo* histological and cytokine assays (Jung *et al.*, 2009b). As shown in Figure 17.2, LU extract showed anti-asthmatic reactive effects with symptoms of increase in



**Figure 17.2** (A) Effects of *L. undulata* extracts (LU) on the recruitment of inflammatory cells into BAL in OVA-induced allergic asthmatic mice. Mice were treated with saline, OVA-inhaled mice administered saline (OVA), and OVA-inhaled mice administered *L. undulata* extract 20 mg/kg (OVA + LU), respectively. BAL cells were separated using a Cytospin, and then stained with Diff-Quik. Differential cell counting was performed using standard morphological criteria. The BAL cells were collected 2 days after the last OVA-challenge. Each value indicates the mean  $\pm$  S.E.M. from five separate experiments ( $n = 6$  per group). \* $p < 0.05$  vs. saline-treated mice; # $p < 0.05$  vs. OVA-treated mice. (B) *L. undulata* extracts (LU) inhibits pathological changes in lung tissues of OVA-sensitized and -challenged mice. Mice were sensitized and challenged as described in methods. Sections were obtained from the rungs of mice receiving saline, OVA-inhaled mice administered saline (OVA), and OVA-inhaled mice administered *L. undulata* extract (20 mg/kg, OVA + LU), respectively. Lungs were removed 2 days after the last airway challenge. Sections were stained by hematoxylin and eosin staining. Six animals were assigned to each group.

the number of eosinophil in bronchoalveolar lavage (BAL) fluid, a marked influx of inflammatory cells into the lung around blood vessels and airways, and airway luminal narrowing; the development of airway hyper-responsiveness. In addition, it exhibited inhibition of TNF- $\alpha$  and Th2 (T helper 2) cytokines, such as IL-4 and IL-5 in the BAL fluid and allergen-specific immunoglobulin E (IgE) in the serum.

Kim *et al.* (2008) investigated the anti-inflammatory activity of the ethanolic extracts from *E. cava* against OVA-stimulated asthma in mice. It was showed that the extracts inhibit OVA-induced airway inflammation in a murine asthma model. Mechanistically, these extracts inhibited the levels of influx of inflammatory cells into the lung, mucus hypersecretion and airway occlusion, and the thicknesses of the bronchial wall and the area of the smooth muscle. The administration of the *E. cava* extracts exhibited significant inhibition of all asthmatic reactions and the mediated inhibition of asthmatic reactions appear to be attributable to the initial suppression of an allergen specific IgE response. The treatment of *E. cava* extracts resulted in significant reduction of matrix metalloproteinase-9 (MMP-9) and suppression of cytokine signaling-3 (SOCS-3) expression and a reduction in increased eosinophil peroxidase (EPO) activity. The *E. cava* extract also resulted in a reduction in the concentrations of the Th2 cytokines (IL-4 and IL-5) in the airways. This study has shown the critical anti-asthmatic effects of *E. cava* extract.

#### 17.4.2 Anti-rheumatoid arthritis (RA)

Arthritis occurs due to inflammation that appears around the joint, causing damage to the joint. Arthritis also is one of the chronic inflammatory diseases. Ryu *et al.* (2009) performed the significant anti-inflammatory activities from the marine brown alga *E. cava*. The methanol extract of brown alga *E. cava* and phlorotannin derivatives of the dieckol and 1-(3',5'-dihydroxyphenoxy)-7-(2',4',6'-trihydroxyphenoxy)2,4,9-trihydroxydibenzo-1,4,-dioxin promoted iNOS and COX-2 and matrix metalloproteinases (MMP-1, MMP-3, and MMP-13). The phlorotannin derivatives exhibited the suppression of phosphorylation of JNK and p38 MAPK in human osteosarcoma cell. The brown alga *E. cava* phlorotannin derivatives should be potent agents in chronic study in the future.

Shin *et al.* (2006) investigated an anti-inflammatory agent from the marine brown algae *E. cava* for potential treatment of osteoarthritis. The phlorotannins in the extract, including LDA103, exhibited inhibition of the downstream degenerative inflammatory development of PGE<sub>2</sub> generation in LPS-stimulated RAW264.7 cells. It

also inhibited IL-1 $\alpha$ -induced proteoglycan degradation that involves the pathophysiological development of osteoarthritis. LDA103 displayed significant therapeutic potentials in arthritic treatment via *in vitro* experiments. However, the mechanism was not investigated in this study.

Ventol, known as a phlorotannin-rich natural compound isolated from *E. cava*, was investigated on cartilage explant culture-inferred to osteoarthritis development that induces loss of homeostatic balance around cartilage tissue (Kang *et al.*, 2004). Inflammatory mediators like cytokines and prostaglandins, and reactive oxygen species, can lead to overproduction of tissue that induces physiological diseases in human body. In this study, ventol exhibited a significant inhibition of PGE<sub>2</sub> generation in LPS-treated RAW246.7 macrophage cells. Ventol also suppressed the human recombinant IL-1 $\alpha$  that leads to proteoglycan degradation-involved osteoarthritis. However, the mechanistic details were not investigated in this study.

*Eisenia bicyclis* is a common brown alga utilized as food and industrial material of alginic acid. Shibata *et al.* (2003) had identified the inhibitory effects of brown algal *Eisenia bicyclis* phlorotannins on phospholipase A<sub>2</sub>s, lipoxygenases and COXs that determined the *in vitro* assay. Oligomers of phloroglucinol; eckol (a trimer), phlorofucofuroeckol A (a pentamer), dieckol (a hexamer) and 8,8'-bieckol (a hexamer) inhibited sPLA<sub>2</sub> production from porcine pancreas and bee venom. The effects of dieckol and eckol on COX-1 and COX-2 had also reported. As we know, high levels of sPLA<sub>2</sub> are indicative of the presence of pathological properties in synovial fluids, articular cartilage and blood from patients with rheumatic diseases. However, the pharmacological activity was not investigated in this study.

MMPs have been related to disease progressions such as metastasis, arthritis, wrinkle formation, and chronic inflammation. Tan *et al.* (2006) reported that MMPs promote inflammation and fibrosis in asbestos-induced lung injury in mice. Kim *et al.* (2006) reported the inhibitory effects of phlorotannin isolated from brown algae *E. cava* on MMP activities in cultured human cell lines. This study is a predominant evidence of further investigation for disease-related inflammation.

#### 17.4.3 Other phlorotannins

A M/C (methanol:chloroform = 1:2, v/v) extract and six phlorotannins (eckol, 6,6'-bieckol, 6,8'-bieckol, 8,8'-bieckol, phlorofucofuroeckol-A, phlorofucofuroeckol-B) isolated from the edible brown alga *Eisenia arborea* were investigated for their anti-allergic activity (Sugiura *et al.*,

2007). The M/C extract suppressed the histamine release from rat basophilic leukemia cells (RBL-2H3) and human basophilic cell line (KU812) in a dose-dependent manner. The isolated phlorotannins, eckol, 6,6'-bieckol, 6,8'-bieckol, 8,8'-bieckol, phlorofucofuroeckol-A, and phlorofucofuroeckol-B, showed inhibitory effect to beta-hexosaminidase release, which responsible for the allergy, from the rat basophilic leukemia-2H3 cells.

Three potential candidates of phlorotannin derivatives of phloroglucinol, dieckol, 6,6'-dieckol, and 1-(3',5'-dihydroxyphenoxy)-7-(2'',4'',6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin were used to clarify antiallergy activity of crude extract isolated from brown alga *E. cava* (Le *et al.*, 2009). These phlorotannin derivatives displayed inhibitory effects on human basophilic leukemia (KU812) and rat basophilic leukemia (RBL-2H3) cells to release histamine stimulated by antibodies of goat anti-human IgE and calcium ionophore A23187. The anti-allergic mechanism also showed the inhibition of binding activity between IgE and Fc $\epsilon$  RI. The compounds of dieckol, 6,6'-dieckol, 1-(3',5'-dihydroxyphenoxy)-7-(2'',4'',6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin are suggested to be potential candidates for pharmaceutical and food industry. However, other mechanisms should be investigated more to provide more evidence for this study.

## 17.5 Conclusion

We demonstrated the effective phlorotannin derivatives in immune system to be: phloroglucinol, eckol, phlorofucofuroeckol A, dieckol, dieckol, bieckol, diphlorethohydroxycarmalol, venol, 1-(3',5'-dihydroxyphenoxy)-7-(2'',4'',6'-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4,-dioxin, 1(3',5'dihydroxyphenoxy)-7-(2'',4'',6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin, phlorofucofuroeckol-A, phlorofucofuroeckol-B, triphloroethol-A, 7-phloroethol, dioxinodehydroeckol. According to this study we can see that phlorotannin derivatives play an important role in mechanisms against inflammatory mediators that might link to cancer, inflammatory diseases, septic shock, viral infection, and correction of immune development due to incorrect regulation of signaling pathways in immune system.

In our review, some marine brown algae species were clarified the potentially active phlorotannins, though there are about 1500–2000 species of brown algae worldwide. Most research has been focused on order Laminariales, including *Ecklonia cava*, exposing the strong activity. In addition, many researchers have characterized and isolated many of the chemical structures of phlorotannin derivatives from the marine macroalga *Ecklonia cava*. According

to this study, marine macroalgae are promising treasure and the potential subjects for anti-inflammatory development and other bioactivities.

## Acknowledgments

This work was supported by the Technology Development Program for Fisheries, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea, and by the New & Renewable Energy of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Korea government Ministry of Knowledge Economy (No. 20103020090020). This research was also supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010–2003111).

## References

- Altena, I.A.V. and Steinberg, P.D. (1992) Are differences in the responses between north American and Australasian marine herbivores to phlorotannins due to differences in phlorotannins structure. *Biochem. Syst. Ecol.*, **20**, 493–499.
- Artan, M., Li, Y., Karadeniz, F., *et al.* (2008) Anti-HIV-1 activity of phloroglucinol derivative, 6,6'-bieckol, from *Ecklonia cava*. *Bioorg. Med. Chem.*, **16**, 7921–7926.
- Athukorala, Y., Kim, K.N. and Jeon, Y.J. (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food Chem. Toxicol.*, **44**, 1065–1074.
- Boettcher, A.A. and Targett, N.M. (1993) Role of polyphenolic molecular-size in reduction of assimilation efficiency in *Xiphister mucosus*. *Ecology*, **74**, 891–903.
- Glombitza, K.W. and Li, S.M. (1991) Hydroxyphlorethols from the brown alga *Carpophyllum maschalocarpum*. *Phytochemistry*, **30**, 2741–2745.
- Heo, S.J., Hwang, J.Y., Choi, J.I., *et al.* (2009) Diphlorethohydroxycarmalol isolated from *Ishige okamurae*, a brown algae, a potent  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. *Eur. J. Pharmacol.*, **615**, 252–256.
- Heo, S.J. and Jeon, Y.J. (2009) Evaluation of diphlorethohydroxycarmalol isolated from *Ishige okamurae* for radical scavenging activity and its protective effect against H<sub>2</sub>O<sub>2</sub>-induced cell damage. *Proc. Biochem.*, **44**, 412–418.
- Jung, H.A., Oh, S.H., and Choi, J.S. (2010) Molecular docking studies of phlorotannins from *Eisenia bicyclis* with

- BACE1 inhibitory activity. *Bioorg. Med. Chem. Lett.*, **20**, 3211–3215.
- Jung, W.K., Ahn, Y.W., Lee, S.H., *et al.* (2009a) *Ecklonia cava* ethanolic extracts inhibit lipopolysaccharide-induced cyclooxygenase-2 and inducible nitric oxide synthase expression in BV2 microglia via the MAP kinase and NF- $\kappa$ B pathways. *Food Chem. Toxicol.*, **47**, 410–417.
- Jung, W.K., Choi, I., Oh, S., *et al.* (2009b) Anti-asthmatic effect of marine red alga (*Laurencia undulata*) polyphenolic extracts in a murine model of asthma. *Food Chem. Toxicol.*, **47**, 293–297.
- Kang, K., Hwang, H.J., Hong, D.H., *et al.* (2004) Anti-oxidant and anti-inflammatory activities of ventol, a phlorotannin-rich natural agent derived from *Ecklonia cava*, and its effect on proteoglycan degradation in cartilage explant culture. *Res. Commun. Molec. Pathol. Pharmacol.*, **115/116**, 77–95.
- Kim, A.R., Shin, T.S., Lee, M.S. *et al.* (2009) Isolation and identification of phlorotannins from *Ecklonia stolonifera* with antioxidant and anti-inflammatory properties. *J. Agric. Food Chem.*, **57**, 3483–3489.
- Kim, M.M. and Kim, S.K. (2010) Effect of phloroglucinol on oxidative stress and inflammation. *Food Chem. Toxicol.*, **48**, 2925–2933.
- Kim, M.M., Ta, Q.V., Mendis, E., *et al.* (2006) Phlorotannins in *Ecklonia cava* extract inhibit matrix metalloproteinase activity. *Life Sci.*, **79**, 1436–1443.
- Kim, S.K., Lee, D.Y., Jung, W.K., *et al.* (2008) Effects of *Ecklonia cava* ethanolic extracts on airway hyperresponsiveness and inflammation in a murine asthma model: Role of suppressor of cytokine signaling. *Biomed. Pharmacother.*, **62**, 289–296.
- Kong, C.S., Kim, J.A., Yoon, N.Y., Kim, S.K. (2009) Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cancer cells. *Food Chem. Toxicol.*, **47**, 1653–1658.
- Le, Q.T., Li, Y., Qian, Z.J. *et al.* (2009) Inhibitory effects of polyphenols isolated from marine alga *Ecklonia cava* on histamine release. *Proc. Biochem.*, **44**, 168–176.
- Li, Y., Qian, Z.J., Ryu, B.M., *et al.* (2009) Chemical components and its antioxidant properties in vitro: An edible marine brown alga, *Ecklonia cava*. *Bioorg. Med. Chem.*, **17**, 1963–1973.
- McClintock, J.B. and Baker, B.J. (2001) *Marine Chemical Ecology*. CRC Press, Boca Raton, FL.
- Nagayama *et al.* (2002) Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. *J. Antimicrob. Chemother.*, **50**, 889–893.
- Paul, V.J. (1992) *Ecological Roles of Marine Natural Products*. Cornell Press, Ithaca, New York.
- Ragan, M.A. and Glombitza, K.W. (1986) *Handbook of Physiological Methods*. Cambridge University Press, Cambridge, UK.
- Ragan, M.A. and Glombitza, K.W. (1986) Phlorotannins, brown algal polyphenols. In: *Progress in Phycological Research* (eds F.E. Round and D.J. Chapman). Biopress Ltd, Bristol, pp. 129–241.
- Ryu, B.M., Li, Y., Qian, Z.J., *et al.* (2009) Differentiation of human osteosarcoma cells by isolated phlorotannins is subtly linked to COX-2, iNOS, MMPs, and MAPK signaling: Implication for chronic articular disease. *Chem.-Biol. Interact.*, **179**, 192–201.
- Shibata, T., Nagayama, K., Tanaka, R., *et al.* (2003) Inhibitory effects of brown algal phlorotannins on secretory phospholipase A<sub>2</sub>s, lipoxygenases and cyclooxygenases. *J. Appl. Phycol.*, **15**, 61–66.
- Shibata, T., Kawaguchi, S., Hama, Y., *et al.* (2004) Local and chemical distribution of phlorotannins in brown algae. *J. Appl. Phycol.*, **16**, 291–296.
- Shibata, T., Ishimaru, K., Kawaguchi, S., *et al.* (2007) Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *J. Appl. Phycol.*, **20**, 705–711.
- Shin, H.C., Hwang, H.J., Kang, K.J. and Lee, B.H. (2006) An anti-oxidative and anti-inflammatory agent for potential treatment of osteoarthritis from *Ecklonia cava*. *Arch. Pharm. Res.*, **29**, 165–171.
- Singh, I.P. and Bharate, S.B. (2006) Phloroglucinol compounds of natural origin. *Nat. Prod. Rep.*, **23**, 558–591.
- Sugiura, Y., Matsuda, K., Yamada, Y., *et al.* (2007) Anti-allergic phlorotannins from the edible brown alga, *Eisenia arborea*. *Food Sci. Technol. Res.*, **13**, 54–60.
- Tan, R.J., Fattman, C.L., Niehouse, L.M., *et al.* (2006) Matrix metalloproteinases promote inflammation and fibrosis in asbestos-induced lung injury in mice. *Am. J. Resp. Cell Molec. Biol.*, **35**, 289–297.
- Targett, N.M. and Arnold, T.M. (1998) Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. *J. Phycol.*, **34**, 195–205.
- Yoon, N.Y., Lee, S.H., Li, Y. and Kim, S.K. (2009) Phlorotannins from *Ishige okamurae* and their acetyl- and butyryl-cholinesterase inhibitory effects. *J. Funct. Foods*, **1**, 331–335.
- Zhang, R., Kang, K.A., Piao, M.J., *et al.* (2008) Ecklon protects V79–4 lung fibroblast cells against gamma-ray radiation-induced apoptosis via the scavenging of reactive oxygen species and inhibiting of the c-Jun NH<sub>2</sub>-terminal kinase pathway. *Eur. J. Pharmacol.*, **591**, 114–123.

# 18

## *In Vivo* and *In Vitro* Studies of Seaweed Compounds

**Raquel Domínguez Gonzalez, Vanessa Romaris Hortas and  
Pilar Bermejo Barrera**

*Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry,  
University of Santiago de Compostela, Santiago de Compostela, Spain*

### 18.1 Introduction

Seaweeds are a marine product consumed in Asian countries since ancient times. In the West, seaweed has been used mainly as a source of components in the food industry (colloids, gelling, and thickness agents). Nevertheless, the current interest in health foods has led to an increase of seaweed availability in food markets. Due to the use of seaweed as a foodstuff, further studies of the nutrient composition are necessary (Chen and Jiand, 2001). Seaweeds have been well recognized as a natural source of essential elements because of their ability to concentrate inorganic species from seawater (Chapman and Chapman, 1980). However, metallic pollutants are also absorbed (Rupérez, 2002); thus, the seaweed industry requires data on the concentration levels of the essential and the toxic elements before commercializing these products. As an example, Aquaron *et al.* (2002) studied three types of table salt supplemented with marine algae (in the French market) and found that one contained a high amount of arsenic, while none of the three commercial salts were in accordance with French legislation because the iodine content was higher than the amount permitted. To know the possible effects of trace elements present in foods, knowledge of the total content in the food is not sufficient; it is also necessary to know the element bioavailable fraction for humans.

The first stage toward the study of the availability of any compound (element) from food, is the evaluation of the fraction of this element that is soluble in the gastrointestinal medium (Salovaara *et al.*, 2002). This fraction is called bioaccessible and represents the portion available for subsequent processes of absorption into intestinal mucosa (Laparra *et al.*, 2003). Bioavailability involves the release of this element, the chemical process that takes place in the gastrointestinal tract (bioaccessibility), and the absorption into the circulatory system (Argyri *et al.*, 2009; Dufaille *et al.*, 2008). The bioavailability of an element in a food depends on gastrointestinal conditions (Bosscher *et al.*, 2001; Wolfgor *et al.*, 2002), on the chemical form of the element (Aquaron *et al.*, 2002; Domínguez *et al.*, 2004; Muñiz *et al.*, 2006; Haro *et al.*, 2006; Perales *et al.*, 2007; Robberecht *et al.*, 2009; Zhu *et al.*, 2009; Tripathi and Platel, 2010; Aherne *et al.*, 2010), as well as on the presence of other components of the diet that can improve or inhibit the absorption process (Perales *et al.*, 2007; Robberecht *et al.*, 2009; Tripathi and Platel, 2010; Aherne *et al.*, 2010; López *et al.*, 2001; Pérez *et al.*, 2002; Solomons, 1986; Chan, Black and Hale, 2007; O'Dell, 1989; Yu *et al.*, 2010; Zheng *et al.*, 2010; Jin *et al.*, 2009; Cámara *et al.*, 2005; Pachon *et al.*, 2008; Wolters *et al.*, 1993).

The whole bioavailability process involves digestion, absorption, transport, utilization, and elimination. Therefore,

bioavailability studies are unable to include all these aspects, especially the absorption and utilization of the elements, stages that are highly dependent on the physiological conditions of the individual, such as trace element status, age, or presence of disease (Favier, 1993).

## 18.2 Methods to study compound bioaccessibility

Estimating compounds bioavailability in food can be performed by *in vivo* (dosing experimental humans or animals with several concentrations of the target compound) or *in vitro* methods. Due to ethical and practical (high cost, time consuming, and large interindividual variations) questions *in vitro* methods are preferred in initial studies of bioavailability.

### 18.2.1 In vivo methods

The most realistic evaluation of the bioavailability of an element can be obtained using *in vivo* studies because these take place under physiological conditions. These studies consist in feeding a group of subjects (human or animal) with the food or target compound under study. After feeding (times vary with the studied element or compound, food type and subject) different biological samples are taken and analyzed for the target. The *in vivo* trials should be done with numerous groups of carefully chosen subjects. The main drawback of *in vivo* studies with humans is the inherent heterogeneity of the subjects and the control of external conditions during the study. In addition, these studies are time consuming and expensive. Some of these problems are easily solved when the subjects are animals, especially small animals like rats.

In some *in vivo* studies, radiolabeled elements (stable isotopes) are used to determine the percentage of absorbed or excreted elements. These kinds of trials in humans are considered unethical, and are therefore performed mainly with animals.

The samples used to perform *in vivo* human studies can be blood (Van Hulle *et al.*, 2004), serum (Van Hulle *et al.*, 2004; Robberecht *et al.*, 2009; Gotelli *et al.*, 1996), urine (Van Hulle *et al.*, 2004; Robberecht *et al.*, 2009; Aquaron *et al.*, 2002). In animals the samples are blood, target organs (kidney, spleen, skin), and feces or urine (Sarriá and Vaquero, 2001).

#### In vivo studies with humans

Aquaron *et al.* (2002) have studied iodine bioavailability from seaweed, after selecting those seaweeds with the high-

est bioabsorption properties for elements. For this purpose they selected two seaweed samples, *Gracilaria verrucosa* (a red seaweed with high organic iodine content) and *Laminaria hyperborean* (a brown seaweed with high mineral iodine content). The results obtained show different iodine bioavailability depending on the basal iodine levels of each subject. Iodine bioavailability was higher from *Gracilaria verrucosa* (high organic iodine content) than in *Laminaria hyperborean* (high inorganic iodine content). This fact can be explained as a result of the differences in seaweed composition, mainly due to the higher alginate content in the brown seaweed, which decreases iodine absorption. In addition, iodine bioavailability when administering aqueous potassium iodide was lower than when administering an organic iodine solution (monoiodotyrosine).

Van Hulle *et al.* (2004) have applied an *in vivo* method with humans (Chinese and European) to study arsenic species bioavailability in boiled or raw seaweed (*Laminaria*). The researchers concluded that all arsenosugars are possibly first transformed into a compound of mass 254 Da before being further metabolized. They found different arsenic concentrations in the different blood compartments of each volunteer.

#### In vivo studies with animals

Although several *in vivo* studies with animals have been performed for different foodstuffs (collards, spinach, soybeans, breakfasts with and without milk, infant formulas, and other foods) (Wien and Schwartz, 1983; Vaquero *et al.*, 1993; Schricker *et al.*, 1981; Sarriá and Vaquero, 2001; Ellickson *et al.*, 2001), and also for soils (Juhász *et al.*, 2007), studies for trace elements in seaweed have not yet been reported.

### 18.2.2 In vitro methods

*In vitro* methods are another approach for studying the bioavailability of trace elements in food. These methods are based on the simulation of gastric and intestinal digestion conditions and the measurement of the concentration of the trace element after this treatment. *In vitro* methods involve conditions (temperature, agitation, pH, and enzyme and chemical composition) similar to those found in the human body during digestion. There are different *in vitro* approaches:

- 1 The study of the maximum soluble concentration of the target compounds in the simulated gastrointestinal solution after filtration or centrifugation (bioaccessible fraction).

- 2 The study of the soluble fraction of the compound (bioaccessible fraction) achieved by using human gastrointestinal microbiota (simulator of the human intestinal microbial ecosystem, SHIME).
- 3 The study of the dialyzable fraction of the compound, which can dialyze through a semi-permeable membrane with a specified pore size (dialyzate or bioavailable fraction) at equilibrium or non-equilibrium conditions.
- 4 The study of the fraction of the compound capable of being retained or transported through a solid or micro-porous support (bioavailable fraction) in which human Caco-2 cells grown are incorporated (intestinal epithelial model).

In this chapter we study methods to study the bioaccessible fraction, the dialyzable fraction and the bioavailable fraction.

#### **Methods to study the bioaccessible fraction (bioaccessibility)**

The simplest approach for *in vitro* methods to perform the estimation of bioavailability of trace elements is the study of the bioaccessible fraction (Hocquellet and L'Hotellier, 1997). The soluble fraction is measured after simulating gastrointestinal digestion using pepsin (gastric digestion) and pancreatin-bile salts (intestinal digestion). Different gastrointestinal conditions can be designed depending on the food to be studied. Lönnerdal and Glazier (1989), Bermejo *et al.* (2002) and Peña *et al.* (2004) used neonatal gastrointestinal conditions to study the bioaccessibility of essential trace elements in human milk, cow milk and infant formulas; while Torres-Escribano *et al.* (2010) have studied mercury and methyl mercury bioavailability from fish.

Bioaccessibility from seaweed has been studied for total arsenic (Laparra *et al.*, 2003) and also for different arsenic species (Laparra *et al.*, 2004; Almela *et al.*, 2005; Koch *et al.*, 2007). Laparra *et al.* (2003) studied three different seaweeds, *Hizikia fusiforme* (brown seaweed), *Porphyra* sp. (red seaweed) and *Enteromorpha* sp. (green seaweed). They found extremely high As levels in *Hizikia fusiforme* (Hijiki). Bioaccessibility percentages of total As in red seaweed (67.2%) was slightly higher than in brown seaweed (62.3%), and double that in green seaweed (32%). The highest bioaccessibility of inorganic arsenic (As (III) + As (V)) in the same samples was found in green seaweed (77.2%); in brown seaweed it was slightly lower (74.7%), and the lowest was in red seaweed (48.6%). The bioaccessibility of As (total and inorganic) increases in *Porphyra* sp. and in *Hizikia fusiforme* after cooking. It is important to mention that the toxic

fraction of As (inorganic As) remains accessible for absorption into intestinal mucosa (over 40% in raw and 70% in cooked seaweed). Studies dealing with cooked seaweed show that the cooking water contained more As(V) than As(III) (Laparra *et al.*, 2004). Hanaoka *et al.* (2001) concluded that treatments like washing and soaking in water before cooking the seaweed decrease As content. Bioaccessibility of total and organic arsenic from seaweed (kelp powder from Canada, *Hizikia fusiforme*, *Undaria pinnatifida* and *Porphyra* sp. from Spain) was studied by Almela *et al.* (2005). Results showed no degradation of arsenosugars (glycerol ribose, phosphate ribose, sulfonate ribose and sulfate ribose) during the *in vitro* digestion procedure. Bioaccessibility of total As varies from 38% (*Undaria pinnatifida*) to 87% (*Porphyra* sp.). However, organic species (arsenosugars) bioaccessibility was found to be very high, from 98% (kelp powder) to 119% (*Porphyra* sp.) for glycerol ribose; 89% (*Undaria pinnatifida* and *Porphyra* sp.) to 120% (*Hizikia fusiforme*) for phosphate ribose; from 81% (*Undaria pinnatifida*) to 120% (*Hizikia fusiforme*) for sulfonate ribose. The bioaccessibility of As sulfonate ribose in *Porphyra* sp. and As sulfate ribose in *Undaria pinnatifida* and *Porphyra* sp. could not be determined because As was not detected in these bioaccessible fractions. The same authors studied the effects of cooking according to manufacturer recommendations (boiling or baking) on the bioaccessibility of As from *Hizikia fusiforme* and *Undaria pinnatifida*. For total As, the bioaccessibility from cooked seaweed increases from 53% to 57% in *Hizikia fusiforme*, and from 87% to 106% in *Porphyra* sp. On the other hand, organic arsenic bioaccessibility decreases after cooking, except for the arsenosugar phosphate ribose in *Porphyra* sp. (from 89% when raw to 93% after cooking). The levels of sulfonate ribose and sulfate ribose in the bioaccessible fraction from *Porphyra* sp. were undetectable.

Koch *et al.* (2007) used an *in vitro* digestion method different from the studies previously mentioned (using glycine instead of pepsin). This method was used to study As bioaccessibility from clams and seaweed harvested in polluted and unpolluted marine environments. Total and inorganic As bioaccessibility was assessed and results were consistent with previous studies (Laparra *et al.*, 2003). No difference was observed for samples from polluted and unpolluted locations.

Other studies included a first stage (mouth digestion with artificial saliva) such as that developed by Dufaille *et al.* (2008). Results show that the solubility of As begins in this stage. On the other hand, dynamic *in vitro* models have also been proposed to assess trace element bioaccessibility. This approach consists of a dynamic multicompartment computer-controlled system with four computer-controlled chambers simulating the conditions of the

stomach, duodenum, jejunum, and ileum. The dynamic development by Minekus *et al.* (1995) was used by Torres-Escribano *et al.* (2011) to assess As, Cd, Pb and Hg bioaccessibility from food reference materials (including IAEA-140/TM *Fucus* sp.), and to compare them with a static *in vitro* model proposed by Laparra *et al.* (2003). Significant differences between the bioaccessibility obtained from the static and dynamic methods were obtained (higher bioaccessibility for As, Cd, and Hg from the dynamic approach, and lower for Pb).

### Methods to study the dialyzable fraction (dialyzability)

Another approach for bioavailability studies is the use of semipermeable membranes to simulate the diffusion process during the intestinal stage. With this approach it is possible to estimate the bioavailable fraction (dialyzable) – the element fraction that can cross the intestinal walls. Miller *et al.* (1981) developed a method based on element dialyzability to determine iron bioavailability. This method has been used with several modifications to predict trace element bioavailability from different foods such as milk (Perez *et al.*, 2002; Muñoz *et al.*, 2006), infant formulas (Jovani *et al.*, 2001; Sarriá and Vaquero, 2001; Domínguez *et al.*, 2004), lettuce and wheat (Chan *et al.*, 2007), finger millet (Tripathi and Platel, 2010), and fast foods (Cabrera-Vique and Bouzas, 2009).

Dialyzability can also be assessed by a dynamic mode. Miniñane *et al.* (1993) used an Amicon stirred cell for a continuous dialysis process. The pressure of this cell was adjusted using oxygen-free nitrogen and maintained using distilled water. Taking into account that pH changes from acid in the stomach to neutral in the duodenum (which is an important site for mineral absorption), a further modification by Shen *et al.* (1994) used a gradual pH change during the dialysis process (instead of adjusting the pH before dialysis). Another continuous dialysis method was used by Bosscher *et al.* (2001) to simulate first-year infant gastrointestinal conditions for Fe, Ca, and Zn availability from infant formulas and human milk.

The pH adjustment during the intestinal digestion has been identified as one of the critical parameters in these studies. All dialyzability studies mentioned above used  $\text{NaHCO}_3$  for filling the dialysis membranes for pH adjustment during the intestinal digestion. This approach requires the calculation of titratable acidity for each sample (number of equivalents of NaOH needed to fix the gastric digest at the desirable pH). To avoid this laborious step and for a better pH control, Kapsukefalou and Miller (1991) proposed the use of PIPES buffer to adjust pH during intestinal digestion. A comparison between the use of  $\text{NaHCO}_3$  and

PIPES in dialyzability studies was performed by a number of authors. For example, Wolfgor *et al.* (2002) assessed iron from fortified foods; while trace elements from edible seaweed were assessed by Domínguez-González *et al.* (2010) for Cr, Co, Mn and V, Romarís-Hortas *et al.* (2011) for iodine and bromine, and by García-Sartal *et al.* (2011) for arsenic. With the use of PIPES, results showed that the pH in the inner and the outer solutions (separated by the dialysis membranes) remained constant during the entire intestinal process.

The bioavailability of each element in seaweeds, estimated as dialyzability percentage, varies depending on the type of seaweed studied. Nine different types of seaweeds harvested on the Galician coast (northwestern Spain) obtained from a local manufacturer were studied by Domínguez-González *et al.* (2010) and Romarís-Hortas *et al.* (2011). One of these samples is commercialized as cooked and canned in brine, and is a mixture of two brown seaweeds: sea spaghetti (*Himanthalia elongata*) and furbelows (*Saccorhiza polyschides*). The remaining samples are red seaweed dulse (*Palmaria palmata*) and nori (*Porphyra umbilicalis*); brown seaweed kombu (*Laminaria ochroleuca*), wakame (*Undaria pinnatifida*) and sea spaghetti (*Himantaria elongata*); green seaweed sea lettuce (*Ulva rigida*); the microalgae *Spirulina platensis*, commonly used in human and animal nutrition; and agar-agar, which is a hydrocolloid obtained from the red seaweed *Gelidium sesquipedale*. Manganese is the element with the highest value of dialyzability for all seaweed samples. The highest dialyzability percentages were found in wakame for Co ( $77.5 \pm 0.22\%$ ), Cr ( $66.7 \pm 0.24\%$ ) and Mn ( $100.0 \pm 0.03\%$ ); in kombu for iodine ( $17 \pm 1.9\%$ ), and in nori for bromine ( $47 \pm 3.0\%$ ). There is no value of vanadium dialyzability because the total vanadium content found in wakame was lower than the limit of quantification. The lowest dialyzability percentages were obtained for agar-agar and cooked canned seaweed due to Co, Cr and V not being detected in the dialyzable fraction, and for iodine in *Spirulina platensis*. For Br the lowest values of dialyzability were obtained for sea lettuce and agar-agar (18%). The results obtained for Co, Cr, Mn and V in *Spirulina platensis* are very interesting: the percentage of dialyzability was very similar for all of these elements ( $20.6 \pm 0.47\%$ ); while in the rest of the samples this value depends on the element studied. This difference can be due to *Spirulina* being a microscopic seaweed. García-Sartal *et al.* (2011) found very similar dialyzability percentages of As (14–17%) for wakame (*Undaria pinnatifida*), kombu (*Laminaria ochroleuca*), sea lettuce (*Ulva rigida*) and nori (*Porphyra umbilicalis*). These authors found that cooking seaweed decreases As bioavailability.

### 18.3 *In vivo* versus *in vitro* methods

The greatest problem in the use of the *in vivo* and *in vitro* methods to study the bioavailability of trace elements in foods is the comparison of the results obtained by both methods. Although for the seaweeds there is no comparative study we include some references about other foods.

Schricker *et al.* (1981) found substantial agreement for Fe in foodstuffs between the results obtained for *in vivo* (using humans) and *in vitro* (using dialyzability) methods, and less agreement between *in vivo* (using rat) and *in vivo* (using humans), but did not find correlation between the results obtained using *in vivo* (using rats) and *in vitro* (using dialyzability) methods.

Other authors such as Wien and Schwartz (1983) and Ellickson *et al.* (2001) did not find correlation between methods *in vivo* (using rats) and *in vitro* (using solubility) for Ca in test meals, and for Pb and As in a certified reference material of soil (NIST). In the comparison between results obtained from an *in vivo* study (using rats) and *in vitro* (using dialyzability) for Fe and Zn in infant formulas, Sarriá and Vaquero (2001) did not find any correlation either. Nevertheless, Juhasz *et al.* (2007) found statistical correlation between results obtained after an *in vivo* study using swine and an *in vitro* method (SBET) for As in contaminated soils.

### 18.4 Methods with cell culture models

The *in vitro* methods have been improved by incorporating a cell culture model to simulate the transport and nutrient retention process that occurs in the small intestine. Caco-2 cell monolayers (human colon carcinoma cell line) have been used to simulate morphological properties of mature human enterocytes in bioavailability studies. Glahn *et al.* (1998) and Jovaní *et al.* (2001) used this method to study Fe bioavailability in infant formulas. Perales *et al.* (2007) studied milk-based formulas and fruit juices containing milk and cereals. Pachon *et al.* (2008) studied meat products. Pynaert *et al.* (2006) studied complementary food for infants. Bering *et al.* (2006) studied rye bread. Chan *et al.* (2007) studied cadmium in lettuce and durum wheat. Jovaní *et al.* (2001) studied Ca and Zn in infant formulas. This method was also used to study Fe bioavailability in *Spirulina* (*Spirulina platensis*) by Puyfoulhoux *et al.* (2001), and Mg bioavailability in *Spirulina* by Planes *et al.* (2002).

Puyfoulhoux *et al.* (2001) compared Fe bioaccessibility from *Spirulina* (blue-green algae) fortified with iron sulfate to yeast, wheat flour and beef and found significant differ-

ences among the four iron sources, with *Spirulina* offering the highest bioavailability. *Spirulina* contains a highly available form of Fe (Bougle *et al.*, 1996), twice as absorbable as the form of Fe found in vegetables and most meats, and 60% more absorbable than other iron supplements such as iron sulfate (Puyfoulhoux *et al.*, 2001). For magnesium, Planes *et al.* (2002) did not find significant differences among *Spirulina* and other magnesium sources such as magnesium chloride (the form found in the therapeutic formula Magnogene) and foods such as Kellogg's All-Bran and Banania (an instant breakfast preparation containing cereals, banana, and honey). Nevertheless, as the magnesium content of the fortified *Spirulina* is very high, this seaweed is considered an excellent source of magnesium.

Mahler *et al.* (2009) have proposed an improvement for correlating *in vitro* and *in vivo* data incorporating HT29-MTX mucus-producing cells that form a mucus layer like that secreted by goblet cells in the intestinal epithelium (Powell *et al.*, 1994). Mahler *et al.* propose cocultures of Caco-2 and HT29-MTX because they represent the two major cell types (absorptive and goblet) found in the human intestinal epithelium and may give more accurate results in bioavailability studies.

### 18.5 Conclusions

*In vitro* methods are useful tools to estimate nutrient bioavailability prior to developing expensive and time consuming *in vivo* studies. These methods allow us to plan and develop bioavailability and/or bioaccessibility studies that include different physiological conditions, inhibitors and enhancers of absorption, and interactions into metal absorption. The results of both methods can then be compared with each other. Before commercializing a new food, a fortified food, or a new nutritional supplement based on seaweed, an *in vitro* digestion study must be performed to obtain an estimation of the bioavailability of essential and toxic trace elements. The effects of cooking on bioaccessibility and/or bioavailability must also be considered; furthermore, speciation studies in elements such as Hg, As, Se and I are essential for assessing chemical risk (Hg, As) and/or benefits (Se, I) of the element to humans (Moreda-Piñeiro *et al.* 2011).

### References

Aherne, S.A., Daly, T.; Jiwan, M.A., O'Sullivan, L. and O'Brien, N.M. (2010) Bioavailability of  $\beta$ -carotene isomers from raw and cooked carrots using an *in vitro*

- digestion model coupled with a human intestinal Caco-2 cell model. *Food Res. Int.*, **43**, 1449–1454.
- Almela, C., Laparra, J.M., Vélez, D., Barberá, R., Farré, R. and Montoro, R. (2005) Arsenosugars in raw and cooked edible seaweed: characterization and bioaccessibility. *J. Agric. Food Chem.*, **53**, 7344–7351.
- Aquaron, R., Delange, F., Marchal, P., Lognoné, V. and Niane, L. (2002) Bioavailability of seaweed iodine in human beings. *Cell. Molec. Biol.*, **48**, 563–569.
- Argyri, K., Birba, A., Miller, D.D., Komaitis, M. and Kapsokefalou, M. (2009) Predicting relative concentrations of bioavailable iron in foods using *in vitro* digestion: new developments. *Food Chem.*, **113**, 602–607.
- Bering, S., Bukhave, K., Henriksen, M., *et al.* (2006) Development of a three-tier *in vitro* system, using Caco-2 cells, to assess the effects of lactate on iron uptake and transport from rye bread following *in vitro* digestion. *J. Sci. Food Agric.*, **86**, 2438–2844.
- Bermejo, P., Peña, E.M., Domínguez, R., Bermejo, A., Cocho, J.A. and Fraga, J.M. (2002) Iron and Zinc in hydrolysed fractions of human milk and infant formulas using an *in vitro* method. *Food Chem.*, **77**, 361–369.
- Bosscher, D., Lu, Z., Van Cauwenbergh, R., Van Caille-Bertrand, M., Robberecht, H. and Deelstra, H. (2001) A method for *in vitro* determination of calcium, iron and zinc availability from first-age infant formula and human milk. *Int. J. Food Sci. Nutr.*, **52**, 173–182.
- Bougle, D., Boudy, M., Arhan, P., Bureas, F., Neuville, D. and Drosowsky, D. (1996) *In vivo* study of the absorption of seaweed minerals by perfused rat intestine. *Photother. Res.*, **10**, 325–326.
- Cabrera-Vique, C. and Bouzas, P.R. (2009) Chromium and manganese levels in convenience and fast foods: *in vitro* study of the dialyzable fraction. *Food Chem.*, **117**, 757–763.
- Cámara, F., Amaro, M.A., Barberá, R. and Lagarda, M.J. (2005) Speciation of bioaccessible (heme, ferrous and ferric) iron from school menus. *Eur. Food Res. Technol.*, **221**, 768–773.
- Chan, D.Y., Black, W.D. and Hale, B.A. (2007) Cadmium bioavailability and bioaccessibility as determined by *in vitro* digestion, dialysis and intestinal epithelial monolayers, and compared to *in vivo* data. *J. Env. Sci. Health, Part A*, **42**, 1283–1291.
- Chapman, V.J. and Chapman, D.J. (1980) *Sea Vegetables (Algae as Food for Man)*. Chapman & Hall, London.
- Chen, F. and Jiand, Y. (eds) (2001) *Algae and their Biotechnological Potential*. Kluwer Academic Publishers, Dordrecht.
- Domínguez, R., Barreiro, T., Sousa, E., *et al.* (2004) Study of the effect of different iron salts used to fortify infant formulas on the bioavailability of trace elements using ICP-OES. *Int. Dairy J.*, **14**, 1081–1087.
- Domínguez-González, R., Romarís-Hortas, V., García-Sartal, C., Moreda-Piñeiro, A., Barciela-Alonso, M.C. and Bermejo-Barrera, P. (2010) Evaluation of an *in vitro* method to estimate trace elements bioavailability in edible seaweeds. *Talanta*, **82**, 1668–1673.
- Dufaille, V., Guérin, T., Noël, L., Frémy, J. and Beauchemin, D. (2008) A simple method for the speciation analysis of bioaccessible arsenic in seafood using on-line continuous leaching and ion Exchange chromatography coupled to inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.*, **23**, 1263–1268.
- Ellickson, K.M., Meeker, R.J., Gallo, M.A., Buckley, B.T. and Lioy, P.J. (2001) Oral bioavailability of lead and arsenic from a NIST standard reference soil material. *Arch. Env. Contam. Toxicol.*, **40**, 128–135.
- Favier, A.E. (1993) Nutritional and clinical factors affecting the bioavailability of trace elements in humans. In: *Bioavailability '93 Nutritional, Chemical and Food Processing Implications of Nutrient Availability (Proceedings, Part 2)*. (ed. U. Schlemmer). Karlsruhe, Germany, pp. 202–212.
- García-Sartal, C., Romarís-Hortas, V., Barciela-Alonso, M.C., Moreda-Piñeiro, A., Domínguez-González, R. and Bermejo-Barrera, P. (2011) Use of an *in vitro* digestion method to evaluate the bioaccessibility of arsenic in edible seaweed by inductively coupled plasma-mass spectrometry. *Microchem. J.*, **98**, 91–96.
- Glahn, R.P., Lai, C., Hsu, J., Thompson, J.F., Guo, M. and Van Campen, D.R. (1998) Decreased citrate improves iron bioavailability from infant formula: application of an *in vitro* digestion/caco-2 cell culture model. *J. Nutr.*, **128**, 257–264.
- Gotelli, C.A., Gotelli, M.J., Boccio, J.R., *et al.* (1996) Bioavailability of microencapsulated ferrous sulphate in fluid milk studies in human beings. *APPTLA*, **46**, 239–245.
- Hanaoka, K., Yosida, K., Tamano, M., Kuroiwa, T., Kaise, T. and Maeda, S. (2001) Arsenic in the prepared edible brown alga hijiki hizikia fusiforme. *Appl. Organomet. Chem.*, **15**, 561–566.
- Haro, J.F., Martínez, C. and Ros, G. (2006) Optimisation of *in vitro* measurement of available iron from different fortificants in citric fruit juices. *Food Chem.*, **98**, 639–648.
- Hocquellet, P. and L'Hotellier, M.D. (1997) Bioavailability and speciation of mineral micronutrients: the enzymolysis approach. *J. AOAC Int.*, **80**, 920–927.
- Jin F., Frohman C., Thannhauser T.W., Welch R.M. and Glahn R.P. (2009) Effects of ascorbic acid, phytic acid and tannic acid on iron bioavailability from reconstituted

- ferritin measured by an in vitro digestion-Caco-2 cell model. *Br. J. Nutr.*, **101**, 972–981.
- Jovaní, M., Barberá, R., Farré, R. and Martín de Aguilera, E. (2001) Calcium, iron, and zinc uptake from digests of infant formulas by Caco-2 cells. *J. Agric. Food Chem.*, **49**, 3480–3485.
- Juhasz, A.L., Smith, E., Weber, J., *et al.* (2007) Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soils. *Chemosphere*, **69**, 961–966.
- Kapsokefalou, M., Miller, D.D. (1991) Effects of meat and selected food components on the valence of nonheme iron during in vitro digestion. *J. Food Sci.*, **56**, 352–355.
- Koch, I., Mc. Pherson, K., Smith, P., Easton, L., Doe, K.G. and Reimer, K.J. (2007) Arsenic bioaccessibility and speciation in clams and seaweed from a contaminated marine environment. *Mar. Poll. Bull.*, **54**, 586–594.
- Laparra, J.M., Vélez, D., Montoro, R., Barberá, R. and Farré, R. (2003) Estimation of Arsenic bioaccessibility in edible seaweed by an in vitro digestion method. *J. Agric. Food Chem.*, **51**, 6080–6085.
- Laparra, J.M., Vélez, D., Montoro, R., Barberá, R. and Farré, R. (2004) Bioaccessibility of inorganic arsenic species in raw and cooked *Hizikia fusiforme* seaweed. *Appl. Organomet. Chem.*, **18**, 662–669.
- Lönnerdal, B. and Glazier, C. (1989) An approach to assessing trace element bioavailability from milk in vitro. *Biol. Trace Element Res.*, **19**, 57–59.
- López, J.C., Lozano, A., Alegría, A. and Barberá, R. (2001) Mathematic predictive models for calculating copper, iron and zinc dialysability in infant formulas. *Eur. Food Res. Technol.*, **212**, 608–612.
- Mahler, G.J., Shuler, M.L. and Glahn, R.P. (2009) Characterization of Caco-2 and HT29-MTX cocultures in an in vitro digestion/cell culture model used to predict iron bioavailability. *J. Nutr. Biochem.*, **20**, 494–502.
- Miller, D.D., Schricker, B.R., Rasmussen, R.R. and Van Campen, D. (1981) An in vitro method for estimation of iron availability from meals. *Am. J. Clin. Nutr.*, **34**, 2248–2256.
- Minekus, M., Marteau, P., Havenaar, R. and Huis in't Veld, J.H.J. (1995) A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. *Atla*, **23**, 197–209.
- Minihane, A.M., Fox, T.E. and Fairweather-Tait, S.J. (1993) A continuous flow in vitro method to predict bioavailability of Fe from foods. In: *Bioavailability '93 Nutritional, Chemical and Food Processing Implications of Nutrient Availability (Proceedings, Part 2)* (ed. U. Schlemmer). Karlsruhe, Germany, pp. 175–179.
- Moreda-Piñeiro, J., Moreda-Piñeiro, A., Romarís-Hortas, V., *et al.* (2011) In-vivo and in-vitro testing to assess the bioavailability of arsenic, selenium and mercury species in food samples. *Trends Anal. Chem.*, **30**, 324–345.
- Muñiz, O., Domínguez, R., Bermejo, A., Bermejo, P., Cocho, J.A. and Fraga, J.M. (2006) Study of the bioavailability of selenium in cow's milk after a supplementation of cow feed with different forms of selenium. *Anal. Bioanal. Chem.*, **385**, 189–196.
- O'Dell, B.L. (1989) Mineral interactions relevant to nutrient requirements. *J. Nutr.*, **119**, 1832–1838.
- Pachon, H., Stoltzfus, R.J. and Glahn, R.P. (2008) Chicken thigh, chicken liver, and iron-fortified wheat flour increase iron uptake in an in vitro digestion/Caco-2 cell model. *Nutr. Res.*, **28**, 851–858.
- Perales, S., Barberá, R., Lagarda, M.J. and Farré, R. (2007) Availability of iron from milk-based formulas and fruit juices containing milk and cereals estimated by in vitro methods (solubility, dialysability) and uptake and transport by Caco-2 cells. *Food Chem.*, **102**, 1296–1303.
- Pérez, A., Lorenzo, L., Cabrera, C. and López, M.C. (2002) Influence of enrichment with vitamins and minerals on the bioavailability of iron in cow's milk. *J. Dairy Res.*, **69**, 473–481.
- Peña, E., Domínguez, R., Bermejo, A., Cocho, J.A., Fraga, J.M. and Bermejo, P. (2004) Enzymolysis approach to compare Cu availability from human milk and infant formulas. *J. Agric. Food Chem.*, **52**, 4887–4892.
- Planes, R., J.M., Laurenta, C., Baccoub, J.C., Besançon, P. and Caporiccio, B. (2002) Magnesium bioavailability from magnesium-fortified spirulina in cultured human intestinal Caco-2 cells. *Food Chem.*, **77**, 213–218.
- Powell, J.J., Whitehead, M.W., Lee, S. and Thompson, R.P.H. (1994) Mechanisms of gastrointestinal absorption: dietary minerals and the influence of beverage ingestion. *Food Chem.*, **51**, 381–388.
- Puyfoulhoux, G., Rouanet, J.M., Besançon, P., Baroux, P., Baccou, J.C. and Caporiccio, B. (2001) Iron availability from iron-fortified Spirulina by an in vitro digestion/Caco-2 cell culture model. *J. Agric. Food Chem.*, **49**, 1625–1629.
- Pynaert, I., Armah, C., Fairweather-Tait, S., Kolsteren, P., Van Camp, J. and De Henauw, S. (2006) Iron solubility compared with in vitro digestion–Caco-2 cell culture method for the assessment of iron bioavailability in a processed and unprocessed complementary food for Tanzanian infants (6–12 months). *Br. J. Nutr.*, **95**, 721–726.
- Robberecht, H., Van Cauwenbergh, R., Van Vlaslaer, V. and Hermans, N. (2009) Dietary silicon intake in Belgium: Sources, availability from foods, and human serum levels. *Science of the Total Environment*, **407**, 4777–4782.
- Romarís-Hortas, V., García-Sartal, C., Barciela-Alonso, M.C., Domínguez-González, R., Moreda-Piñeiro, A. and Bermejo-Barrera, P. (2011) Bioavailability study using an

- in Vitro method of iodine and bromine in edible seaweed. *Food Chem.*, **124**, 1747–1752.
- Rupérez, P. (2002) Mineral content of edible marine seaweeds. *Food Chem.*, **79**, 23–26.
- Salovaara, S., Sandberg, A.S. and Andlid, T. (2002) Organic acids influence iron uptake in the human epithelial cell line Caco-2. *J. Agric. Food Chem.*, **50**, 6233–6238.
- Sarriá, B. and Vaquero, M.P. (2001) Zinc and iron bioavailability in a powder or in-bottle-sterilized infant formula estimated by in vitro and in suckling rats. *J. Nutr. Biochem.*, **12**, 266–273.
- Schricker, B.R., Miller, D.D., Rasmussen, R.R. and Van Campen, D. (1981) A comparison of in vivo and in vitro methods for determining availability of iron from meals. *Am. J. Clin. Nutr.*, **34**, 2257–2263.
- Shen, L., Luten, J., Robberecht, H., Bindels, J. and Deelstra, H. (1994) Modification of an in vitro method for estimating the bioavailability of zinc and calcium from foods. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, **199**, 442–445.
- Solomons, N.W. (1986) Competitive interactions of iron and Zn in the diet. Consequences for human nutrition. *J. Nutr.*, **116**, 927–935.
- Torres-Escribano, S., Denis, S., Blanquet-Diot, S., *et al.* (2011) Comparison of a static and a dynamic in vitro model to estimate the bioaccessibility of As, Cd, Pb and Hg from food reference materials *Fucus* sp. (IAEA-140/Tm) and lobster hepatopancreas (TORT-2). *Science of the Total Environment*, **409**, 604–611.
- Torres-Escribano, S., Vélez, D. and Montoro, R. (2010) Mercury and methylmercury bioaccessibility in swordfish. *Food Additives and Contaminants*, **27**, 327–337.
- Tripathi, B. and Platel, K. (2010) Finger millet (*Eleusine coracana*) flour as a vehicle for fortification with zinc. *J. Trace Elements Med. Biol.*, **24**, 46–51.
- Van Hulle, M., Zhang, C., Schotte, B., *et al.* (2004) Identification of some arsenic species in human urine and blood after ingestion of Chinese seaweed *Laminaria*. *J. Anal. Atom. Spectrom.*, **19**, 58–64.
- Vaquero, M.P., Van Dokkum, W., Van den Hamer, C.J.A. and Schaafsma, G. (1993) Bioavailability of calcium from breakfast containing coffee. In vitro and in vivo determinations. In: *Bioavailability'93 Nutritional, Chemical and Food Processing Implications of Nutrient Availability* (Proceedings, Part 2). (ed. U. Schlemmer). Karlsruhe, Germany, pp. 249–253.
- Wien, E.M. and Schwartz, R. (1983) Comparison of in vitro and in vivo measurements of dietary calcium exchangeability and bioavailability. *J. Nutr.*, **113**, 388–393.
- Wolfgor, R., Drago, S.R., Rodriguez, V., Pellegrino, N.R., Valencia, M.E. (2002) In Vitro measurement of available iron in fortified foods. *Food Res. Int.*, **35**, 85–90.
- Wolters, M.G.E., Schreuder, H.A.W., Van den Heuvel, G., Van Lonkhuijsen, H.J., Hermus, R.J.J. and Voragen, A.G.J. (1993) A continuous in vitro method for estimation of the bioavailability of minerals and trace elements in foods: application to breads, varying in phytic acid content. *Br. J. Nutr.*, **69**, 849–861.
- Yu, Y.X.; Li, J.L., Zhang, X.Y., *et al.* (2010) assessment of the bioaccessibility of polybrominated diphenyl ethers in foods and the correlations of the bioaccessibility with nutrient contents. *J. Agric. Food Chem.*, **58**, 301–308.
- Zheng L., Cheng Z., Ai C., *et al.* (2010) Nicotianamine, a novel enhancer of rice iron bioavailability to humans. *PLoS ONE*, **5**, e10190.
- Zhu, L., Glahn, R.P., Nelson, D. and Miller, D.D. (2009) Comparing soluble ferric pyrophosphate to common iron salts and chelates as sources of bioavailable iron in a Caco-2 cell culture model. *J. Agric. Food Chem.*, **57**, 5014–5019.

# 19

## Brown Seaweed-Derived Phenolic Phytochemicals and Their Biological Activities for Functional Food Ingredients with Focus on *Ascophyllum nodosum*

Emmanouil Apostolidis and Chong M. Lee

University of Rhode Island, Kingston RI, USA

### 19.1 Introduction: seaweed-derived functional food ingredients

For many years seaweeds, in the form of extracts, have been used as thickeners, gelling agents, stabilizers and texturizing agents for the food, cosmetic, pharmaceutical and consumer product industry; this includes dairy desserts, ice creams, processed meats, sauces, prepared meals, gel capsules, toothpaste, shampoo, and lotion. However, in recent years, seaweeds have gained much attention in terms of interest in its nutraceutical potential and development of new seaweed-based functional foods and nutraceuticals worldwide, but most actively in China, Japan, and Korea.

Functional foods are one of the major consumer trends in the food industry. Consumers continue to seek multiple ways to enhance their health to prevent diseases and promote healthy aging by paying attention to what they are eating and how it benefits their health. Today consumers are more concerned about their weight, cardio-health, digestive

health and immunity than ever before. As health becomes a major consumer's concern, a new trend has emerged that nutraceutical ingredients are incorporated into food products for health benefits.

Marine macroalgae, which are better known as seaweeds, are widely used as a major diet component in East Asia. Seaweeds are classified according to their pigmentation into red (Rhodophyta), green (Chlorophyta) and brown (Phaeophyta), and consumed as food and used in production of hydrocolloids and recently for cosmetic and functional food ingredients. More specifically, brown seaweeds are receiving significant attention for functional food ingredient development due to the presence of health beneficial compounds such as fucoidan, fucosterol, fucoxanthin and phlorotannins (phloroglucinol). Phlorotannins belong to the tannin group; they consist of polymers of phloroglucinol units, and are found only in brown seaweeds. Phlorotannins are large molecular weight phenolic compounds and have been linked with a various phenolic antioxidant-mediated health

benefits. Furthermore, the presence of smaller phenolic compounds, such as simple phenols and phenolic acids, has been reported but not extensively studied.

The most popular commercial nutraceutical ingredient derived from brown seaweeds is fucoxanthin, and recently several companies in Korea and Japan are producing standardized phenolic (phlorotannin) extracts from brown seaweeds. The major target of phlorotannin-containing supplements is cardiovascular disease management including atherosclerosis prevention and an increase of the protective high-density lipoproteins (HDL). This chapter covers the global variation of brown seaweeds and their major characteristics, as well as the phenolic-derived health benefits of brown seaweeds with focus on *Ascophyllum nodosum*, a native brown seaweed of the Northern Atlantic Ocean.

## 19.2 Major commercial brown seaweeds

Seaweeds are abundant and ancient autotrophic organisms that can be found in virtually all near-shore aquatic ecosystems and some may attain a length of 50 m or longer (Pereira and Yarish, 2008). Despite the variety of life forms and the thousands of seaweed species described, seaweed aquaculture is presently based in a relatively small group of about 100 taxa. Of these, five genera (*Laminaria*, *Undaria*, *Porphyra*, *Euclima*/*Kappaphycus*, and *Gracilaria*) account for about 98% of world seaweed production (Pereira and Yarish, 2008). Among them, *Laminaria* and *Undaria* are brown seaweeds and account for almost 50% of world seaweed produced by aquaculture (FAO, 2006). In addition to the above widely cultured brown seaweed genera, other important brown seaweeds used for dietary and functional food purposes include the species of *Ecklonia cava* and *Ascophyllum nodosum*.

### 19.2.1 Ecology and characteristics

#### *Laminaria*

According to the FAO, 4 074 415 million tonnes (wet weight) of *Laminaria* was harvested globally in 2004, mainly through aquaculture (FAO, 2006). The major industrial use of *Laminaria* is for alginate production. *Laminaria digitata* in France is the main raw material for the alginate industry. *L. saccharina* often grows in close association with *L. digitata*, and is sometimes harvested at the same time. In Norway, *L. digitata* grows in masses at the lower end of the eulittoral zone and was previously an important source for the Norwegian industry. In France, it is in the upper sublittoral zone and is harvested around the coast of Brittany and

adjacent islands. Iceland is also a source of *L. digitata* for the alginate industry in Scotland (United Kingdom). *L. hyperborea* is found on the west coast of Ireland, and the Outer Hebrides and the Orkney Islands, Scotland (United Kingdom). On the west coast of Norway, it forms dense forests, 1–2 m high. There are estimates of large quantities growing around the coast of Brittany (France), but commercial harvesting has not yet occurred. *Saccharina japonica* (previously called *Laminaria japonica*) (Lane *et al.*, 2006) is the most important economic seaweed in China. *S. japonica* grows naturally in the Republic of Korea and is also cultivated, but on a much smaller scale; the demand is lower because Koreans prefer *meey-erk* (*Undaria pinnatifida*) (Tseng, 1987).

*Laminaria* species contain about 10% protein, 2% fat, 35% dietary fiber and useful amounts of minerals and vitamins (Yuan, 2008) and has been used as a “health” vegetable in China (Tseng, 1988). Additionally, *Laminaria* species contain large amounts of polysaccharides such as fucoidan (fucose rich polysaccharides) and laminarin (1,3 linked beta glucan) (Fitton *et al.*, 2008).

#### *Undaria*

*Undaria* is the second most widely cultivated brown seaweed and 2 519 905 million tonnes (wet weight), valued at more than 1.0 billion USD, was produced in 2004 (FAO, 2006), mainly via aquaculture. Native to cold temperate coastal areas of Japan, Korea and China, in recent decades it has become established in New Zealand, the United States, France, United Kingdom, Spain, Italy, Argentina and Australia (Torres *et al.*, 2004). It was nominated as one of the 100 worst invasive species in the world (Lowe *et al.*, 2000). *Undaria* is an important food delicacy in Japan and Korea, traditionally known as “wakame” and “meey-erk”, respectively. It is sold wet or dried and is especially appreciated as an ingredient for soybean paste soup (“misoshiru”) and seaweed salad, while in Korea a soup called “meeyerk-guk” is loaded with this seaweed. *Undaria* has three species (*Undaria pinnatifida* [Harvey] Suringar, *Undaria undarioides* [Yendo] Okamura, and *Undaria peterseniana* [Kjellman] Okamura), and of these, *U. pinnatifida* is the most important (Pereira and Yarish, 2008).

*Undaria* species contain about 20% protein (d.w.), 5% fat (d.w.) and 51% dietary fiber (d.w.) (Dawczynski *et al.*, 2007). Fucoxanthin is a xanthophyll that contains an allenic bond and two epoxy groups and is reported to be present at high levels in *Undaria* (Czczuga and Taylor, 1987). *Undaria* lipids, which could be byproducts from polysaccharide or other water soluble compound production, were found to contain 5–10% fucoxanthin (Miyashita and Hosokawa, 2008).

## 19.2.2 Health benefits

Both *Laminaria* and *Undaria* genera have been linked with nutraceutical effects due to the presence of unique biological compounds, such as fucoidan and laminarin polysaccharides (*Laminaria*) and fucoxanthin (*Undaria*).

### Fucoidan and laminarin polysaccharides

Most marine-derived polysaccharides, including fucoidan and laminarin, are not digestible by humans and therefore are regarded as dietary fibers. The health benefits from dietary fiber consumption have been well documented (Lahaye and Thibault, 1990; Lahaye, 1991). Fitton *et al.* (2008) reported that fucoidan polysaccharides have tumor inhibition and immune modulation activities. Further studies on this topic suggest that fucoxanthin-containing brown seaweeds *Laminaria*, *Undaria*, and *Cladosiphon*, enhance innate immunity by increasing T helper 1 (Th1) cytokine profile (interferon-gamma; IFN $\gamma$ ) and natural killer (NK) cell activity (Maruyama *et al.*, 2003; Mao *et al.*, 2005). Fitton *et al.* (2008), reported that a fucoidan preparation known as Algosol T128 was used to treat leukemia and Matsumoto *et al.* (2004) showed that *Cladosiphon* fucoidan downregulated interleukin-6 (a Th2 cytokine) and ameliorated colitis. In addition, fucoidans have been shown to have antithrombotic and blood anticoagulant effects (Berteau and Mulloy, 2003; Blondin *et al.*, 1996; Soeda *et al.*, 1992) and stem cell modulation effects (Vintila *et al.*, 2001; Frenette and Weiss, 2000; Sweeney *et al.*, 2002; Luyt *et al.*, 2003; Irhimeh *et al.*, 2007).

A wide variety of marine-derived polysaccharides are found to control hypertension, serum lipids and sugar metabolism (Fitton *et al.*, 2008). In a clinical study, it was shown that low sulfated laminarians had serum lipid reduction effect in patients with ischemic heart disease (Besterman and Evans, 1957). Additionally, Mori *et al.* (1982) reported that an *Undaria* fucoidan fraction was shown to result in rapid clearance of serum lipids.

### Fucoxanthin

Various natural carotenoids seem to be valuable for cancer prevention and these carotenoids may be more suitable in combinational use rather than single use (Murakoshi *et al.*, 1992; Nishino *et al.*, 2009). Specifically for fucoxanthin, it was shown that this seaweed-derived carotenoid has an inhibitory effect on cancer cells (Miyashita and Hosokawa, 2008). Fucoxanthin from brown algae *U. pinnatifida* was found to be an active component having antiproliferative activity observed in extract against human prostate cancer cells (PC-3, DU 145 and LNCap) (Kotake-Nara *et al.*, 2001). In the same study, the effect of 15 carotenoids were evaluated

and although acyclic carotenoids (such as phytofluene,  $\beta$ -carotene and lycopene) significantly reduced prostate cancer cell viability, the effect was lower compared to those of fucoxanthin and neoxanthin (Kotake-Nara *et al.*, 2001). Another study showed that fucoxanthin at concentrations as low as 22.6  $\mu$ M, completely inhibited proliferation of HL-60 cancer cells and this activity was higher than that of  $\beta$ -carotene (Compton, 1992; Solary *et al.*, 1993; Bino *et al.*, 1991). Furthermore, a study by Okuzumi *et al.* (1993) revealed that oral administration of 0.005% fucoxanthin inhibited duodenal carcinogenesis induced by *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine in mice.

Several possible mechanisms of action of fucoxanthin against cancer cells have been elucidated. Quantitative sandwich enzyme-linked immunosorbent assay, using an antihistone antibody and an anti-DNA antibody, showed a dose-dependent cellular DNA fragmentation increase in Caco-2 cells treated with fucoxanthin (Hosokawa *et al.*, 2004). It was shown that fucoxanthin suppresses the level of Bcl-2 protein, which is responsible for suppression of programmed cell death as a survival factor (Hockenbery *et al.*, 1990; Levy *et al.*, 2003). Das *et al.* (2005) reported that fucoxanthin inhibited the proliferation of human colon carcinoma WiDr and HCT116 cells by cell cycle arrest through upregulation of p21<sup>WAF1/Cip1</sup> which inhibit cyclin-dependent kinases (cdks) that regulate the eukaryotic cell progression.

Recently, a series of studies described the potential effect of fucoxanthin on obesity and type 2 diabetes management. Initial studies have shown that fucoxanthin inhibits the adipocyte differentiation of 3T3-L1 cells through downregulation of peroxisome proliferator activator gamma (Maeda *et al.*, 2006). Furthermore, Maeda *et al.* (2008b) reported the indirect antiobesity effect of fucoxanthin in mice, where the administration of fucoxanthin-containing *Undaria* oil increased the levels of total DHA (a marine algal fatty acid that is strongly correlated with antiobesity effects) in the liver of the subjects. A similar effect was observed when fucoxanthin was administrated to KKAY obese/diabetic mice (Tsukui *et al.*, 2007). Additionally, fucoxanthin administration to KKAY obese mice showed reduction in the white adipose tissue (WAT) weight (Maeda *et al.*, 2005; Miyashita, 2009). Another interesting study revealed that fucoxanthin administration in KKAY obese/diabetic mice results in the reduction of WAT weight and blood glucose (Maeda *et al.*, 2007).

From the above studies, the mechanism of action of fucoxanthin for the observed antiobesity is suggested as follows. Uncoupling protein (UCP) is inner-membrane mitochondrial protein that has the ability to dissipate energy through uncoupling of oxidative phosphorylation which, instead of ATP, produces heat. A great deal of interest has focused on adaptive thermogenesis by UCP families (UCP1,

2, and 3) in several tissues and organs as a physiological defense against obesity. UCP expression in brown adipose tissue (BAT) is known as a significant component of whole body energy expenditure, however, adult humans have very little BAT, making it unlikely to be a major contributor to human weight regulation. In humans, most of fat is stored in white adipose tissue (WAT) (Maeda *et al.*, 2008b) and nutrigenomic study revealed that fucoxanthin induces uncoupling protein 1 expression in WAT mitochondria leading to oxidation of fatty acids and heat production in WAT (Maeda *et al.*, 2008a,b; Miyashita 2009).

## 19.3 Brown seaweeds and phenolic phytochemicals

Polyphenols are secondary metabolites present in various terrestrial and sea plants. These metabolites in marine plants include tannins, phenolic acids and phlorotannins. Phlorotannins are found only in marine plants (Ragan and Glombitza, 1986). Among seaweeds, the brown seaweeds contain the highest levels of phenols, mainly as phlorotannins such as phloroglucinol polymers with size up to 650 kDa (Ragan and Glombitza, 1986).

### 19.3.1 Brown seaweed phenolic phytochemicals and health benefits

Phlorotannins have a diversity of roles such as protection of the thallus against grazers/pathogens/epiphytes (Geiselman and McConnell, 1981; Pavia and Toth, 2000; Ragan and Glombitza, 1986; Jennings and Steinberg, 1997) and photo-protection against cytotoxic effects of UV-radiation (Pavia *et al.*, 1997). During the early stages of development in Fucales, phenols are involved in both the formation of the cell wall (Schoenwaelder and Clayton, 1998) and the elaboration of adhesive cement (Vreeland *et al.*, 1998).

Although phlorotannins are known to be formed biosynthetically via the acetate-malonate pathway, also known as the polyketide pathway (Arnold and Targett, 2002), the exact biosynthetic pathway for phlorotannin production is unknown and so are the methodologies to monitor phlorotannin synthesis at the genetic or enzymatic levels. Discovery of the exact biosynthetic pathway could lead to the clear understanding of the phlorotannin biosynthesis (Amsler and Fairhead, 2006).

Another commonly found phenolic in marine plant is catechin (Yuan, 2008). Catechin is widely found in all three classes of seaweeds (red, green, and brown), but at higher levels in brown seaweeds, with *Eisenia bicyclis* having the highest amounts (Yoshie *et al.*, 2000). Water soluble simple phenolic acids present in all three classes of seaweeds

include caffeic acid and cinnamic acid (Yuan, 2008). The literature concerning the phenolic profile and the biological effect of marine plants is limited since most effort has been targeted towards terrestrial plants. However, the abundance and underutilization of certain marine plant species leads to a global interest in the marine plant research.

As most tannins, phlorotannins have potential for cardiovascular disease management through increase in HDL cholesterol and prevention of atherosclerosis (Jung *et al.*, 2006; Fukuyama *et al.*, 1989, 1990; Uhm *et al.*, 2003; Kang *et al.*, 2003; Ruehl *et al.*, 2002). Phlorotannins, even though they have higher antioxidant properties than individual simple phenolics, are usually not bioavailable, and are to some extent anti-nutritive due to their ability to bind and precipitate biological macromolecules such as proteins and carbohydrates (Chung *et al.*, 1998). A more simple water- and ethanol-based extraction from seaweeds is more suitable for simple phenolic acid extractions and recently such studies have received attention. Initially, water and ethanol extracts of green seaweed *Posidonia oceanica* are shown to contain significant amounts of hydroxybenzoic acid, coumaric acid, cinnamic acid, and caffeic acid (Dumay *et al.*, 2004). Furthermore, simple water extracts of 27 common seaweeds are shown to have significant antioxidant activity (Yan *et al.*, 1998).

The major sources for phlorotannin production at the moment are *Fucus vesiculosus* ("bladderwrack", found in North Sea, Baltic Sea, and the Atlantic and Pacific Oceans) (Ragan and Glombitza, 1986; Pavia and Toth, 2000), *Ascophyllum nodosum* (found only in North Atlantic Ocean) (Ragan and Glombitza, 1986; Pavia and Toth, 2000), and *Ecklonia cava* (a native seaweed in Korea and Japan) (Artan *et al.*, 2008). *E. cava* in particular has been extensively studied for polyphenol-linked health benefits, since it has been shown to contain large amounts of phlorotannins (Artan *et al.*, 2008).

### 19.3.2 Ecklonia cava health benefits

*E. cava*, an edible marine brown seaweed, is widely distributed in the southern coasts of Korea and Japan. It is abundantly produced in Jeju Island of Korea (30 000 tonnes per year) for commercial purposes that include food ingredients, animal feed, fertilizers and folk medicine (Li *et al.*, 2009). In Japan, it was estimated that the annual net production of *E. cava* is 2.9 kg(d.w.)/m<sup>2</sup>/year (Yokohama *et al.*, 1987).

Li *et al.* (2009) reported that *E. cava* contains plenty of phlorotannin derivatives with interesting bioactivities. A study on chemical composition and antioxidant activity of *E. cava* showed that the major phlorotannins present

are phloroglucinol, eckol, fucodiphloroethol G, phlorofucuroeckol, 1-(3',5'-dihydro-xyphenoxy)-7-(2'',4'',6''-trihydro-xyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin, dieckol and 6,6 bieckol (Li *et al.*, 2009). In the same study, it was shown that *E. cava* has strong antioxidant properties based on a wide variety of assays due to the presence of fucodiphloroethol G, dieckol and 6,6 bieckol (Li *et al.*, 2009).

The immunomodulatory effect of *E. cava* was observed (Ahn *et al.*, 2008). Jung *et al.* (2009) showed that treatment of BV2 microglia with *E. cava* can decrease levels of proinflammatory mediators following lipopolysaccharide stimulation by inhibiting the release of nitric oxide, prostaglandin E<sub>2</sub>, tumor necrosis factor- $\alpha$ , and interleukin-1 $\beta$  at the transcriptional level in a dose-dependent manner. The anti-inflammatory activity of *E. cava* was further mediated by the down-regulation of mitogen-activated protein kinases (MAPKs), nuclear factor (NF)- $\kappa$ B, and the inhibition of reactive oxygen species (ROS) accumulation (Jung *et al.*, 2009). Chronic inflammation is a condition linked to the hypersensitivity of the immune system. One of the most prevalent chronic inflammation diseases is arthritis, which is characterized by structural and biochemical changes in major tissues of the joint, including degradation of the cartilage matrix and insufficient synthesis of extracellular matrix (ECM). Ryu *et al.* (2009) reported that *E. cava*-derived phlorotannins can prevent arthritis incidence by increasing collagen production and promoting cell differentiation, and at the same time had direct anti-inflammatory effect via attenuation of matrix metalloproteinase (MMP-1, MMP-2, MMP-13) gene expressions and MAPK pathway in chronic articular diseases. In addition to arthritis, allergy is a disorder linked with immune system hypersensitivity. Basophils and mast cells express Fc $\epsilon$ RI (a high affinity receptor for immunoglobulin E) on the cell surface and act as effector cells in allergic reactions. A study by Shim *et al.* (2009) showed that *E. cava* extract may exert anti-allergic activity via downregulation of Fc $\epsilon$ RI gene expression and subsequent decrease in histamine release. The above studies (Ahn *et al.*, 2008; Jung *et al.*, 2009; Ryu *et al.*, 2009; Shim *et al.*, 2009) provide strong evidence that *E. cava* has an anti-inflammatory activity.

Many compounds of plant origin that inhibit human immunodeficiency virus (HIV) during various stages of its life cycle have been described, including alkaloids, coumarins, carbohydrates, flavonoids, lignans, phenolics, quinines, phospholipids, terpenes, and tannins (Artan *et al.*, 2008). A phloroglucinol isolated from *E. cava* (6,6'-bieckol) showed strong inhibition against HIV-1-induced syncytia formation, lytic effects, and viral p24 antigen production (Artan *et al.*, 2008). In the same study, it was shown that 6,6-bieckol, unlike most tannins, selectively inhibited HIV-1

reverse transcriptase (RT) enzyme and HIV-1 entry, without exhibiting any cytotoxicity (Artan *et al.*, 2008). A similar study by Ahn *et al.* (2004) demonstrated the complete inhibitory effect of *E. cava* derived phlorotannins (8,8'-bieckol and 8,4'''-dieckol) against HIV-1 reverse transcriptase (RT). *E. cava* phlorotannin-rich extracts could be a novel and safe natural strategy to significantly inhibit HIV-1 replication and reverse transcriptase activity, without exhibiting any side-effects (Ahn *et al.*, 2006; Artan *et al.*, 2008).

Epidemiological evidence of cancer-protective effects of fruits and vegetables, as well as the basic mechanisms by which phytochemicals in fruits and vegetables protect against cancer development has been compiled (Wargovich, 2000). A crude polyphenolic fraction of *E. cava* showed strong selective cell proliferation inhibition in murine colon cancer cell line (CT-26), two human leukemia cell lines (THP-1 and U-937), mouse melanoma cell line (B-16), and Chinese hamster fibroblast cell line (V79-4) (Athukorala *et al.*, 2006). A recent study by Kong *et al.* (2009) showed that *E. cava* phloroglucinol derivative, dioxinodihydroeckol, significantly induced proliferation inhibition and apoptosis in a dose-dependent manner on MCF-7 human breast cancer cells. The mechanism of action suggested was via the specific phloroglucinol-dependent down-regulation of the NF- $\kappa$ B pathway, which is a protein complex that controls the transcription of DNA, and its incorrect regulation is linked to cancer development (Kong *et al.*, 2009).

Recent studies showed that phenolic phytochemicals from botanical sources can be used for the management of type 2 diabetes via  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition and the alleviation of related complications (hypertension and oxidative stress) via angiotensin converting enzyme-I (ACE-I) inhibition and their natural antioxidant activity (Apostolidis *et al.*, 2006; Kwon *et al.*, 2006, 2007). Phlorotannins from *E. cava* were purified and evaluated for the *in vitro* ability to inhibit the carbohydrate hydrolyzing enzymes relevant to type 2 diabetes (Lee *et al.*, 2009). Most phlorotannins tested showed significant non-competitive inhibitory activities in a dose-dependent manner against rat  $\alpha$ -glucosidase and porcine  $\alpha$ -amylase (Lee *et al.*, 2009). Another study by Okada *et al.* (2004) showed that *E. cava*-derived phlorotannins could inhibit  $\alpha$ -amylase with concurrent reduction of glycated proteins (advanced glycation endproducts – AGE), accumulation of which is responsible for diabetic complications such as cataracts, neuropathy and retinopathy. Additional studies have revealed the beneficial effect of *E. cava* and *E. stolonifera* against hypertension, another complication of type 2 diabetes (Hong *et al.*, 2006; Jung *et al.*, 2009). Initially, Hong *et al.* (2006) showed that crude methanolic *E. cava* extract had significant ACE-I inhibitory activity *in vitro* and *in vivo*, in rats. The same study showed that *E. cava* supplementation to

Goldblatt hypertensive rats (GB-HT) resulted in the reduction of systolic blood pressure to levels similar to the control at a dose of 50 mg/kg. Jung *et al.* (2009) determined that the observed antihypertensive effect of *Ecklonia* species is phlorotannin-dependent. More specifically, six different phlorotannins and fucosterol were isolated from *E. stolonifera* and all phlorotannins tested had strong ACE-I inhibitory activity compared to fucosterol (Jung *et al.*, 2009).

The above findings clearly indicate the potential of the unique *E. cava*-derived phenolic phytochemicals as nutraceuticals. It is not surprising that Asian researchers are the leaders in the research concerning seaweed-derived health benefits since seaweed consumption has been a long tradition in Asia.

## 19.4 *Ascophyllum nodosum*: importance and health benefits

In recent years, there have been increasing interest in the brown seaweed, *Ascophyllum nodosum*, for its unique nutraceutical properties. *A. nodosum* grows mostly in the Atlantic coasts, including Ireland, Spain, France and North America. Rockweed (*A. nodosum*) is a dominant rocky intertidal brown seaweed species belonging to the class Phaeophyceae. *A. nodosum* grows on sheltered sites on shores in the mid-littoral where it can become the dominant species in the littoral zone (Morton, 2003; Lewis, 1964). It is found on the northeastern coast of North America and the northwestern coast of Europe (Taylor, 1962). The landings of *A. nodosum* in the Gulf of Maine increased from 5 million pounds in 2005 to 7 million pounds in 2006 and 2007, with steady price (around 4¢/lb – 8.8¢/kg) for the last 10 years (Maine Seaweed Council, personal communication).

Currently, *A. nodosum* is harvested for use in alginates, fertilizers and for the manufacture of seaweed meal for animal and human consumption (FAO, 2011; Chan *et al.*, 2006). It has long been used as an organic fertilizer for many varieties of crops due to its combination of macronutrients (e.g., N, P, K, Ca, Mg, S), micronutrients (e.g., Mn, Cu, Fe, Zn, etc.), and a variety of other compounds beneficial to plant growth (cytokinins, auxin-like gibberellins, betaines, mannitol, organic acids, polysaccharides, amino acids) (Craigie, 2010).

### 19.4.1 Health benefits

*A. nodosum*, similarly to other brown seaweeds, contains fucoxanthin (Marais and Joseleau, 2001), a carotenoid that has anti-obesity (Maeda *et al.*, 2006) and antioxidant poten-

tial (Yan *et al.*, 1999), phlorotannins (Ragan and Glombitza, 1986), large molecular weight phenolics that have antioxidant (Yan *et al.*, 1996) and antidiabetic (Zhang *et al.*, 2007) potential, and simple phenolics, like catechins (Yoshie *et al.*, 2000), hydroxybenzoic acid, coumaric acid, cinnamic acid and caffeic acid (Dumay *et al.*, 2004) that have antioxidant and antidiabetic potential (Apostolidis and Lee, 2010; Apostolidis *et al.*, 2010).

The effect of a phlorotannin-enriched *A. nodosum* extract (220 mg/g phlorotannins, prepared through fractionations) on differentiation and fatty acid accumulation was evaluated using 3T3-L1 adipocytes (He *et al.*, 2009). The results of this study showed that the *A. nodosum* enriched medium increased the accumulation of monounsaturated fatty acids (MUFA) and increased the ratio of MUFA to SFA (saturated fatty acids) in a dose-dependent manner (He *et al.*, 2009). Decreasing dietary SFA or increasing the ratio of USFA to SFA could lower the plasma total LDL-cholesterol (Abbey *et al.*, 1994) and this may lower the incidence of heart attack, cardiac death, heart failure or stroke (De Lorgeril *et al.*, 1999). It was further observed that a high-MUFA diet could be an alternative to a low-fat diet for dietary therapy of type 2 diabetes (Ros, 2003). Therefore, consumption of the phlorotannin-rich *A. nodosum* extract may benefit human health by increasing the ratio of MUFA to SFA in the adipocytes that was observed by He *et al.* (2009).

Lipogenesis and lipolysis concurrently exist in mature adipocytes and glycerol-3-phosphate dehydrogenase (GPDH) is the key enzyme for controlling the lipogenic synthesis rate in adipocytes during differentiation (Spiegelman *et al.*, 1993). He *et al.* (2009) found that the addition of phlorotannin-containing *A. nodosum* extract to adipocytes resulted in reduced cellular GPDH activity. The inhibition of GPDH activity affects the lipogenesis process and may result in less *de novo* fatty acids synthesis since it is related to the inhibition of adipocyte differentiation in the early differentiating adipocytes and lipogenesis in mature adipocytes (Spiegelman *et al.*, 1993), and might contribute to obesity management.

The phenolic-mediated antiobesity potential of *A. nodosum* was further confirmed in a weight management study with high fat-fed rats (Terpend *et al.*, 2011). An oral administration of spray-dried *A. nodosum* water extract (390 mg/g phloroglucinol eq.) to high fat-fed rats at a dose of 400 mg/kg resulted in a reduction of mass body weight gain (MBWG) and body fat mass (BFM) by 32% and 19%, respectively (Terpend *et al.*, 2011). In the same study, *A. nodosum* extract reduced the *in vitro* lipase and  $\alpha$ -amylase digestive enzyme activities (Terpend *et al.*, 2011), possibly leading to a lower absorption of lipids and carbohydrates and eventually a reduction of the MBWG and BFM.

The above studies suggest the potential preventive effect of *A. nodosum* against obesity which according to the Centers for Disease Control (CDC) is the leading dietary-linked disease in the United States (CDC, 2010b). Thirty-three states had prevalence equal to or greater than 25% and nine of these states had a prevalence of obesity equal to or greater than 30% (CDC, 2010b). Type 2 diabetes is the second leading diet-linked disease in the United States and according to CDC estimates in 2007, 23.7 million people (10% of American adults) had diabetes and by 2050 this figure is expected to jump to 33%, or one-third of all American adults (CDC, 2010a). Diabetes cost Americans \$174 billion to manage in 2007 – a figure that is expected to skyrocket based on the CDC's latest estimates (CDC, 2010a).

### 19.4.2 *Ascophyllum nodosum* phenolic phytochemical-mediated type 2 diabetes management

Apostolidis and Lee (2010) evaluated the phenolic-mediated potential of *A. nodosum* for type 2 diabetes management. Among four seaweeds harvested in Rhode Island (United States) (*A. nodosum*, *Ceramium virgatum*, *Ulva lactuca* and *Saccharina latissima*), *A. nodosum* was chosen for its highest phenolic content and was subjected to water extraction. Among extraction ratios of 50 g to 100–1000 ml at room temperature, 50 g/400 ml yielded the highest phenolic content of 4.5 mg/g wet weight. The effect of temperature (20–80 °C) on phenolic content and  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities, relevant to type 2 diabetes management, was further evaluated (Apostolidis and Lee, 2010). Among temperatures studied, extraction at 80 °C resulted in the highest total phenolic contents (4.2 mg/g wet weight). The 80 °C extract had the highest  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activity with  $IC_{50}$  of 0.24  $\mu$ g and 1.34  $\mu$ g phenolics, respectively, compared to the  $IC_{50}$  of acarbose, reference inhibitor, being 0.37  $\mu$ g and 0.68  $\mu$ g (Figure 19.1, Apostolidis and Lee, 2010).

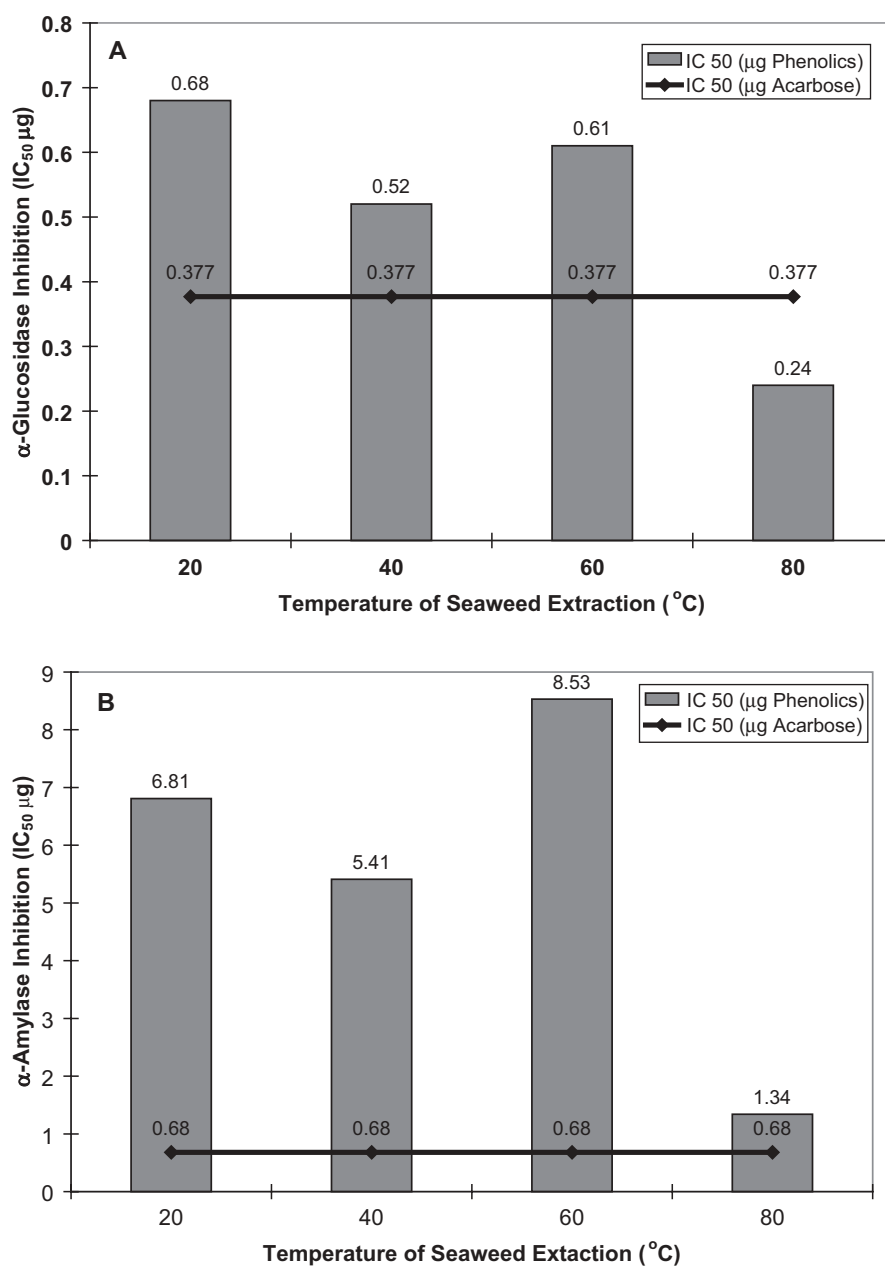
The results showed that fresh *A. nodosum* has strong  $\alpha$ -glucosidase and mild  $\alpha$ -amylase inhibitory activities which correlated with phenolic contents (Figure 19.2, Apostolidis and Lee, 2010).

The phenolic-mediated potential of *A. nodosum* for type 2 diabetes management was also evaluated *in vitro* and *in vivo* in streptozotocin-diabetic mice (Zhang *et al.*, 2007). Initially, fractionated aqueous ethanolic extract of *A. nodosum* was shown to have a strong  $\alpha$ -glucosidase inhibitory activity and stimulate basal glucose uptake into 3T3-L1 adipocytes (Zhang *et al.*, 2007). A crude polyphenol extract (PPE) and a polyphenolic enriched fraction (PPE-F1) from *A. nodosum* were prepared and assayed for blood glu-

cose levels reduction in streptozotocin-diabetic mice and the results showed that both PPE and PPE-F1 improved fasting serum glucose levels and reduced the levels of glycated serum protein (Zhang *et al.*, 2007), accumulation of which is responsible for diabetic complications (Okada *et al.*, 2004).

*E. cava* has been widely investigated for its nutraceutical potential including type 2 diabetes management as outlined previously in the chapter. Recently, Kazantzis *et al.* (2011) compared the type 2 diabetes potential of *E. cava* and *A. nodosum*. Both seaweeds were collected, dried and extracted in hot water (90 °C). *E. cava* had a higher phenolic content (16 mg/g DW) than *A. nodosum* (12 mg/g DW) as determined by Folin–Ciocalteu assay. The antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and oxygen radical absorbance capacity (ORAC) based on Trolox equivalent. The results correlated with total phenolic contents with *E. cava* having 72% free radical scavenging activity (56% for *A. nodosum*) and 11  $\mu$ M Trolox eq. (7  $\mu$ M for *A. nodosum*). However, *A. nodosum* had higher  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities (58% and 64%, respectively) than *E. cava* (40% and 20%, respectively) (Figure 19.3, Kazantzis *et al.*, 2011) at a dose of 10  $\mu$ g. It is clear that although *E. cava* has higher total phenolic content and antioxidant activity, *A. nodosum* has higher potential for carbohydrate hydrolyzing enzyme inhibition relevant for type 2 diabetes management. This could be due to the presence of unique bioactive phenolic compounds in *A. nodosum* which are not found in *E. cava*.

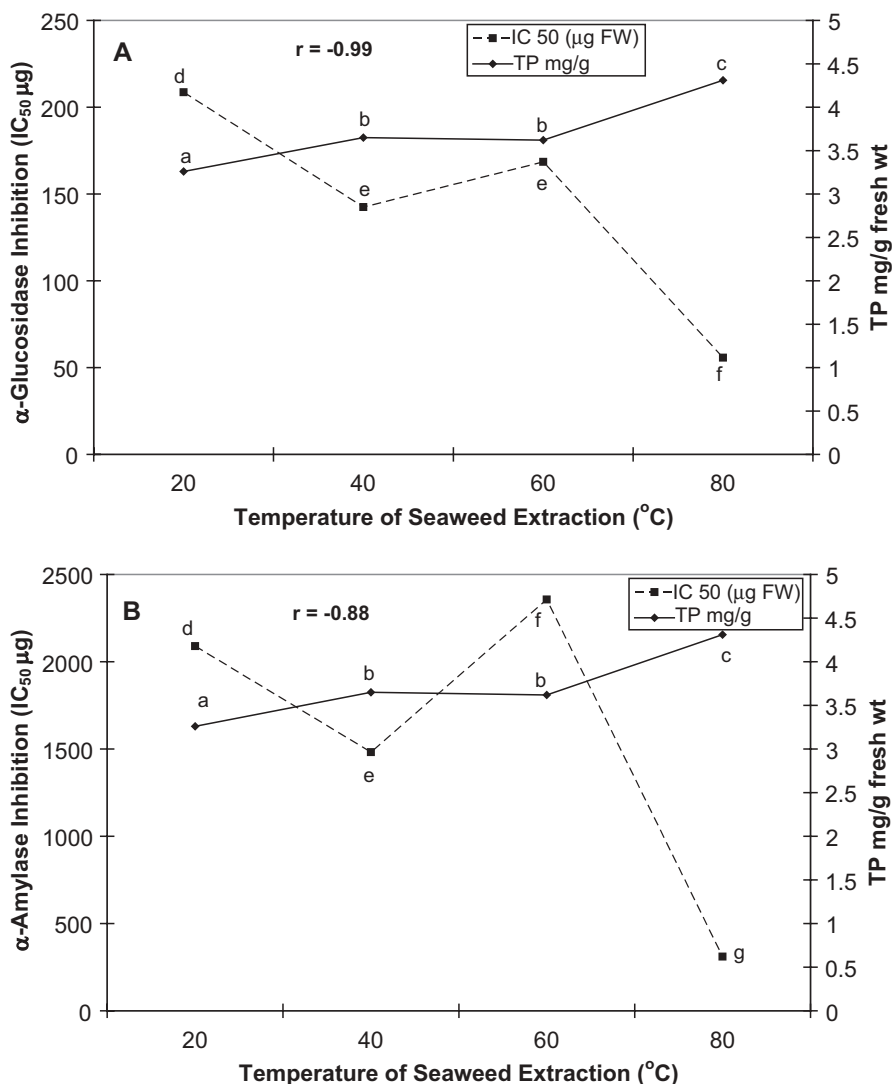
Marine plants produce phenolic phytochemicals as a byproduct of internal resource balances (Mooney, 1972; Mattson, 1980; Bryant *et al.*, 1983; Ilvessalo and Tuomi, 1989) in response to nutrient stress (Mattson, 1980; Bryant *et al.*, 1983; Ilvessalo and Tuomi, 1989; Gershenzon, 1984; Fajer *et al.*, 1992) or as a result of severe defoliation (Tuomi *et al.*, 1984; Bryant *et al.*, 1991a, b). The seasonal and geographical variations of phenolic content in *A. nodosum* and other brown seaweeds have been reported in the past (Yates and Peckol, 1993; Steinberg, 1989; Parys *et al.*, 2009) and was linked to observed potential for type 2 diabetes management (Apostolidis *et al.*, 2010). A study by Apostolidis *et al.* (2010) examined the seasonal variation of *A. nodosum* in phenolic contents and subsequent antioxidant,  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities relevant for type 2 diabetes management. *A. nodosum* was harvested monthly and extracted in hot water (1 kg in 8 l water) at 80 °C for 30 min and the resulting extracts were dried using a spray dryer. The final powder was assayed for total phenolic content, antioxidant activity and inhibition of carbohydrate digesting enzymes. The results indicate clear seasonal variation in terms of phenolic content with June, July, and October



**Figure 19.1** Comparison of acarbose and *Ascophyllum nodosum*  $\alpha$ -glucosidase and  $\alpha$ -amylase  $IC_{50}$  values at varying extraction temperatures. Comparison was made with the single drug compound acarbose on the phenolic base  $IC_{50}$  value of *A. nodosum* (Apostolidis and Lee, 2010).

being the highest (36.4, 37 and 36 mg/g respectively) and May the lowest (21.8 mg/g) (Apostolidis *et al.*, 2010). The antioxidant activities, in terms of DPPH free radical scavenging activity, correlated with the phenolic contents observed ( $r = 0.89$ ) with the month of July being the highest (58%) and April the lowest (26%) (Apostolidis *et al.*, 2010).

Similarly, in terms of Trolox equivalent, July had the highest activity (15.53  $\mu$ M) and April and May the lowest (8.40 and 8.27  $\mu$ M, respectively). The highest  $\alpha$ -glucosidase inhibitory activity ( $IC_{70}$ ) was observed in the month of July followed by October and June (2.2, 4.3, and 4.5  $\mu$ g, respectively, Figure 19.4, Apostolidis *et al.*, 2010). Additionally, a



**Figure 19.2** Relationship between  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition ( $IC_{50}$ ) of *A. nodosum* ( $\mu$ g fresh weight) and total phenolic (TP) content (mg/g). Values with different letters are significantly different,  $p < 0.05$  (Apostolidis and Lee, 2010).

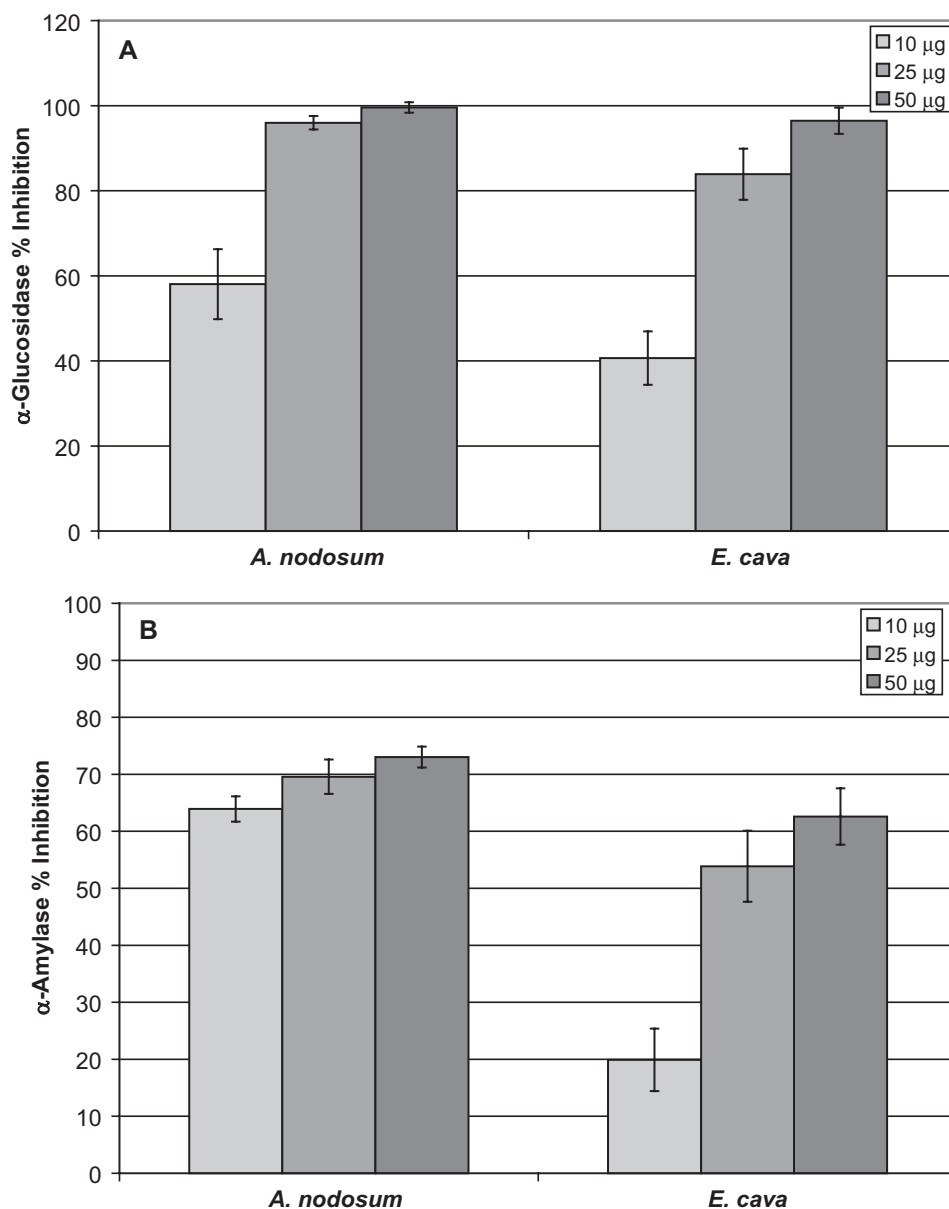
reverse correlation was observed between  $IC_{70}$  values and total phenolic content ( $r = -0.89$ ) indicating a potential phenolic-dependent  $\alpha$ -glucosidase inhibitory activity (Figure 19.4, Apostolidis *et al.*, 2010). No  $\alpha$ -amylase inhibitory activity was observed at the tested concentrations. Such seasonal variation is believed to be caused by temperature-related stress considering that *A. nodosum* is a cold water species, and evidently this influenced the bioactivities.

The above studies support the potential of phenolic-mediated type 2 diabetes management potential of *A. nodosum*. These studies also suggest that *A. nodosum* phenolics have a possible “dual” mode of action both in the gastrointestinal tract, via delay of glucose absorption by inhibition

of carbohydrate hydrolyzing enzymes (Zhang *et al.*, 2007; Apostolidis and Lee, 2010; Apostolidis *et al.*, 2010; Kazantzis *et al.*, 2011) and in cell level via stimulation of basal glucose uptake into 3T3-L1 adipocytes (Zhang *et al.*, 2007). Following this direction, more research efforts are needed to further identify the exact biological signatures for the observed effects and further verify the observed mechanisms of action.

### 19.4.3 Future directions

The phenolic-mediated health benefits of *A. nodosum* have been until recently overlooked. However, the studies



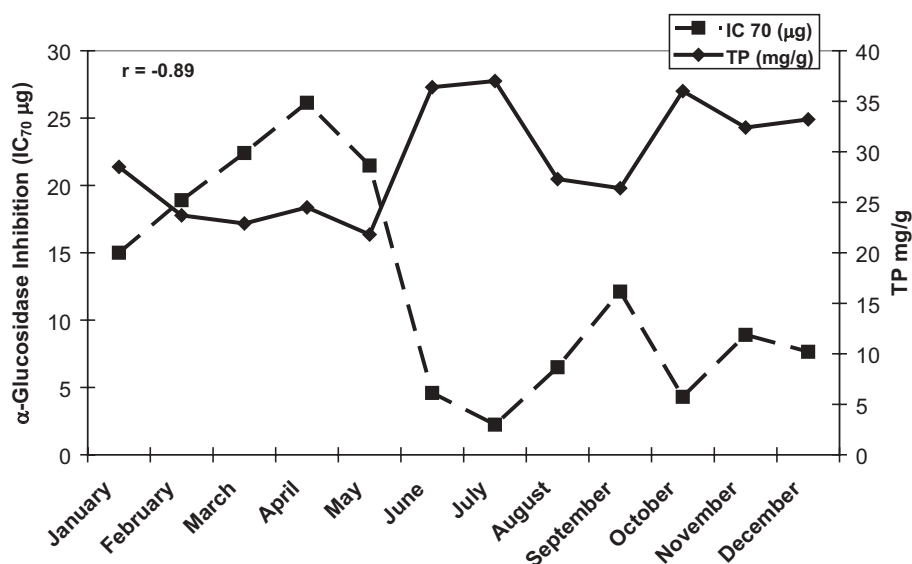
**Figure 19.3** Comparison of  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities of *A. nodosum* and *E. cava* (Kazantzis *et al.*, 2011).

outlined above provide a strong rationale for the in-depth investigation of *A. nodosum* to the same extent with *E. cava*. *E. cava* has received much attention and was extensively investigated because of the fact that it grows in Korea and Japan, countries that have a long history of consuming seaweeds, in comparison to *A. nodosum* that is mainly used for animal and plant nutrition. *A. nodosum* is an abundant unique resource found in the Atlantic ocean and offers po-

tential value addition leading to commercial development in the region.

## 19.5 Conclusions

For many years, in Europe and the United States the commercial use of seaweeds has been limited to industrial



**Figure 19.4** Correlation between  $\alpha$ -glucosidase inhibitory activity ( $IC_{70}$ ) and total phenolic (TP) content of *A. nodosum* spray dried powder (Apostolidis *et al.*, 2010)

applications as hydrocolloids such as thickening, gelling, stabilizing and texturizing aids, for food, the cosmetic, and pharmaceutical industries. However, in recent years, seaweeds have gained much attention in terms of interest in its nutraceutical potential and development of new seaweed-based functional foods and nutraceuticals worldwide, but most actively in China, Japan, and Korea.

Initially, various types of microalgae were utilized for nutraceutical ingredient development. However, the most popular commercial nutraceutical ingredient derived from seaweeds (macroalgae) is fucoxanthin. The major market of fucoxanthin products is weight loss through the thermogenic action that was previously discussed. The retail dollars spent on weight loss is \$84.7 billion in 2002 making this market very appealing to new product entries (Nutraingredients-USA, 2010). Recently, several companies produce standardized phenolic (phlorotannin) extracts from brown seaweeds. The major target of phenolic-containing seaweed supplements is cardiovascular disease management since these companies are claiming atherosclerosis prevention and an increase in protective HDL cholesterol. Some of the phlorotannin products in the market include HealSea™ (produced by Diana Naturals in France), IdAlg™ (produced by Bio Serac in France), and Seanol™ (produced by LiveChem in South Korea and distributed by Simply Health, USA).

Seaweed phenolics is an emerging research topic in marine nutraceutical exploration. The most well studied field of phenolic phytochemicals are terrestrial plants; however,

increased research efforts are being given to abundant marine plants (seaweeds) by making full use of the available terrestrial plant research data. Based on the above information, we anticipate more research activities and phenolic-based commercial products from brown seaweeds in the global market.

## References

- Abbey, M., Noakes, M., Belling, G.B. and Nestel, P.J. (1994) Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low-density-lipoprotein cholesterol. *Am. J. Clin. Nutr.*, **59**, 995–999.
- Ahn, G., Hwang, I., Park, E., *et al.*, (2008) Immunomodulatory effects of an enzymatic extract of *Ecklonia cava* on murine splenocytes. *Mar. Biotechnol.*, **10**, 278–289.
- Ahn, M.J., Yoon, K.-D., Min, S.-Y., *et al.*, (2004) Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from brown alga *Ecklonia cava*. *Biol. Pharm. Bull.*, **27**, 544–547.
- Amsler, C.D. and Fairhead, V.A. (2006) Defensive and sensory chemical ecology of brown algae. *Adv. Bot. Res.*, **43**, 1–91.
- Apostolidis, E., Kwon, Y.-I. and Shetty, K. (2006) Potential of cranberry - based herbal synergies for diabetes and hypertension management. *Asia Pacific J. Clin. Nutr.*, **15**, 433–441.

- Apostolidis, E. and Lee, C.M. (2010) *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant mediated  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition. *J. Food Sci.*, **75**, 97–102.
- Apostolidis, E., Karayannakidis, P.D., Kwon, Y.I., *et al.*, (2010) *Seasonal variation of phenolic antioxidant mediated type 2 diabetes management potential of Ascophyllum nodosum*. Annual Meeting of Institute of Food Technology, Chicago, USA.
- Arnold, T.M. and Targett, N.M. (2002) Marine tannins: The importance of mechanistic framework for predicting ecological roles. *J. Chem. Ecol.*, **28**, 1919–1934.
- Artan, M., Li, Y., Karadeniz, F., *et al.*, (2008) Anti-HIV-1 activity of phloroglycinol derivative, 6,6'-bieckol, from *Ecklonia cava*. *Bioorg. Med. Chem.*, **16**, 7912–7926.
- Athukorala, Y., Kim, K.-N. and Jeon, Y.-J. (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food Chem. Toxicol.*, **44**, 1065–1074.
- Berteau, O. and Mulloy, B. (2003) Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active towards this class of polysaccharide. *Glycobiology*, **13**, 29–40.
- Besterman, E.M. and Evans, J. (1957) Antilipaemic agent without anticoagulant action. *Br. Med. J.*, **51**, 310–312.
- Bino, G.D., Skierski, J.S. and Darzynkiewicz, Z. (1991) The concentration dependent diversity of effects of DNA topoisomerase I and II inhibitors on the cell cycle of HL-60 cells. *Exp. Cell Res.*, **195**, 485–491.
- Blondin, C., Chaubet, F., Nardella, A., *et al.*, (1996) Relationship between chemical characteristics and anticomplementary activity of fucans. *Biomaterials*, **17**, 597–603.
- Bryant, J.P., Chapin, F.S. III. and Klein, D.R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**, 357–368.
- Bryant, J. P., Danell, K., Provenza, F., *et al.*, (1991a) Effects of mammal browsing on the chemistry of deciduous woody plants. In: *Phytochemical Induction by Herbivores* (eds D. W. Tallamy and M. J. Raupp). John Wiley & Sons, New York, pp. 135–154.
- Bryant, J. P., Heitkonig, I., Kuropat, P. and Owen-Smith, N. (1991b) Effects of severe defoliation on the long-term resistance to insect attack and on leaf chemistry in six woody species of the southern African savanna. *Am. Nat.*, **137**, 50–63.
- Centers for Disease Control (2010a) Website <http://www.cdc.gov/diabetes/pubs/pdf/ndfs'2007.pdf> (accessed 11 April 2011).
- Centers for Disease Control (2010b) Website <http://www.cdc.gov/obesity/data/index.html> (accessed 11 April 2011).
- Chan, C.-X., Ho, C.-L. and Phang, S.-M. (2006) Trends in seaweed research. *Trends Plant Sci.*, **11**, 165–66.
- Chung, K.T., Wong, T.Y., Wei, C.I., *et al.*, (1998) Tannins and human health: a review. *Crit. Rev. Food Sci. Nutr.*, **38**, 421–464.
- Compton, M.M. (1992) A biochemical hallmark of apoptosis: internucleosomal degradation of the genome. *Cancer Metastasis Rev.*, **11**, 105–119.
- Craigie, J.S. (2010) Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.*, (Online First, 28 July 2010).
- Czeczuga, B. and Taylor, F.J. (1987) Carotenoid content in some species of the brown and red algae from the coastal area of New Zealand. *Biochem. Syst. Ecol.*, **15**, 5–8.
- Das, S.K., Hashimoto, T., Shimizu, K., *et al.*, (2005) Fucoxanthin induces cell cycle arrest at G0/G1 phase in human colon carcinoma cells through up-regulation of p21<sup>WAF1/Cip1</sup>. *Biochim. Biophys. Acta*, **1726**, 328–335.
- Dawczynski, C., Schubert, R. and Jahreis, G. (2006) Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chem.*, **103**, 891–899.
- De Lorgeril, M., Salen, P., Martin, J.L., *et al.*, (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon diet heart study. *Circulation*, **99**, 779–785.
- Dumay, O., Costa, J., Desjobert, J.-M. and Pergent, G. (2004) Variations in the concentration of phenolic compounds in the seagrass *Posidonia oceanica* under conditions of competition. *Phytochemistry*, **65**, 3211–3220.
- Fajer, E.D., Bowers, M.D. and Bazzaz, F.A. (1992) The effect of nutrients and enriched CO<sub>2</sub> environments on production of carbon-based allelochemicals in *Plantago*: a test of the carbon/nutrient balance hypothesis. *Am. Nat.*, **140**, 707–723.
- Food and Agriculture Organization Yearbook (2006) *Fishery Statistics*. FAO, Rome, Italy.
- Food and Agriculture Organization (2011) Website <http://www.fao.org/docrep/005/ac860e/ac860e02.htm> (accessed 28 April 2011).
- Fitton, H.J., Irhimeh, M.R., and Teas, J. (2008) Marine algae and polysaccharides with therapeutic applications. In: *Marine Nutraceuticals and Functional Foods* (eds C. Barrow and F. Shahidi). CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 345–365.
- Frenette, P.S. and Weiss, L. (2000) Sulfated glycans induce rapid hematopoietic progenitor cell mobilization: evidence for selectin-dependent and independent mechanisms. *Blood*, **96**, 2460–2468.
- Fukuyama, Y., Kodama, M., Miura, I., *et al.*, (1989) Structure of an anti-plasmin inhibitor, eckol, isolated from the brown alga *Ecklonia kurome* okamura and inhibitory

- activities of its derivatives on plasmin inhibitors. *Chem. Pharm. Bull.*, **37**, 349–353.
- Fukuyama, Y., Kodama, M., Miura, I., *et al.*, (1990) Anti-plasmin inhibitor, structure of phlorofueckol a, a novel phlorotannin with both dibenzo-1,4-dioxin and dibenzofuran elements from *Ecklonia kurome* okamura. *Chem. Pharm. Bull.*, **38**, 133–135.
- Gershenson, J. (1984) Changes in the levels of plant secondary metabolites under water and nutrient stress. In: *Phytochemical Adaptations to Stress* (eds B.N. Timmermann, C. Steelink and F.A. Loewus). Plenum, New York, pp. 273–320.
- Geiselman, J.A. and McConnell, O.J. (1981) Polyphenols in brown algae *Fucus vesiculosus* and *Ascophyllum nodosum*: chemical defenses against the marine herbivorous snail, *Littorina littorea*. *J. Chem. Ecol.*, **7**, 1115–1133.
- He, M.L., Wang, Y., You, J.S., *et al.*, (2009) Effect of a seaweed extract on fatty acid accumulation and glycerol-3-phosphate dehydrogenase activity of 3T3-L1 adipocytes. *Lipids*, **44**, 125–132.
- Hockenbery, D., Nunez, G., Millman, C., *et al.*, (1990) Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature*, **348**, 334–336.
- Hong, J.H., Son, B.S., Kim, B.K., *et al.*, (2006) Antihypertensive effect of *Ecklonia cava* extract. *Korean J. Pharmacogn.*, **37**, 200–205.
- Hosokawa, M., Kudo, M., Maeda, H., *et al.*, (2004) Fucoxanthin induces apoptosis and enhances the antiproliferative effect of PPAR $\gamma$  ligand, troglitazone, on colon cancer cells. *Biochim. Biophys. Acta*, **1675**, 113–119.
- Irhimeh, M.R., Fitton, J.H. and Lowenthal, R.M. (2007) Fucoidan ingestion increases the expression of CXCR4 on human CD 34+ cells. *Exp. Hematol.*, **35**, 989–994.
- Ilvessalo, H. and Tuomi, J. (1989) Nutrient availability and accumulation of phenolic compounds in the brown alga *Fucus vesiculosus*. *Mar. Biol.*, **101**, 115–119.
- Jennings, J.G. and Steinberg, P.D. (1997) Phlorotannins versus other fractions affecting epiphyte abundance on the kelp *Ecklonia radiata*. *Oecologia*, **109**, 461–473.
- Jung, H.A., Hyun, S.K., Kim, H.R. and Choi, J.S. (2006) Angiotensin-converting enzyme I inhibitory activity of phlorotannins from *Ecklonia stolonifera*. *Fish. Sci.*, **72**, 1292–1299.
- Jung, W.-K., Ahn, Y.-W., Lee, S.-H., *et al.*, (2009) *Ecklonia cava* ethanolic extracts inhibit lipopolysaccharide-induced cyclooxygenase-2 and inducible nitric oxide synthase expression in BV2 microglia via MAP kinase and NF- $\kappa$ B pathways. *Food Chem. Toxicol.*, **47**, 410–417.
- Kang, K., Park, Y., Hwang, H.J., *et al.*, (2003) Antioxidative properties of brown algae polyphenolics and their perspectives as chemopreventive agents against vascular risk factors. *Arch. Pharm. Res.*, **26**, 286–293.
- Kazantzis, C., Apostolidis, E., Karayannakidis, P.D. and Lee, C. (2011) *Potential of Ascophyllum nodosum and Ecklonia cava for phenolic antioxidant-mediated type 2 diabetes management*. Annual Meeting of Institute of Food Technology, New Orleans, USA
- Kong, C.-S., Kim, J.-A., Yoon, N.-Y. and Kim, S.-K. (2009) Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cells. *Food Chem. Toxicol.*, **47**, 1653–1658.
- Kotake-Nara, E., Kushiro, M., Zhang, H., *et al.*, (2001) Carotenoids affect proliferation of human prostate cancer cells. *J. Nutr.*, **131**, 3303–3306.
- Kwon, Y.-I., Vatter, D. A. and Shetty, K. (2006) Clonal herbs of *Lamiaceae* species against diabetes and hypertension. *Asia Pacific J. Clin. Nutr.*, **15**, 107–118.
- Kwon, Y.-I., Apostolidis, E., Kim, Y.-C. and Shetty, K. (2007) Health benefits of traditional corn, beans and pumpkin; *In vitro* studies for hyperglycemia and hypertension management. *J. Med. Foods*, **10**, 266–75.
- Lahaye, M. and Thibault, J.F. (1990) Chemical and physicochemical properties of fibers from algal extraction by-products. In: *Dietary Fibre: Chemical and Biological Aspects* (eds D.A.T. Southgate, K. Waldron, I.T. Johnson and G.R. Fenwick). Royal Society of Chemistry, Cambridge, pp. 68–72.
- Lahaye, M. (1991) Marine algae as sources of fibres: determination of soluble and insoluble dietary fiber contents in some sea vegetables. *J. Sci. Food Agric.*, **54**, 587–594.
- Lane, C.E., Mayes, C., Druehl, L.D. and Saunders, G.W. (2006) A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. *J. Phycol.*, **43**, 493–512.
- Lee, S.H., Li, Y. and Karadeniz, F. (2008)  $\alpha$ -Glucosidase and  $\alpha$ -amylase inhibitory activities of phloroglucinol derivatives from edible marine brown alga, *Ecklonia cava*. *J. Sci. Food Agric.*, **89**, 1552–1558.
- Levy, P., Robin, H., Bertrand, F., *et al.*, (2003) Butyrate-treated colonic Caco-2 cells exhibit defective integrin-mediated signaling together with increased apoptosis and differentiation. *J. Cell Physiol.*, **197**, 336–347.
- Lewis, J.R. (1964) *The Ecology of Rocky Shores*. English Universities Press, London.
- Li, Y., Qian, Z.-J., Ryu, B., *et al.*, (2009) Chemical components and its antioxidative properties in vitro: An edible marine brown alga, *Ecklonia cava*. *Bioorg. Med. Chem.*, **17**, 1963–1973.
- Lowe, S., Browne, M., Boudjelas, S. and De Poorter, M. (2000) *100 of the world's worst invasive alien species. A selection from the global invasive species database*. The Invasive Species Specialists Group (ISSG), Auckland, Australia

- Luyt, C.E., Meddahi-Pelle, M.P., Ho-Tin-Noe, B., *et al.*, (2003) Low-molecular-weight fucoidan promotes therapeutic revascularization in a rat model of critical hindlimb ischemia. *J. Pharmacol. Exp. Ther.*, **305**, 24–30.
- Maeda, H., Hosokawa, M., Sashima, T., *et al.* (2005) Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissue. *Biochem. Biophys. Res. Commun.*, **332**, 392–397.
- Maeda, H., Hosokawa, M., Sashima, T., *et al.*, (2006) Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *Int. J. Molec. Med.*, **18**, 147–152.
- Maeda, H., Hosokawa, M., Sashima, T. and Miyashita, K. (2007) Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK- $A^y$  mice. *J. Agric. Food Chem.*, **55**, 7701–7706.
- Maeda, H., Tsukui, T., Sashima, T., *et al.*, (2008a) Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient. *Asia Pacific J. Clin. Nutr.*, **17**, 196–199.
- Maeda, H., Hosokawa, M., Sashima, T. and Miyashita, K. (2008b) Antiobesity effect of fucoxanthin from edible seaweeds and its multibiological functions. In: *Functional food and health* (eds T. Shibamoto, K. Kanazawa, F. Shahidi and C.-T. Ho). American Chemical Society Symposium Series, Washington DC, pp. 376–388.
- Mao, T.K., Van de Water, J. and Gershwin, M.E. (2005) Effects of *Spirulina*-based dietary supplement pm cytokine production from allergic rhinitis patients. *J. Med. Foods*, **8**, 27–30.
- Marais, M.-F. and Joseleau, J.-P. (2001) A fucoidan fraction from *Ascophyllum nodosum*. *Carbohydr. Res.*, **336**, 155–59.
- Maruyama, H., Tamauchi, H., Hashimoto, M. and Nakano, T. (2003) Antitumor activity and immune response of Mekabu fucoidan extracted from sporophyll of *Undaria pinnatifida*. *In Vivo*, **17**, 245–250.
- Matsumoto, S., Nagaoka, M., Hara T. *et al.*, (2004) Fucoidan derived from *Cladosiphon okamuranus* Tokida ameliorates murine chronic colitis through down-regulation of interleukin-6 production on colonic epithelial cells. *Clin. Exp. Immunol.*, **136**, 432–439.
- Mattson, W.J., Jr. (1980) Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.*, **11**, 119–161.
- Miyashita, K. (2009) The carotenoid fucoxanthin from brown seaweed affects obesity. *Lipid Technol.*, **21**, 186–190.
- Miyashita, K. and Hosokawa, M. (2008) Beneficial health effects of seaweed carotenoid, fucoxanthin. In: *Marine Nutraceuticals and Functional Foods* (eds C. Barrow and F. Shahidi). CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 297–319.
- Mooney, H.A. (1972) The carbon balance of plants. *Ann. Rev. Ecol. Syst.*, **3**, 315–346.
- Mori, H., Kamei, H., Nishide, E. and Nisizawa, K. (1982) Sugar constituents of some sulphated polysaccharides from sporophylls of Wakame (*Undaria pinnatifida*) and their biological activities. In: *Marine Algae and Pharmaceutical Science* (eds H.A. Hoppe and T. Levring). Walter de Gruyter, NY, pp. 109–121.
- Morton, O. (2003) *Marine Algae of Northern Ireland*. Ulster Museum, Belfast, Ireland
- Murakoshi, M., Nishino, H., Satomi, Y., *et al.*, (1992) Potent preventive action of  $\alpha$ -carotene against carcinogenesis: Spontaneous liver carcinogenesis and promoting stage of lung and skin carcinogenesis in mice are suppressed more effectively by  $\alpha$ -carotene than  $\beta$ -carotene. *Cancer Res.*, **52**, 6583–6587.
- Nishino, H., Murakoshi, M., Tokuda, H. and Satomi, Y. (2009) Cancer prevention by carotenoids. *Arch. Biochem. Biophys.*, **483**, 165–168.
- Nutraingredients-USA. Available from <http://www.nutraingredients-usa.com/smartlead/view/200250/4/Bellalean-Fucoxanthin-the-Next-Weight-Loss-Trend> (accessed 11 April 2011).
- Okada, Y., Ishimaru, A., Suzuki, R. and Okuyama, T. (2004) A new phloroglucinol derivative from brown alga *Eisenia bicylis*. Potential for the effective treatment of diabetic complications. *J. Nat. Prod.*, **67**, 103–105.
- Okuzumi, J., Takahashi, T., Yamane, T., *et al.*, (1993) Inhibitory effects of fucoxanthin, a natural carotenoid, on *N*-ethyl-*N'*-nitro-*N*-nitrosoguanmidine-induced mouse duodenal carcinogenesis. *Cancer Lett.*, **68**, 159–168.
- Parys, S., Kehraus, S., Pete, R., *et al.*, (2009) Seasonal variation of polyphenolics in *Ascophyllum nodosum* (Phaeophyceae). *Eur. J. Phycol.*, **44**, 331–338.
- Pavia, H. and Toth, G.B. (2000) Inducible chemical resistance to herbivory in the brown seaweed *Ascophyllum nodosum*. *Ecology*, **81**, 3212–3225.
- Pavia, H., Cervin, G., Lindgren, A. and Aberg, P. (1997) Effect of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. *Mar. Ecol. Progr. Series*, **157**, 139–146.
- Pereira, R. and Yarish, C. (2008) Mass production of marine macroalgae. In: *Encyclopedia of Ecology* (eds S.E. Jørgensen and B.D. Fath). Elsevier, Oxford, pp. 2236–2247.
- Ragan, M.A. and Glombitza, K.W. (1986) Phlorotannins, brown algal polyphenols. In: *Progress in Phycological Research* (eds F.E. Round and D.J. Chapman). Biopress Ltd, Bristol, pp. 129–241.

- Ros, E. (2003) Dietary *cis*-monounsaturated fatty acids and metabolic control in type 2 diabetes. *Am. J. Clin. Nutr.*, **78**(suppl), 617S–625S.
- Ruehl, M.L., Orozco, J.A., Stoker, M.B., *et al.*, (2002) Protective effects of inhibiting both blood and vascular selectins after stroke and reperfusion. *Neurol. Res.*, **24**, 226–232.
- Ryu, B., Li, Y., Qian, Z.-J., *et al.*, (2009) Differentiation of human osteosarcoma cells by isolated phlorotannins is subtly linked to COX-2, iNOS, MMPs, and MAPK signaling: Implications for chronic articular disease. *Chem.-Biol. Interactions*, **179**, 192–201.
- Schoenwaelder, M.E.A. and Clayton, M.N. (1998) Secretion of phenolic substances into the zygote was and cell plate in embryos of *Hormosira* and *Acrocarpia* (Fucales, Phaeophyceae). *J. Phycol.*, **34**, 969–980.
- Shim, S.-Y., Le, Q.-T., Lee, S.-H. and Kim, S.-K. (2009) *Ecklonia cava* extract suppresses the high affinity IgE receptor, FcεRI expression. *Food Chem. Toxicol.*, **47**, 555–560.
- Soeda, S., Sakaguchi, S., Shimeno, H. and Nagamatsu, A. (1992) Fibrinolytic and anticoagulant activities of highly sulfated fucoidan. *Biochem. Pharmacol.*, **43**, 1853–1858.
- Solary, B.E., Bertrand, R., Kohn, K.W. and Pommier, Y. (1993) Differential induction of apoptosis in undifferentiated and differentiated HL-60 cells by DNA topoisomerase I and II inhibitors. *Blood*, **81**, 1359–1368.
- Spiegelman, B.M., Frank, M. and Green, H. (1983) Molecular cloning of mRNA from 3T3 adipocytes. Regulation of mRNA content for glycerophosphate dehydrogenase and other differentiation-dependent proteins during adipocyte development. *J. Biol. Chem.*, **258**, 10083–10089.
- Steinberg, P.D. (1989) Biogeographical variation in brown algal polyphenolics and other secondary metabolites: comparison between temperate Australia and North America. *Oecologia*, **78**, 373–382.
- Sweeney, E.A., Lortat-Jacob, H., Priestley, G.V. *et al.*, (2002) Sulfated polysaccharides increase plasma levels of SDF-1 in monkeys and mice: involvement in mobilization of stem/progenitor cells. *Blood*, **99**, 44–51.
- Taylor, W.R. (1962) *Marine Algae of the Northeastern Coast of North America*. Ann Arbor, University of Michigan Press, Michigan, USA.
- Terpend, K., Bisson, J.F., Le Gall, C. and Linares, E. (2011) Effect of ID-*alg*<sup>TM</sup> on weight management in high-fat-fed rats through inhibitory activities against digestive enzymes. *Phytother. Res.*, in press.
- Torres, A.I., Gil, M.N. and Esteves, J.L. (2004) Nutrient uptake rates by the alien alga *Undaria pinnatifida* (Phaeophyta) (Nuevo Gulf, Patagonia, Argentina) when exposed to diluted sewage effluent. *Hydrobiologia*, **520**, 1–6.
- Tseng, C.K. (1987) Laminaria mariculture in China. In: *Case Studies of Seven Commercial Seaweed Resources* (eds M.S. Dotty, J.F. Caddy and B. Santelices). FAO, Rome, Italy.
- Tsukui, T., Konno, K., Hosokawa, M., *et al.*, (2007) Fucoxanthin and fucoxanthinol enhance the amount of doco-haexanoic acid in liver of KKAY obese/diabetic mice. *J. Agric. Food Chem.*, **55**, 5025–5029.
- Tuomi, J., Niemela, P., Haukioja, E., *et al.*, (1984) Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. *Oecologia*, **61**, 208–210.
- Uhm, C.S., Kim, K.B., Lim, J.H., *et al.*, (2003) Effective treatment with fucoidin for perinatal ischemic encephalopathy in rats. *Neurosci. Lett.*, **353**, 21–24.
- Vintila, C.D., Schneider, J., Pollack, S. and Farley, T. (2001) Heparin anticoagulation promotes CD34 positive hematopoietic progenitor cells (HPC) mobilization into peripheral blood (PB). In: Proceedings of Annual Meeting of American Society of Clinical Oncology, Abstract 46.
- Vreeland, V., Waite, J.H. and Epstein, L. (1998) Polyphenols and oxidases in substratum adhesion by marine algae and mussels. *J. Phycol.*, **34**, 1–8.
- Wargovich, M.J. (2000) Anticancer properties of fruits and vegetables. *HortScience*, **35**, 573–575.
- Yan, X., Nagata, T. and Fan, X. (1998) Antioxidative activities of some common seaweeds. *Plant Foods Hum. Nutr.*, **52**, 253–262.
- Yan, X.J., Li, X.C., Zhou, C.X. and Fan, X. (1996) Prevention of fish oil rancidity by phlorotannins from *Sargassum kjellmanianum*. *J. Appl. Phycol.*, **8**, 201–203.
- Yan, X.J., Chuda, Y., Suzuki, M. and Nagata T. (1999) Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Biosci. Biotechnol. Biochem.*, **63**, 605–07.
- Yates, J.L. and Peckol, P. (1993) Effects of nutrient availability and herbivory on polyphenolics in the seaweed *Fucus vesiculosus*. *Ecology*, **74**, 1757–1766.
- Yokohama, Y., Tanaka, J. and Chihara, M. (1987) Productivity of *Ecklonia cava* community in a bay of Izu peninsula on the Pacific coast of Japan. *Bot. Mag., Tokyo*, **100**, 129–141.
- Yoshie, Y., Wang, W., Petillo, D. and Suzuki, T. (2000) Distribution of catechins in Japanese seaweeds. *Fish. Sci.*, **66**, 998–1000.
- Yuan, Y.V. (2008) Marine algal constituents. In: *Marine Nutraceuticals and Functional Foods* (eds C. Barrow and E. Shahidi). CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 259–295.
- Zhang, J., Tiller, C., Shen, J., *et al.*, (2007) Antidiabetic properties of polysaccharide and polyphenolic enriched fractions from the brown seaweed *Ascophyllum nodosum*. *Can. J. Physiol. Pharmacol.*, **85**, 1116–23.

# 20

## Antiobesity and Antidiabetic Effects of Seaweeds

**Chang-Suk Kong<sup>1</sup> and Se-Kwon Kim<sup>2</sup>**

<sup>1</sup>*Department of Food and Nutrition, College of Medical and Life Science, Silla University, Busan, Republic of Korea*

<sup>2</sup>*Department of Chemistry, Pukyong National University, Busan, Republic of Korea*

### 20.1 Introduction

The rates of obesity in the population as well as insulin resistance or type 2 diabetes have been increasing with rapidly growing prevalence worldwide. Obesity develops from burning fewer calories due to overeating, irregular meals and lack of daily physical activity. It is defined as an excessive body weight (in the form of fat) to such a serious degree and characterized by increments in the number and size of fat cells and their stored lipid levels (Kong *et al.*, 2010; Kim and Kong, 2010; Matsuo *et al.*, 2001; Xavier and Sunyer, 2002). The possible mechanisms were suggested with reference to antiobesity actions as follows: reducing the incorporation of glucose and free fatty acids into triglyceride, increasing the oxidation of glucose and/or fatty acids, or increasing lipolysis (Evans *et al.*, 2002). Many people think of obesity as one of the serious socioeconomic health problems with increasing prevalence, as it is associated with numerous pathological disorders such as type 2 diabetes, hypertension, cardiovascular disease, cancer, etc. (Giri *et al.*, 2006; Kong *et al.*, 2010; Lee *et al.*, 2005; Xavier and Sunyer, 2002). For these reasons, many studies have been conducted to search for new health benefit substances for obesity or weight control.

Diabetes mellitus is a highly prevalent metabolic disease, when the pancreas does not produce enough insulin to meet its need or when the body does not effectively use the insulin it produces; it then increases the levels of blood glucose, inducing glucose excretion in the urine (Hayashi and Ito, 2002; Hayashi *et al.*, 2006). Diabetes mellitus can be classified into two major types, type 1 and type 2. Type 2 diabetes is often caused from excess body weight and physical inactivity, which is defined by a raised fasting or postchallenge blood glucose level (Do *et al.*, 2008). Diabetes in clinical diagnosis usually accompanies the symptoms of hypercholesterolemia with hyperglycemia, which can damage blood vessels. This is referred to as microvascular disease, and increases the risk of heart attack, stroke and kidney failure (Dieterle *et al.*, 2006). Therefore, hyperglycemia and hypercholesterolemia are well-known major cardiovascular risk factors in type 2 diabetes. The hyperglycemic and hypercholesterolemic activities in diabetic animal models have been adopted for antidiabetic study. Streptozotocin (STZ)-induced diabetes has been well recognized in animal studies for type 1 and type 2 diabetes. STZ is particularly toxic to the insulin-producing beta cells of the pancreas in mammals.

Since prehistoric times seaweeds have been consumed traditionally as an alternative medicine and food staple item in Asian countries such as Korea, Japan, and China (Ali *et al.*, 2000). In the West and European countries, seaweeds have been largely regarded as a health food to diversify the sources of dietary fiber over the past few decades (Lahaye and Kaeffer, 1997; Dawczynski *et al.*, 2007). They belong to one of the marine algae such as the red, green, or brown algae (based on pigmentation; Kim *et al.*, 2010). They are rich in calcium, magnesium, and iodine. For the past few decades, a wealth of evidence on the various biological activities of seaweeds and their chemical components has been reported (Riou *et al.*, 1996; Hwang *et al.*, 2006; Maeda *et al.*, 2006; Turner *et al.*, 2002; Saker *et al.*, 2001; Saker *et al.*, 2004; Lim *et al.*, 2002; Huang and Wang, 2004; Horikawa *et al.*, 1999). In this chapter, we describe the antiobesity and antidiabetic effects of seaweeds and their active components. In fact, seaweeds extracts are used in some diet pills, which exhibit the same effect as gastric banding or expansion in the stomach to make the body feel more full (Maeda *et al.*, 2005; Fox News 2009; Softpedia, 2009).

## 20.2 Antiobesity and antidiabetic effects of seaweed

Seaweeds are rich in dietary fiber (non-starch polysaccharides) as well as proteins, minerals and vitamins (Jurkovic *et al.*, 1995; Urbano and Goni, 2002). They have a low lipid content and provide fewer calories, and interfere with the bioavailability of other dietary components (Lahaye, 1991; Wong *et al.*, 1999). In addition, seaweeds cannot be entirely digested by human intestinal enzymes (Kim MS *et al.*, 2008a) as a source of dietary fiber, which is essentially derived from the following polysaccharides: alginates, cellulose, fucans and laminarans (Jimenez-Escrig and Sanchez-Muniz, 2000; Renn, 1990).

### 20.2.1 Brown seaweed

There are approximately 2000 species of brown seaweeds, which are generally found in cold temperate coastal areas. They have been known as the resource of bioactive compounds than either green or red seaweeds (Prabhasankar *et al.*, 2009). Their antiobesity and antidiabetic effects have been reported in the literature.

#### *Undaria pinnatifida*

*Undaria pinnatifida* has been one of the widely consumed edible seaweeds or sea vegetable in Korea, Japan, and China from ancient times. Recently, other countries including the

United States, New Zealand, France, Spain, Italy, the United Kingdom, Argentina, and Australia have introduced this seaweed into their menus (Torres *et al.*, 2004), which is served in soups and salads, as a side dish with salad vegetables like cucumber. It is called “*miyeok*” in Korea, “*wakame*” in Japan, “*qundaicai*” (Abbott, 1989) in China, “*fougère des mers*” in French, and “sea mustard” in English. It contains high levels of calcium, iodine, thiamine, and niacin, and is a rich source of eicosapentaenoic acid, omega-3 fatty acid and soluble fraction dietary fiber (acidic polysaccharides such as alginates), and its lipid content is normally <1.0%. Recent research has identified antiobesity and antidiabetic compounds such as fucoxanthin and fucoidan from brown seaweed lipids (Miyashita, 2009).

In an *in vivo* experiment (Gudiel-Urbano and Goni, 2002; Cho and Bang, 2004) the intake of seaweeds neither affected body weight gain nor food intake; however, the *U. pinnatifida* diet-fed group induced a higher reduction in  $\beta$ -glucosidase, which is an inducible enzyme by indigestible substrates (Gudiel-Urbano and Goni, 2002). The dried diet-fed group (as a 20% food mixture) induced slight reduction in blood glucose, decrease of serum concentrations of triglycerides, and an increase of high density lipoprotein (HDL)-cholesterol levels in STZ-induced diabetic rats (Cho and Bang, 2004).

Kim MS *et al.* (2008) reported the physiological effects of seaweed (*U. pinnatifida* and *Laminaria japonica*) supplementation on blood glucose levels and lipid profile in subjects with type 2 diabetes. Pills with dry powdered sea mustard were provided three times a day for 4 weeks (total daily consumption, 48 g). The seaweed-supplemented group intakes of total dietary fiber (30.1 g/day) was 2.5 times higher than in the control group (12.3 g/day). Consistent with the increased dietary fiber intake, seaweed supplementation significantly decreased the fasting blood glucose levels, 2-hour postprandial blood glucose levels, and the serum concentrations of triglycerides. In addition, seaweed supplementation increased HDL-cholesterol, but did not affect the concentrations of total cholesterol and low-density lipoprotein (LDL)-cholesterol.

A number of studies have been focused on fucoxanthin and fucoxanthin-rich seaweeds to discover antiobesity and antidiabetic components from brown seaweeds. In an *in vivo* study on the antiobesity effect of brown seaweed lipids from *U. pinnatifida*, the seaweed lipid intake decreased the weight gain of abdominal white adipose tissue (WAT), the blood glucose and plasma insulin concentration in obese/diabetic rats and mice, compared with perirenal and epidermal abdominal adipose tissue. Brown seaweed lipids are rich in fucoxanthin and are potential sources for omega-3 and -6 unsaturated fatty acids (HUFA) (Maeda *et al.*, 2007).

### ***Laminaria japonica* (konbu)**

*Laminaria japonica*, as well as *Undaria pinnatifida*, are rich in indigestible polysaccharides and appear to be good sources of soluble dietary fiber. The biological components of *L. japonica* are polysaccharides (Wang *et al.*, 2008; Qiong *et al.*, 2010), and are composed of algin, laminarin, fucoidan, and different proportions of galactose, xylose, glucuronic acid, with a little protein (Zvyagintseva *et al.*, 2005; Qiong *et al.*, 2010). Its extract has also been used for weight loss (Wang *et al.*, 2008) and a seaweed mixture supplementation, including *Laminaria japonica*, significantly reduced blood glucose levels and the serum concentrations of triglycerides and increased HDL-cholesterol in subjects with type 2 diabetes (Kim MS *et al.*, 2008).

### ***Petalonia binghamiae***

*Petalonia binghamiae* (J. Agaradh) Vinogradova is an edible brown alga and is consumed as a traditional food in fishery areas of Northeast Asia. Galactosyldiacylglycerol (Mizushima *et al.*, 2001) and fucoxanthin-related compounds (Mori *et al.*, 2004) have been reported as bioactive compounds from *Petalonia binghamiae*. Its potent antiobesity and antidiabetic activities were reported in several studies.

The water-soluble extract of *P. binghamiae*, prepared by enzymatic digestion (PBEE), suppressed adipocyte differentiation and adipogenesis via inhibition of adipogenic specific gene expression and insulin-stimulated uptake of glucose in an *in vitro* study. In rat model with high-fat diet (HFD)-induced obesity, PBEE exhibited a potent antiobesity effect. PBEE supplementation reduced body weight, fat storage, serum levels of glutamic pyruvic and glutamic oxaloacetic transaminases, but increased the serum level of HDL-cholesterol (Kang *et al.*, 2010). Moreover, the dietary administration of its ethanol extract decreased hyperglycemia and improved glucose tolerance in STZ-induced diabetic mice, in which PBEE exerts an *in vivo* antidiabetic effect by mediating both insulin-like and insulin-sensitizing actions in adipocytes (Kang *et al.*, 2008).

### ***Ascophyllum nodosum***

In an *in vitro* experiment, the antiobesity effect of the extract from *Ascophyllum nodosum* was tested by measuring cellular glycerol-3-phosphate dehydrogenase (GPDH) activity and fatty acid accumulation as lipogenesis- and adipogenesis-related factors in 3T3-L1 adipocytes (He *et al.*, 2009). The seaweed extracts increased cellular monounsaturated fatty acids (MUFA) and the ratio MUFA/saturated fatty acids (SFA), while inhibited the GPDH activity. They suggest

phlorotannins as biological factors involved in lipogenesis or lipolysis and in improved MUFA accumulation.

### ***Sargassum yezeense***

Kim SN *et al.* (2008) isolated active compounds, sarguinoic acid (SQA) and sargahydroquinoic acid (SHQA), from *Sargassum yezeense* as novel peroxisome proliferator-activated receptor (PPAR) $\alpha/\gamma$  dual agonists. The binding affinity of SQA with PPAR $\gamma$  was higher than that of the specific PPAR $\gamma$  agonist troglitazone, leading to an activation of PPAR $\gamma$  transcriptional activity. Therefore, they suggest that SQA and SHQA are novel PPAR $\alpha/\gamma$  dual agonists and may be beneficial for reducing insulin resistance through regulation of adipogenesis.

### ***Hizikia fusiforme***

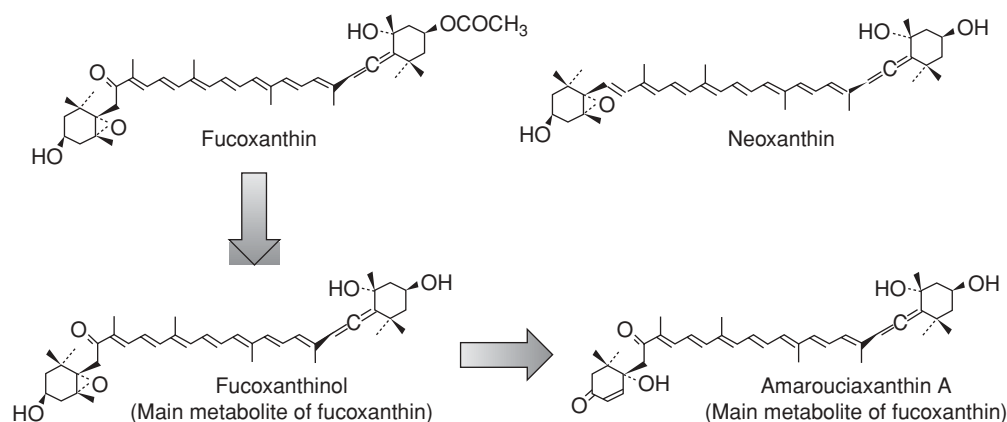
In the animal model fed a cholesterol-rich diet, the seaweed mixture (*Eisenia bicyclis*, *H. fusiformis*, *U. pinnatifida* sporophylls and *Porphyra yezeensis*) exerted antihyperlipidemic activity by lowering triglyceride, serum total cholesterol, LDL-cholesterol, free cholesterol levels (Amano *et al.*, 2005).

## **20.2.2 Active components**

### ***Fucoxanthin***

As an *in vitro* analysis to search for new health-beneficial food/agents for obesity or weight control, many research efforts have been conducted with 3T3-L1 adipocytes (Kong *et al.*, 2010). Screening work for carotenoids revealed that only four kinds of carotenoids, fucoxanthin, fucoxanthinol, amarouciaxanthin A, and neoxanthin (Figure 20.1) showed an encouraging suppressive effect on the of 3T3-L1 adipocyte differentiation and intracellular lipid accumulation (Miyashita, 2009). Among them, fucoxanthin was found at high levels in brown seaweed such as *Sargassum horneri*, *S. thunbergii*, *S. fusiforme*, *U. pinnatifida* and *L. japonica*, as compared with other carotenoids in natural products, although it is not found in green and red seaweeds (Maeda *et al.*, 2007; Terasaki *et al.*, 2009).

A number of studies have shown that fucoxanthin is an effective antiobesity and antidiabetic supplement and fat blocker (Maeda *et al.*, 2005, 2006; Matsumoto *et al.*, 2010; Jeon *et al.*, 2010). Fucoxanthin-rich seaweed extract effectively suppressed body weight gain and improved lipid metabolism for an antiobesity effect based on mode of action in C57BL/6J mice (Jeon *et al.*, 2010). Maeda *et al.* (2005) reported that dietary fucoxanthin reduced abdominal WAT weights and increased mitochondrial uncoupling protein 1 (UCP1) expression in WAT in the diabetic/obese mouse



**Figure 20.1** Chemical structures of allenic carotenoids (Miyashita, 2009).

KK-A<sup>y</sup>, where UCP1 is a key molecule for metabolic thermogenesis to suppress an excess of fat accumulation. Moreover, fucoxanthin intakes (0.2%) induced the reduction of body weight gain, blood glucose concentration of around 170 mg/dl and plasma insulin levels compared with that of control KK-A<sup>y</sup> mice group (Maeda *et al.*, 2007). Moreover, a dietary combination of fucoxanthin and fish oil improved the attenuation of weight gain and blood glucose in diabetic/obese KK-A<sup>y</sup> mice (Maeda *et al.*, 2007). Therefore, Miyashita suggests that one would probably need to eat a lot of brown seaweed daily to induce remarkable weight loss and eating huge amounts of seaweed is not the quickest or most convenient path for losing weight, since fucoxanthin is tightly bound to proteins in the brown seaweed and is not easily absorbed in the form of whole seaweed. However, we can expect that brown seaweed can be developed into a pill as the most active form that one can take daily.

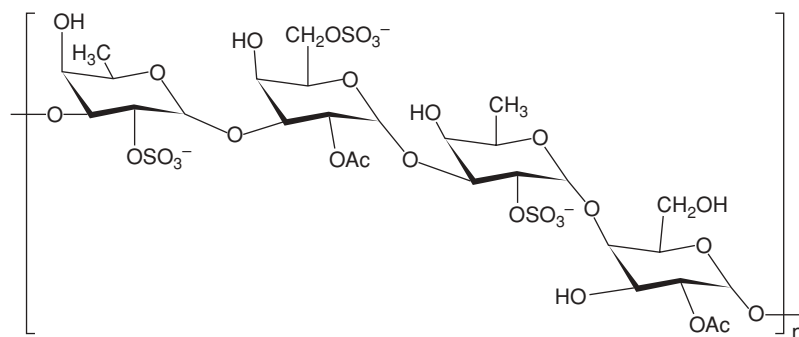
### Fucoxanthin

Fucoxanthin is a group of sulfated fucose-containing polysaccharides extracted from non-mammalian origin such as

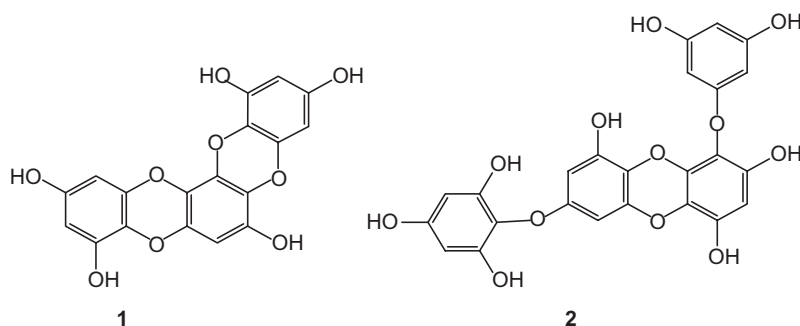
brown algae. Kim *et al.* (2009) reported on its *in vitro* antiobesity effect using adipocyte cells. Treating the 3T3-L1 cells with 100 and 200 µg/ml fucoxanthin (Figure 20.2) effectively decreased fat accumulation and adipocyte-specific gene expression, compared to adipocyte controls. The results suggest that fucoxanthin could be used for inhibiting fat accumulation via mediating adipocyte-specific gene expression.

### Phlorotannins

Phlorotannins are the largest group of polyphenols, which are found at high levels in brown seaweeds. Among them, *in vitro* antiobesity effects of dioxinodehydroeckol and 7'-phloroeckol (Figure 20.3) from *E. cava* have been reported (Kong *et al.*, 2010; Kim and Kong, 2010). Dioxinodehydroeckol and 7'-phloroeckol inhibited intracellular lipid accumulation and downregulated adipocyte specific gene or protein expression in adipocyte cells by suppression of adipocyte differentiation. They suggested that these phlorotannins may be an effective candidate for preventing obesity or obesity-related diseases.



**Figure 20.2** Chemical structure of fucoxanthin (Kim *et al.*, 2009).



**Figure 20.3** Chemical structures of phlorotannins, dioxinoldehydroeckol (1) and 7'-phloroeckol, isolated from *Ecklonia cava*.

## 20.3 Conclusions

Obesity and type 2 diabetes predispose people to a variety of pathological disorders and have become a serious public health problem with fast growing prevalence. A number of clinical trials have been conducted widely on marine seaweeds or marine algae. Even from ancient times, marine seaweeds have been part of the staple diet and an alternative medicine in many Asian countries due to the abundance of natural bioactive substances. Marine seaweeds and their components such as fucoxanthin, fucoidan, and phlorotannins exhibited a great antiobesity effect. Fucoxanthin especially shows a great antiobesity and antidiabetic effects in *in vitro/in vivo* analysis. Therefore, it can be considered as a candidate for slimming supplement.

## References

- Abbott I.A (1989) Food and food products from seaweeds. In: *Algae and Human Affairs* (eds C.A. Lembi and J.R. Waaland). Cambridge University Press, Phycological Society of America, p. 141.
- Ali MS, Jahangir M, Saleen M, Pervez MK, Hameed S and Ahmad VU (2000) Metabolites of marine algae collected from Karachi-coasts of Arabian sea. *Nat. Prod. Sci.*, **6**, 61–65.
- Amano H, Kakinuma M, Coury DA, Ohno H and Hara T (2005) Effect of a seaweed mixture on serum lipid level and platelet aggregation in rats. *Fish. Sci.*, **71**, 1160–1166.
- Cho YJ and Bang MA (2004) Effects of dietary seaweed on blood glucose, lipid and glutathione enzymes in streptozotocin-induced diabetic rats. *J. Korean Soc. Food Sci. Nutr.*, **33**, 987–994.
- Dawczynski C, Schubert R and Jahreis G (2007) Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chem.*, **103**, 891–899.
- Dieterle C, Brendel MD, Seissler J, Eckhard M, Bretzel RG and Landgraf R (2006) Therapy of diabetes mellitus. Pancreas transplantation, islet transplantation, stem cell and gene therapy. *Internist (Berlin)*, **47**, 489–496.
- Do JY, Kwak DM and Kwon OD (2008) Antidiabetic effects of high molecular weight chitosan in streptozotocin-induced type 1 diabetic ICR mice. *Lab. Animal Res.*, **24**, 311–317.
- Evans M, Lin X, Odle J and McIntosh M (2002) *Trans*-10, *cis*-12 conjugated linoleic acid increases fatty acid oxidation in 3T3-L1 preadipocytes. *J. Nutr.*, **132**, 450–455.
- Fox News (2009) <http://www.foxnews.com/story/0,2933,476766,00.html?sPage=fnc/health/nutrition> (accessed 12 April 2011).
- Giri S, Rattan R, Haq E, *et al.* (2006) AICAR inhibits adipocyte differentiation in 3T3L1 and restores metabolic alterations in diet-induced obesity mice model. *Nutr. Metab.*, **3**, 31–50.
- Gudiel-Urbano M and Goni I (2002) Effect of edible seaweeds (*Undaria pinnatifida* and *Porphyra tenera*) on the metabolic activities of intestinal microflora in rats. *Nutr. Res.*, **22**, 323–331.
- Hayashi K and Ito M (2002) Antidiabetic action of low molecular weight chitosan in genetically obese diabetic KK-A<sup>y</sup> mice. *Biol. Pharm. Bull.*, **25**, 188–192.
- Hayashi K, Kojima R and Ito M (2006) Strain differences in the diabetogenic activity of streptozotocin in mice. *Biol. Pharm. Bull.*, **29**, 1110–1119.
- He ML, Wang Y, You JS, Mir PS and McAllister TA (2009) Effect of a seaweed extract on fatty acid accumulation and glycerol-3-phosphate dehydrogenase activity in 3T3-L1 adipocytes. *Lipids*, **44**, 125–132.
- Horikawa M, Noro T and Kamei Y (1999) In vitro antimethicillin resistant *Staphylococcus aureus* activity found in extracts of marine algae indigenous to the coastline of Japan. *J. Antibiotics (Tokyo)*, **52**, 186–189.
- Huang HL and Wang BG (2004) Antioxidant capacity and lipophilic content of seaweeds collected from the Qingdao coastline. *J. Agric. Food Chem.*, **52**, 4993–4997.

- Hwang H, Chen T, Nines RG, Shin HC and Stoner GD (2006) Photochemoprevention of UVB-induced skin carcinogenesis in SKH-1 mice by brown algae polyphenols. *Int. J. Cancer*, **119**, 2742–2749.
- Jeon SM, Kim HJ, Woo MN, *et al.* (2010) Fucoxanthin-rich seaweed extract suppresses body weight gain and improves lipid metabolism in high-fat-fed C57BL/6J mice. *Biotechnol. J.*, **5**, 961–969.
- Jimenez-Escrig AB and Sanchez-Muniz FJ (2000) Dietary fiber from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res.*, **20**, 585–598.
- Jurkovic N, Kolb N and Colic I (1995) Nutritive value of marine algae *Laminaria japonica* and *Undaria pinnatifida*. *Food/Nahrung*, **39**, 63–66.
- Kang SI, Jin YJ, Ko HC, *et al.* (2008) *Petalonia* improves glucose homeostasis in streptozotocin-induced diabetic mice. *Biochem. Biophys. Res. Commun.*, **373**, 265–269.
- Kang SI, Kim MH, Shin HS, *et al.* (2010) A water-soluble extract of *Petalonia binghamiae* inhibits the expression of adipogenic regulators in 3T3-L1 preadipocytes and reduces adiposity and weight gain in rats fed a high-fat diet. *J. Nutr. Biochem.*, **21**, 1251–1257.
- Kim JA, Kong CS and Kim SK (2010) Effect of *Sargassum thunbergii* on ROS mediated oxidative damage and identification of polyunsaturated fatty acid components. *Food Chem. Toxicol.*, **48**, 1243–1249.
- Kim MJ, Chang UJ and Lee JS (2009) Inhibitory effects of fucoidan in 3T3-L1 adipocyte differentiation. *Mar. Biotechnol.*, **11**, 557–562.
- Kim MS, Kim JY, Choi WH and Lee SS (2008) Effects of seaweed supplementation on blood glucose concentration, lipid profile, and antioxidant enzyme activities in patients with type 2 diabetes mellitus. *Nutr. Res. Pract.*, **2**, 62–67.
- Kim SK and Kong CS (2010) Anti-adipogenic effect of dioxinohydroeckol via AMPK activation in 3T3-L1 adipocytes. *Chem.-Biol. Interact.*, **186**, 24–29.
- Kim SN, Choi HY, Lee W, Park GM, Shin WS and Kim YK (2008). Sargaquinoic acid and sargahydroquinoic acid from *Sargassum yezoense* stimulate adipocyte differentiation through PPAR $\alpha$ / $\gamma$  activation in 3T3-L1 cells *FEBS Lett.*, **582**, 3465–3472.
- Kong CS, Kim JA, Ahn BN, Vo TS, Yoon NY and Kim SK (2010) 1-(3',5'-dihydroxyphenoxy)-7-(2'',4'',6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin inhibits adipocyte differentiation of 3T3-L1 fibroblasts. *Mar. Biotechnol.* **12**, 299–307.
- Lahaye M (1991) Marine algae as sources of fibres: determination of soluble and insoluble DF contents in some sea vegetables. *J. Sci. Food Agric.*, **54**, 587–594.
- Lahaye M and Kaeffer B (1997) Seaweed dietary fibres: structure, physic-chemical and biological properties relevant to intestinal physiology. *Sciences des Aliments*, **17**, 563–584.
- Lee WJ, Koh EH, Won JC, Kim MS, Park JY and Lee KU (2005) Obesity: the role of hypothalamic AMP-activated protein kinase in body weight regulation. *Int. J. Biochem. Cell Biol.*, **37**, 2254–2259.
- Lim SN, Cheung PCK, Ooi VEC and Ang PO (2002) Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum*. *J. Agric. Food Chem.*, **50**, 3862–3866.
- Maeda H, Hosokawa M, Sashima T, Funayama K and Miyashita K (2005) Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commun.*, **332**, 392–397.
- Maeda H, Hosokawa M, Sashima T, Takahashi N, Kawada T and Miyashita K (2006) Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *Int. J. Molec. Med.*, **18**, 147–152.
- Maeda H, Hosokawa M, Sashima T and Miyashita K (2007) Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-A<sup>y</sup> mice. *J. Agric. Food Chem.*, **55**, 7701–7706.
- Matsumoto M, Hosogawa M, Matsukawa N, *et al.* (2010) Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. *Eur. J. Nutr.*, **49**, 243–249.
- Matsuo T, Matsuo M, Kasai M and Takeuchi H (2001) Effect of a liquid diet supplement containing structured medium and long-chain triacylglycerols on body fat accumulation in healthy young subjects. *Asia Pacific J. Clin. Nutr.*, **10**, 46–50.
- Miyashita K (2009) The carotenoid fucoxanthin from brown seaweed affects obesity. *Lipid Technol.*, **21**, 186–190.
- Mizushima Y, Sugiyama Y, Yoshida H, *et al.* (2001) Galactosyldiacylglycerol, a mammalian DNA polymerase  $\alpha$ -specific inhibitor from a sea alga, *Petalonia binghamiae*. *Biol. Pharm. Bull.*, **24**, 982–987.
- Mori K, Ooi T, Hiraoka M, *et al.* (2004) Fucoxanthin and its metabolites in edible brown algae cultivated in deep seawater. *Marine Drugs*, **2**, 63–72.
- Prabhasankar P, Ganesan P, Bhaskar N, *et al.* (2009) Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chem.*, **115**, 501–508.
- Qiong L, Jun L, Jun Y, Yin Zhu Z, Xiaoyan C and Minglian Y (2011) The effect of *Laminaria japonica* polysaccharides on the recovery of the male rat reproductive system and mating function damaged by multiple

- mini-doses of ionizing radiations. *Env. Toxicol. Pharmacol.*, **31**, 286–294.
- Renn DW (1990) Seaweeds and biotechnology-inseparable companions. *Hydrobiology*, **204–205**, 7–13.
- Riou D, Collic-Jouault S, Pinczon du Sel D, *et al.* (1996) Antitumor and antiproliferative effects of a fucan extracted from *Ascophyllum nodosum* against a non-small-cell bronchopulmonary carcinoma line. *Anticancer Res.*, **16**, 1213–1218.
- Saker KE, Allen VG, Fontenot JP, *et al.* (2001) Tasco-forage: II. Monocyte immune cell response and performance of beef steers grazing tall fescue treated with a seaweed extract. *J. Animal Sci.*, **79**, 1022–1031.
- Saker KE, Fike JH, Veit H and Ward DL (2004) Brown seaweed-(TascoTM) treated conserved forage enhances antioxidant status and immune function in heat-stressed Wether lambs. *J. Anim. Physiol. Anim. Nutr. (Berl.)*, **88**, 122–130.
- Softpedia (2009) <http://news.softpedia.com/news/Appesat-the-Seaweed-Diet-Pill-that-Expands-in-the-Stomach-101227.shtml> (accessed 12 April 2011).
- Terasaki M, Hirose A, Narayan B, *et al.* (2009) Evaluation of recoverable functional lipid components of several brown seaweeds (Phaeophyta) from Japan with special reference to fucoxanthin and fucosterol contents. *J. Phycol.*, **45**, 974–980.
- Torres AI, Gil MN and Esteves JL (2004) Nutrient uptake rates by the alien alga *Undaria pinnatifida* (Phaeophyta) (Nuevo Gulf, Patagonia, Argentina) when exposed to diluted sewage effluent. *Hydrobiologia*, **520**, 1–3.
- Turner JL, Dritz SS, Higgins JJ and Minton JE (2002) Effects of *Ascophyllum nodosum* extract on growth performance and immune function of young pigs challenged with *Salmonella typhimurium*. *J. Animal Sci.*, **80**, 1947–1953.
- Urbano MG and Goni I (2002) Bioavailability of nutrients in rats fed on edible seaweeds, nori (*Porphyra tenera*) and wakame (*Undaria pinnatifida*), as a source of dietary fibre. *Food Chem.*, **76**, 281–286.
- Wang J, Zhang QB, Zhang ZS and Li Z (2008) Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. *Int. J. Biol. Macromol.*, **42**, 127–132.
- Wong KH, Sam SW, Cheung PCK and Ang PO (1999) Changes in lipid profiles of rats fed with seaweed-based diets. *Nutr. Res.*, **19**, 1519–1527.
- Xavier F and Sunyer PI (2002) The obesity epidemic: pathophysiology and consequences of obesity. *Obesity Res.*, **10**, 97–104.
- Zvyagintseva TN, Shevchenko NM, Nazarenko EL, *et al.* (2005) Water-soluble polysaccharides of some brown algae of the Russian far-east. Structure and biological action of low-molecular mass polyuronans. *J. Exp. Mar. Biol. Ecol.*, **320**, 123–131.

# 21

## Health Beneficial Aspects of Phloroglucinol Derivatives from Marine Brown Algae

Noel Vinay Thomas<sup>1</sup> and Se-Kwon Kim<sup>1,2</sup>

<sup>1</sup>*Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan, Republic of Korea*

<sup>2</sup>*Marine Bioprocess Research Center, Pukyong National University, Busan, Republic of Korea*

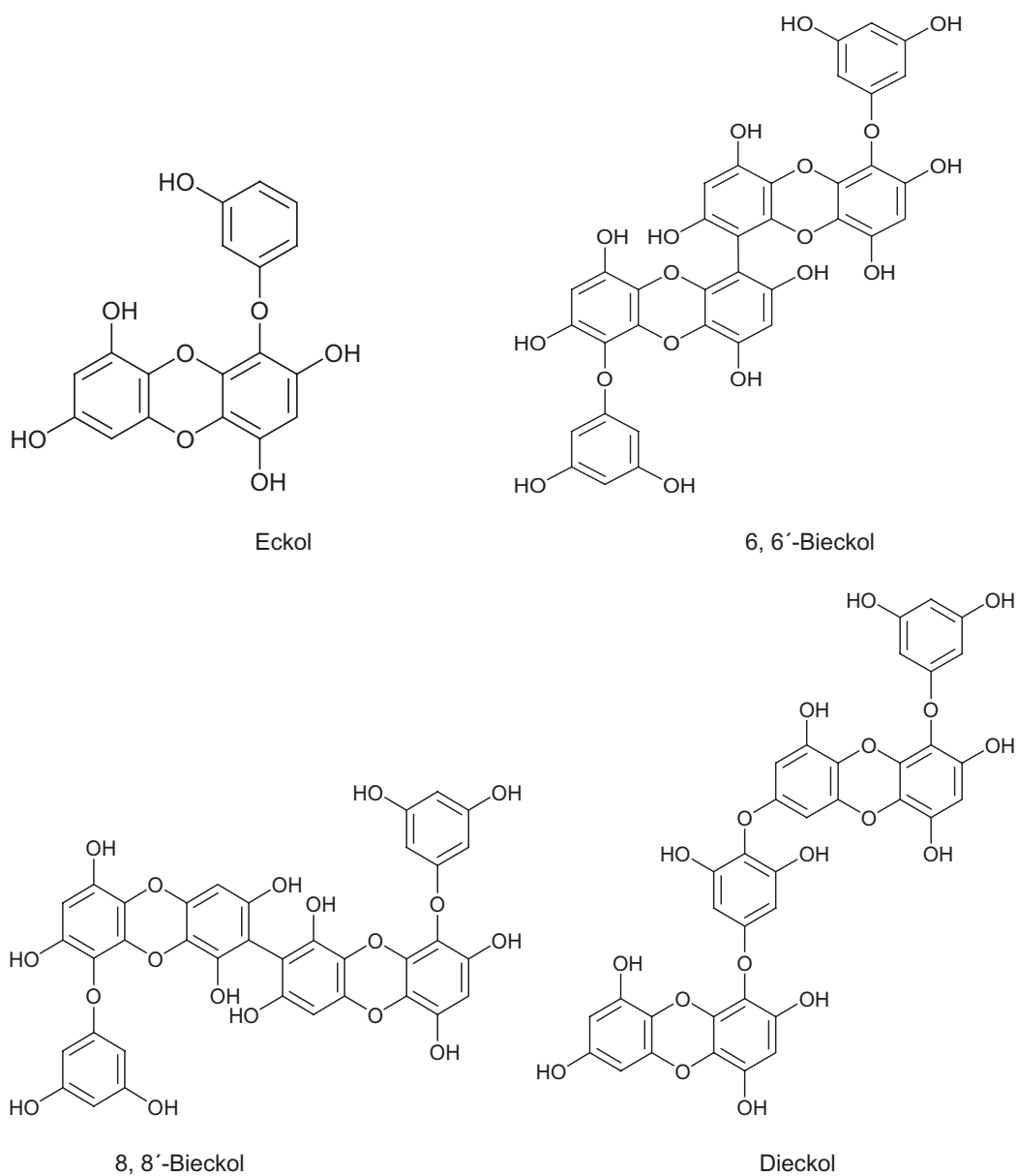
### 21.1 Introduction

The seaweeds possess enormous biologically important and beneficial ingredients that aid human health. Recently, the isolation and characterization of the biologically active components from seaweeds has gained a lot of attention from various research groups across the world. This is due to their role in influencing the health benefits for humans. Out of the varied marine inhabitants, the significance of seaweeds as a source for natural products has been well known for a long time. In Asian countries such as Korea, Japan, and China, marine algae are considered as sea vegetables in the diet and also as an alternative medicine (Ali *et al.* 2000). Among the marine algae, brown algal members (Laminariaceae) have been recognized as the chief sources of biologically active components, most importantly phlorotannins. Marine brown algae *Ecklonia cava* and *Eisenia bicyclis* have been intensively investigated for their human beneficial bioactive components including phlorotannins, polysaccharides such as alginic acid, fucoidans, pyropheophytin, tripeptides, and oxylipin (Kousaka *et al.* 2003) and also the beneficial bioactivities that include anti-inflammation, in-

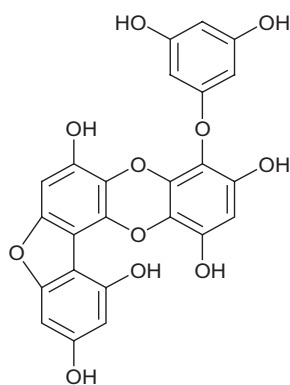
hibition of hyaluronidase activity and antidiabetic activity (Shibata *et al.*, 2002; Okada *et al.*, 2004). In addition to the above mentioned health beneficial aspects, phlorotannins are reported to exhibit biological activities such as antitumor, antihypertensive, antiallergic, and matrix metalloproteinases (MMP) inhibition activities. In this chapter we discuss the health beneficial activities of phlorotannins derived from brown algae and their potential role in pharmaceutical, food and cosmeceutical industries.

### 21.2 Phloroglucinol derivatives (phlorotannins) from marine brown algae

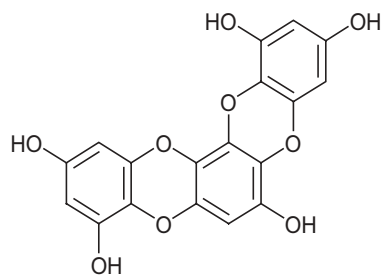
The marine algal phloroglucinol derivatives otherwise known as phlorotannins (Figure 21.1) are exclusively confined to the marine brown algae. Marine algal phlorotannins are usually phloroglucinol-based polyphenols with phenyl and phenoxy units. Phlorotannins, are polymers of phloroglucinols (1,3,5-trihydroxybenzene). They are



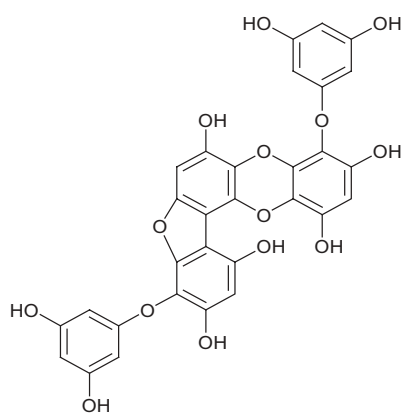
**Figure 21.1** Chemical structures of phlorotannins from marine brown algae.



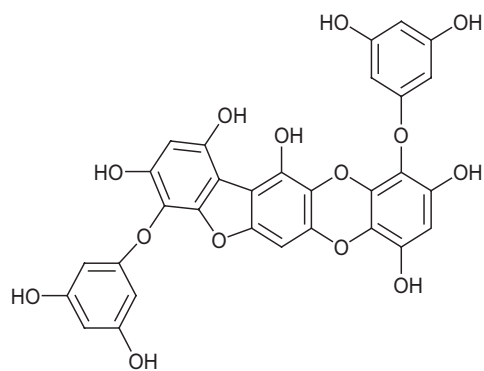
Fucofuroeckol-A



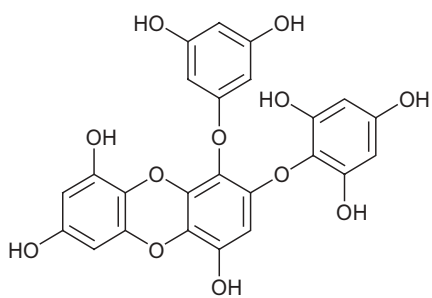
Dioxynodehydroeckol



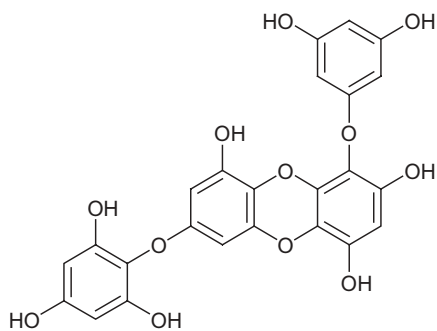
Phlorofucofuroeckol-A



Phlorofucofuroeckol-B

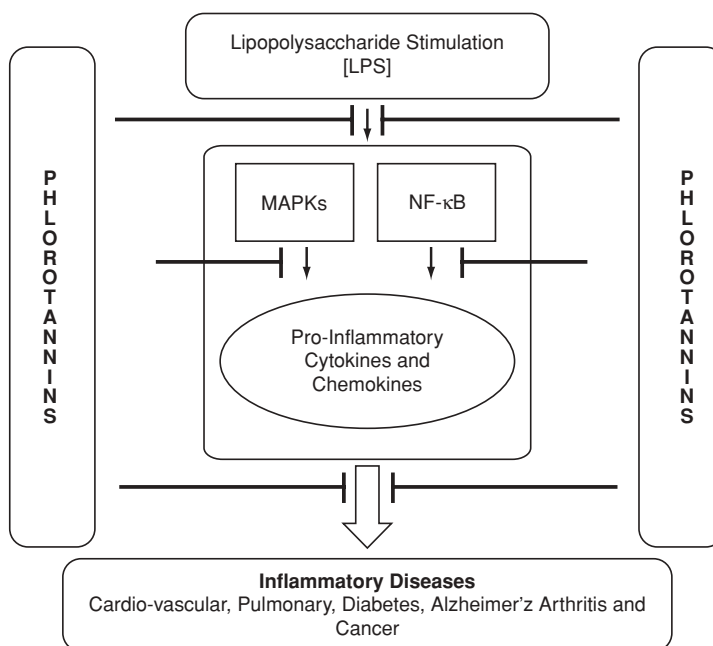


2-Phloroeckol



7-Phloroeckol

Figure 21.1 (Continued)



**Figure 21.2** Generalized anti-inflammatory activity of phlorotannins.

formed biosynthetically via the acetate–malonate pathway, also known as the polyketide pathway (Arnold and Targett, 2002; Ragan and Glombitza 1986). The molecular weights of phlorotannins vary from 126 to 650 Da, but are most commonly found in the 10 to 100 Da range (Boettcher and Targett 1993; McClintock and Baker, 2001). Phlorotannins are unique polyphenolic compounds bearing dibenzo-1,4-dioxin skeletons, which are exclusively confined to some brown algal genera such as *Ecklonia* and *Eisenia*. The members of Laminaraceae are reported to be the rich resources of phlorotannins among other marine algae (Okada *et al.*, 2004). The well-studied phlorotannins from *E. bicyclis* and *E. cava* are phloroglucinol, phloroglucinol tetramer, eckol, phlorofucofuroeckol A, dieckol, 8-8'-bieckol, dioxinodehydroeckol. Few novel phlorotannins from other edible seaweeds have been also reported such as phlorofucofuroeckol A, triphloroethol B, 2-phloroecol, 7-phloroecol, diphlorethol, fucofuroeckol A from *Ecklonia stolonifera* and phlorofucofuroeckol A from *Ecklonia kurome* (Li *et al.*, 2009; Shibata *et al.*, 2004).

## 21.3 Health beneficial aspects of brown algal phlorotannins

### 21.3.1 Anti-inflammatory activity

Inflammation is a complex biological response by the vascular tissues to harmful exo- and endogenous stimuli, like

pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the harmful stimuli and to initiate the healing process. However, prolonged inflammation could be potentially harmful to the body and might lead to several complications like autoimmune disorders, cardiovascular and pulmonary diseases, diabetes, Alzheimer's disease, arthritis and cancer. Over the past few decades, intensive research has provided a better understanding of inflammation-driven diseases. However, the proper treatment of these ailments is still an unresolved issue. Cellular mediators like prostaglandins (PGs), leukotrienes (LTs), nitric oxide (NO), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and cytokines of the interleukin (IL) family add to the complex cascade of inflammatory reactions.

In recent years, there has been increasing interest in the protective biochemical effect of naturally occurring biomolecules on anti-inflammation in biological systems and their mechanism (Figure 21.2). A number of nutritional compounds have been proven to illustrate both antioxidant activity and anti-inflammatory effects. Polyphenolic compounds in particular, have been reported to exert therapeutic effects (Soory, 2009). Phloroglucinol, a phlorotannin monomer extracted from *Ecklonia cava*, reduced the expression levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and PGE<sub>2</sub> in RAW264.7 cells stimulated by lipopolysaccharide (LPS), indicating that chronic inflammation can be repressed by phloroglucinol in macrophages (Kim and Kim, 2010). Their *in vitro* studies revealed that phloroglucinol exhibited inhibitory properties

not only on oxidative stress but also on the production of inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and PGE<sub>2</sub> in RAW264.7 cells stimulated by LPS.

Ethanollic extracts from *E. cava* (EC) shown an anti-inflammatory effect in LPS-stimulated murine BV2 microglia in a concentration-dependent manner. The expression of the proinflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  was reduced considerably at a concentration of 200  $\mu$ g/ml suggesting the potentiality of EC extracts as anti-inflammatory substances (Jung *et al.*, 2009). Phlorofucofuroeckol A from brown algae *E. stolonifera* significantly inhibited the LPS-induced production of NO and PGE<sub>2</sub> through the down-regulation of inducible nitric oxide synthase and cyclo-oxygenase 2 (COX-2) protein expressions in RAW264.7 murine macrophage cells. Treatment with 10 and 20  $\mu$ M phlorofucofuroeckol A suppressed the expression of inducible nitric oxide synthase (iNOS) and COX-2 proteins, and the result showed that the inhibitory effect of phlorofucofuroeckol A on iNOS and COX-2 protein expression was dose dependent (Kim *et al.*, 2009).

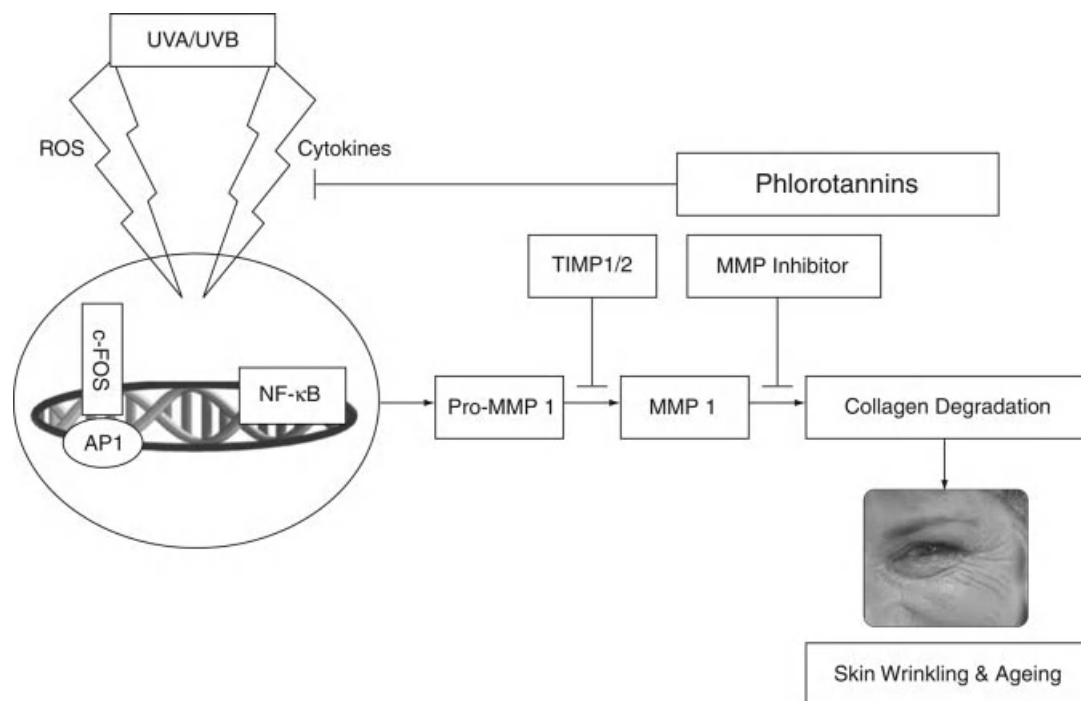
### 21.3.2 Antioxidant activity

Serious human health disorders such as atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, some neurological disorders, and some types of cancer, as well as aging are caused by the uncontrolled production of free radicals such as superoxide anion (O<sub>2</sub><sup>-</sup>), hydroxyl radical (HO<sup>+</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Ruberto *et al.*, 2001). These free radicals are physiological metabolites formed during aerobic life as a result of the metabolism of oxygen. They attack cellular macromolecules such as membrane lipids, proteins, and DNA. To protect cellular biomolecules in biological systems, equilibrium between oxidant formation and endogenous antioxidant defense mechanisms exist. If this balance is disturbed, it can produce oxidative stress. Therefore, antioxidants play a vital role to prevent this oxidative stress. Because of the unwanted side effects of chemical antioxidants, natural antioxidants from marine and terrestrial resources have gained much importance. Recent studies have shown that polyphenolic compounds from marine algae have strong antioxidant activities on free radicals (Shibata *et al.*, 2008; Kang *et al.*, 2003). Polyphenols are electron-rich compounds, which can intervene with efficient electron donation reactions and in turn produce phenoxyl radical species as intermediates in the presence of oxidizing agents. This feature of polyphenols makes them a good choice for natural antioxidants. These phenoxyl radicals are stabilized by resonance delocalization of the unpaired electron to the *ortho*- and *para*- positions of the ring. Moreover, phe-

noxyl radicals are also stabilized by hydrogen bonding with an adjacent hydroxyl group. Oligomers of phloroglucinol (1,3,5-trihydroxybenzene), eckol (a trimer), phlorofucofuroeckol A (a pentamer), dieckol and 8,8'-bieckol (hexamers), isolated from the Laminarian brown algae *Eisenia bicyclis*, *Ecklonia cava* and *Ecklonia kurome*, exhibited potent inhibition of phospholipid peroxidation in the liposome system. These phlorotannins had significant radical scavenging activities against the superoxide anion (50% effective concentration values: 6.5–8.4  $\mu$ M) and 2,2-diphenyl-1-picrylhydrazyl (50% effective concentration values: 12–26  $\mu$ Mol) (Shibata *et al.*, 2008). Enzymatic extracts of *Ishige okamurae* generated by different proteases (except for neutrase) had stronger hydrogen peroxide scavenging effects than the carbohydrase extract. In particular, the Kojizyme extract exhibited cytoprotective effects against H<sub>2</sub>O<sub>2</sub>-induced DNA damage (Heo and Jeon, 2008). Furthermore, several phlorotannins that purified from *E. cava* are responsible for antioxidant activities and showed protective effects against hydrogen peroxide-induced cell damage (Kang *et al.* 2005, 2006). *In vitro* study on the cellucast extract of the enzymatic hydrolysate of *E. cava* has been reported to exhibit a good antioxidant activity against hydrogen peroxide mediated cell damage (KN Kim *et al.*, 2006).

### 21.3.3 Anti-photoaging activity

Photoaging and sunburn are directly linked to the over exposure of skin to ultraviolet (UV) radiation from the Sun. More-over it is known that continuous exposure to UV radiation results in increased occurrence of non-melanoma skin cancer and skin aging. Human skin, when exposed to UV rays for prolonged periods undergoes an oxidative stress that ultimately manifests as erythema (Thiele *et al.*, 1997). Many synthetic and terrestrial plant-derived cosmetics are available to overcome the problem of sunburn. A few functional food researchers strongly recommend that regular intake of dietary antioxidants or treatment of the skin with products containing antioxidant ingredients might be useful in preventing UV-induced skin damage (Messina *et al.* 1994). It is reported that phlorotannins show an ability to absorb UV light and the phenolic compounds from marine brown algae exhibit antioxidant activity (Pavia *et al.*, 1997; Henry and Van Alstyne, 2004; Ragan and Glombitza, 1986). The expression of MMP-1, which is an interstitial collagenase, and is mainly responsible for the degradation of dermal collagen in the human skin aging process was attenuated by eckol and dieckol isolated from *E. stolonifera* in human dermal fibroblasts. This inhibition by *E. stolonifera*-derived phlorotannins was in correlation with the inhibition of both



**Figure 21.3** Schematic representation of the role of phlorotannins in preventing photoaging.

nuclear factor (NF)- $\kappa$ B and activator protein-1 (AP-1) reporter activity (Joe *et al.*, 2006). Dieckol from *E. cava* was found to have exceptional protective activity against photo-oxidative stress (Figure 21.3). It exhibited 57.8% protective properties against UV-B radiation-induced DNA damage in fibroblast cells *in vitro* (Heo *et al.*, 2009).

#### 21.3.4 Antitumor activity

The progression of tumor is a complex, multistage process wherein genetic changes occur in a normal cell that result in phenotypic alterations and the stimulation of the ability to multiply and colonize at distant sites in the body. Even though many factors regulate malignant tumor growth and spread, interactions between a tumor and its surrounding microenvironment result in the production of important protein products that are crucial to each step of tumor progression (Nelson *et al.*, 2000). Phlorotannins derived from brown algae have long been considered to be important secondary metabolites that play a role in chemical deterrence. Importantly, it has been reported that phlorotannins possess anticarcinogenic and antibacterial effects. Scientific investigation suggests that dioxinodehydroeckol's potential antiproliferative activity might be associated with the induction of apoptosis through the NF- $\kappa$ B family and the NF- $\kappa$ B-dependent pathway (Kong *et al.*, 2009). Phlorotan-

nin extract (PE) derived from brown algae *Laminaria japonica* Aresch (*L. japonica*) has shown considerable antiproliferative activity in the human hepatocellular carcinoma cell line (BEL-7402) and on the murine leukemic cell line (P388) in a dose-dependent manner. Microscopic observations have revealed that the morphologic features of tumor cells treated with PE and 5-fluorouracil (a commercial chemotherapy drug) are markedly different from the normal control group suggesting the antiproliferative effect of PE (Yang *et al.*, 2010). The antiproliferative activity depends on the total polyphenolic content in the algae. For example, the antiproliferative effects of red alga, *Palmaria palmata* and three kelp *Laminaria setchellii*, *Macrocystis integrifolia*, *Nereocystis leutkeana* extracts on human cervical adenocarcinoma cells (HeLa cells). Interestingly, HeLa cell proliferation was inhibited ( $p < 0.05$ ) between 0% and 78% by *P. palmata*; 0% and 55% by *L. setchellii* and 0% and 69% by *M. integrifolia* and *N. leutkeana* at 0.5–5 mg/ml algal extract (Yuan and Walsh, 2006). This scientific investigation proves the effectiveness of polyphenolic compounds in controlling tumor growth and brings front a fact that not only marine brown algae are the choice for antitumor compounds but also other marine algae could prove beneficial. Dioxinodehydroeckol isolated from *E. cava* has exhibited a remarkable antiproliferative effect on human breast cancer cells (MCF-7). The enzymatic extract of *E. cava* together with

its crude polysaccharide (CpoF) and crude polyphenolic fractions (CphF) have been reported to possess antiproliferative and antiradical activities. Especially the CphF at an  $IC_{50}$  value of 5.1  $\mu\text{g/ml}$  has successfully inhibited cell proliferation in the murine colon cancer cell line (CT-26). The antiproliferative effect of CphF is believed to be associated with apoptotic cell demise in CT-26, confirmed by the nuclear staining experiment (Athukorala *et al.*, 2006). In pretumor-bearing mice, the dietary feeding (0.1% and 0.5%) of brown algal polyphenols significantly reduced tumor multiplicity (45% and 56%) and tumor volume (54% and 65%), and topical administration (3 and 6 mg) significantly decreased tumor multiplicity (60% and 46%) and tumor volume (66% and 57%), respectively. It is believed that brown algal polyphenols inhibit COX-2 activity and cell proliferation, hence preventing tumor progression (Hwang *et al.*, 2006).

### 21.3.5 MMP inhibition activity

MMP are a family of proteolytic enzymes generally termed endopeptidases. This group of endopeptidases mostly consists of enzymes from metzincin family that include seralysins, astacins, adamalysins (a disintegrin and metalloproteinase domain or ADAMs), and matrixins (MMP). The involvement of the regulated degradation of the extracellular matrix (ECM) is essential for physiological remodeling processes like tissue repair, development, and morphogenesis. Interestingly, the remodeling process was found to be uncontrolled and there was a deleterious immunological response to repair tissue damage, which was credited to cardiorelated ailments, cancer, and arthritis (Murphy and Nagase, 2008; Huxley-Jones *et al.*, 2007). The poor selectivity, improper metabolism, low oral bioavailability, poor solubility, side effects and the risk of increased drug toxicity have strongly eliminated the few synthetic MMP-inhibitory substances (MMPIs) from clinical trials. However, the wide diversity of marine life forms that serves as a chief source for unique biologically active compounds could be beneficial in designing marine derived MMPIs (Noel Vinay and Kim, 2010). Dieckol and 1-(3',5'-dihydroxyphenoxy)-7-(2'',4'',6''-trihydroxyphenoxy) 2,4,9-trihydroxydibenzo-1,4-dioxin from the methanolic extract of *E. cava* has remarkably promoted osteosarcoma differentiation by increasing alkaline phosphatase (ALP) activity, mineralization, total protein and collagen synthesis in human osteosarcoma cells (MG-63). Moreover the inhibition of mRNA gene and protein levels of MMP-1, MMP-3, and MMP-13, iNOS and COX-2 was confirmed by casein zymographic, Western blot and reverse transcriptase-polymerase chain reaction (RT-PCR) assays (Ryu *et al.*, 2009). For the first

time, Kim *et al.* reported a detailed *in vitro* study on the inhibitory effects of phlorotannins derived from *E. cava* on MMPs activities. A novel gelatin digestion assay could visualize complete inhibition of bacterial collagenase-1 activity at 20  $\mu\text{g/ml}$  of *E. cava* extract during preliminary screening assays (MM Kim *et al.*, 2006). *E. bicyclis* derived phlorotannins fucufuroeckol-A (FF) and eckol (EK) have markedly inhibited the expression of MMP-2 and MMP-9 in the human fibrosarcoma cell line HT1080. It is reported that FF and EK have significantly inhibited the NF- $\kappa$ B expression and also they had a significant inhibitory effect on AP-1 expression. Thus the expression of MMP-2 and -9 via blocking the transcription of both NF- $\kappa$ B and AP-1 was inhibited. At present, several MMP inhibitors are undergoing clinical trials and it is expected that the use of these inhibitors would develop a new approach for the treatment of cancer in addition to traditional drugs. However, most of these drugs are reported to exert side effects. Therefore, marine brown algal members would be potent natural sources for the development of pharmaceuticals against MMP and cancer.

### 21.3.6 Additional health beneficial aspects of phlorotannins

Apart from the above discussed potential health beneficial aspects there are several other biological activities of phlorotannins derived from marine brown algae. The enzyme hyaluronidase is known for its involvement in allergic effects, migration of cancer, and inflammation, by depolymerizing the polysaccharide hyaluronic acid in the ECM of the connective tissue. Brown algal phlorotannins such as eckol, phlorofucufuroeckol A, dieckol, and 8,8'-bieckol have the ability to inhibit the hyaluronidase (Shibata *et al.*, 2002) suggesting the possibility of phlorotannins as potential anti-inflammatory agents. Phloroglucinol derivatives from *Eisenia bicyclis* have been reported to exhibit inhibitory effect on glycation and  $\alpha$ -amylase enzyme activities that play a major role in type 2 diabetes (Okada *et al.*, 2004). Phlorofucufuroeckol-A isolated from *Ecklonia stolonifera* has shown preventive effects on diabetic complications by exhibiting significant inhibitory effects against advanced glycation end products. Phloroglucinol derivatives from marine algae have shown a promising effect in controlling human immunodeficiency virus type-1 (HIV-1), the world's catastrophic infection. *In vitro* studies revealed that 6,6'-bieckol, one of the major phloroglucinol derivative that naturally occurs in *E. cava*, has inhibited HIV-1-induced syncytia formation, lytic effects, and viral p24 antigen production (Artan *et al.*, 2008). In addition, some phlorotannins such as 7-phlorphloroekol, phlorofucufuroeckol A, and 6,6'-bieckol have been reported

to show inhibitory activities against the both enzymes acetylcholinesterase and butyrylcholinesterase suggesting the possible role of phlorotannins as a dietary supplement to treat neurological disorders such as Alzheimer's disease (Yoon *et al.*, 2009). Most of the biological activities of marine-derived phlorotannins were assessed by *in vitro* or *in vivo* methods. However, as the results are very promising it is highly recommended to study these aspects up to the clinical trials stage to establish their potentiality as promising drug candidates.

## 21.4 Conclusions and future prospects

Marine brown algae have contributed several novel biologically active components that benefit human health. They are normally used as dietary supplements in Asian countries. The occurrence of phloroglucinol derivatives, phlorotannins, has made them unique sources for medicinal and functional food researchers. However, among the marine brown algae, most of the research is focused on *Ecklonia* and *Eisenia* species. As scientific reports clearly project the abundant chemical distribution of phlorotannins among marine brown algae, it is suggested for future prospects that the main focus should be on the biological and pharmacological activities of phlorotannins from other brown algal species. With the latest advances in the fields of molecular biology and biochemistry, a sophisticated approach to study the interactions of phlorotannins with human cellular systems could prove beneficial in understanding mechanisms to treat various human diseases. On the other hand, it is recommended to screen phlorotannins from other marine macroalgae and evaluate their biological activities as a comparative study. This would broaden the chances of coming up with more biologically efficient phlorotannins that might help in rendering quality biological activities that would provide better and more resourceful drug candidates for pharmacological purposes.

## References

- Ali MS, Jahangir M, Saleem M, Pervez MK, Hameed S and Ahmad VU. (2000) Metabolites of marine algae collected from Karachi-coasts of Arabian sea. *Nat. Prod. Sci.*, **6**(2), 61–65.
- Arnold TM and Targett NM. (2002) Marine tannins: the importance of a mechanistic framework for predicting ecological roles. *J. Chem. Ecol.*, **28**(10), 1919–1934.
- Artan M, Li Y, Karadeniz F, Lee SH, Kim MM, and Kim SK. (2008) Anti-HIV-1 activity of phloroglucinol derivative, 6, 6'-bieckol, from *Ecklonia cava*. *Bioorg. Med. Chem.*, **16**(17), 7921–7926.
- Athukorala Y, Kim KN, and Jeon YJ. (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food Chem. Toxicol.*, **44**(7), 1065–1074.
- Boettcher AA, and Targett NM. (1993) Role of polyphenolic molecular size in reduction of assimilation efficiency in *Xiphister mucosus*. *Ecology*, **74**(3), 891–903.
- Henry BE, and KL Van Alstyne. (2004) Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. *J. Phycol.*, **40**, 527–533.
- Heo SJ, and Jeon YJ. (2008) Radical scavenging capacity and cytoprotective effect of enzymatic digests of *Ishige okamurae*. *J. Appl. Phycol.*, **20**(6), 1087–1095.
- Heo SJ, Ko SC, Cha SH, *et al.* (2009) Effect of phlorotannins isolated from *Ecklonia cava* on melanogenesis and their protective effect against photo-oxidative stress induced by UV-B radiation. *Toxicol. in Vitro*, **23**(6), 1123–1130.
- Huxley-Jones J, TK Clarke, C Beck, G Toubaris, DL Robertson, and RP Boot-Handford. (2007) The evolution of the vertebrate metzincins; insights from *Ciona intestinalis* and *Danio rerio*. *BMC Evol. Biol.*, **7**(1), 63.
- Hwang H, Chen T, Nines RG, Shin HC, and Stoner GD. (2006) Photochemoprevention of UVB induced skin carcinogenesis in SKH 1 mice by brown algae polyphenols. *Int. J. Cancer*, **119**(12), 2742–2749.
- Joe MJ, Kim SN, Choi HY, *et al.* (2006) The inhibitory effects of eckol and dieckol from *Ecklonia stolonifera* on the expression of matrix metalloproteinase-1 in human dermal fibroblasts. *Biol. Pharm. Bull.*, **29**(8): 1735–1739.
- Jung W-K, Ahn Y-W, Lee S-H, *et al.* (2009) *Ecklonia cava* ethanolic extracts inhibit lipopolysaccharide-induced cyclooxygenase-2 and inducible nitric oxide synthase expression in BV2 microglia via the MAP kinase and NF-[kappa]B pathways. *Food Chem. Toxicol.*, **47**(2), 410–417.
- Kang K, Park Y, Hwang HJ, Kim SH, Lee JG, and Shin HC. (2003) Antioxidative properties of brown algae polyphenolics and their perspectives as chemopreventive agents against vascular risk factors. *Arch. Pharm. Res.*, **26**(4), 286–293.
- Kang KA, Lee KH, Chae S, *et al.* (2005) Triphlorethol-A from *Ecklonia cava* protects V79–4 lung fibroblast against hydrogen peroxide induced cell damage. *Free Rad. Res.*, **39**(8), 883–892.
- Kang KA, KH Lee, S Chae, *et al.* (2006) Cytoprotective effect of phloroglucinol on oxidative stress induced cell damage via catalase activation. *J. Cell. Biochem.*, **97**(3), 609–620.
- Kim AR, Shin TS, Lee MS, *et al.* (2009) Isolation and identification of phlorotannins from *Ecklonia stolonifera* with

- antioxidant and anti-inflammatory properties. *J. Agric. Food Chem.*, **57**(9), 3483–3489.
- Kim KN, Heo SJ, Song CB, *et al.* (2006) Protective effect of *Ecklonia cava* enzymatic extracts on hydrogen peroxide-induced cell damage. *Proc. Biochem.*, **41**(12), 2393–2401.
- Kim MM, and Kim SK. (2010) Effect of phloroglucinol on oxidative stress and inflammation. *Food Chem. Toxicol.*, **48**(10), 2925–2933.
- Kim MM, QV Ta, E Mendis, *et al.* (2006) Phlorotannins in *Ecklonia cava* extract inhibit matrix metalloproteinase activity. *Life Sci.*, **79**(15), 1436–1443.
- Kong CS, J Kim, NY Yoon, and SK Kim. (2009) Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cancer cells. *Food Chem. Toxicol.*, **47**(7), 1653–1658.
- Kousaka K, Ogi N, Akazawa Y, *et al.* (2003) Novel oxylipin metabolites from the brown alga *Eisenia bicyclis*. *J. Nat. Prod.*, **66**(10), 1318–1323.
- Li Y, Qian ZJ, Ryu BM, Lee S-H, Kim M-M and Kim S-K (2009) Chemical components and its antioxidant properties in vitro: An edible marine brown alga, *Ecklonia cava*. *Bioorg. Med. Chem.*, **17**(5), 1963–1973.
- McClintock JB, and Baker BJ. (2001) *Marine Chemical Ecology*. CRC Press, Boca Raton, FL.
- Messina MJ, Persky V, Setchell KDR, and Barnes S. (1994) Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr. Cancer*, **21**(2), 113–131.
- Murphy G., and H. Nagase. (2008) Progress in matrix metalloproteinase research. *Molec. Aspects Med.*, **29**(5), 290–308.
- Nelson AR, Fingleton B, Rothenberg ML, and Matrisian LM. (2000) Matrix metalloproteinases: biologic activity and clinical implications. *J. Clin. Oncol.*, **18**(5), 1135.
- Okada Y, Ishimaru A, Suzuki R, and Okuyama T. (2004) A new phloroglucinol derivative from the brown alga *Eisenia bicyclis*: potential for the effective treatment of diabetic complications. *J. Nat. Prod.*, **67**(1), 103–105.
- Pavia H, Cervin G, Lindgren A, and Aaberg P. (1997) Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. *Mar. Ecol. Prog. Ser.*, **157**, 139–146.
- Ragan MA, and Glombitza KW. (1986) Phlorotannins, brown algal polyphenols. In: *Handbook of Phycological Methods* (eds J.A. Hellebust and J.S. Craigie). Cambridge University Press, Cambridge.
- Ruberto G, Baratta MT, Biondi DM, and Amico V. (2001) Antioxidant activity of extracts of the marine algal genus *Cystoseira* in a micellar model system. *J. Appl. Phycol.*, **13**(5), 403–407.
- Ryu BM, Y Li, ZJ Qian, MM Kim, and SK Kim. (2009) Differentiation of human osteosarcoma cells by isolated phlorotannins is subtly linked to COX-2, iNOS, MMPs, and MAPK signaling: Implication for chronic articular disease. *Chem.-Biol. Interact.*, **179**(2–3), 192–201.
- Shibata T, Fujimoto K, Nagayama K, Yamaguchi K, and Nakamura T. (2002) Inhibitory activity of brown algal phlorotannins against hyaluronidase. *Int. J. Food Sci. Technol.*, **37**(6), 703–709.
- Shibata T, Ishimaru K, Kawaguchi S, Yoshikawa H, and Hama Y. (2008) Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *J. Appl. Phycol.*, **20**(5), 705–711.
- Shibata T, Kawaguchi S, Hama Y, Inagaki M, Yamaguchi K, and Nakamura T. (2004) Local and chemical distribution of phlorotannins in brown algae. *J. Appl. Phycol.*, **16**(4), 291–296.
- Soory M. (2009) Relevance of nutritional antioxidants in metabolic syndrome, ageing and cancer: potential for therapeutic targeting. *Infectious Disorders-Drug Targets*, **9**(4), 400–414.
- Thiele JJ, Podda M, and Packer L. (1997) Tropospheric ozone: An emerging environmental stress to skin. *Biol. Chem. Hoppe-Seyler*, **378**(11), 1299–1305.
- Thomas N.V. and Kim S K. (2010) Metalloproteinase inhibitors: status and scope from marine organisms. *Biochem. Res. Int.*, Article ID 845975, 10 pages, doi:10.1155/2010/845975.
- Yang H, M Zeng, S Dong, Z Liu, and R Li. (2010) Anti-proliferative activity of phlorotannin extracts from brown algae *Laminaria japonica* Aresch. *Chinese J. Oceanol. Limnol.*, **28**(1), 122–130.
- Yoon NY, Lee S-H, Yong L and Kim S-K (2009) Phlorotannins from *Ishige okamurae* and their acetyl- and butyrylcholinesterase inhibitory effects. *J. Funct. Foods*, **1**(4), 331–335.
- Yuan YV, and NA Walsh. (2006) Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food Chem. Toxicol.*, **44**(7), 1144–1150.

## Biological Effects of Proteins Extracted from Marine Algae

**Taek-Jeong Nam**

*College of Fisheries Science, Pukyong National University, Busan, Republic of Korea*

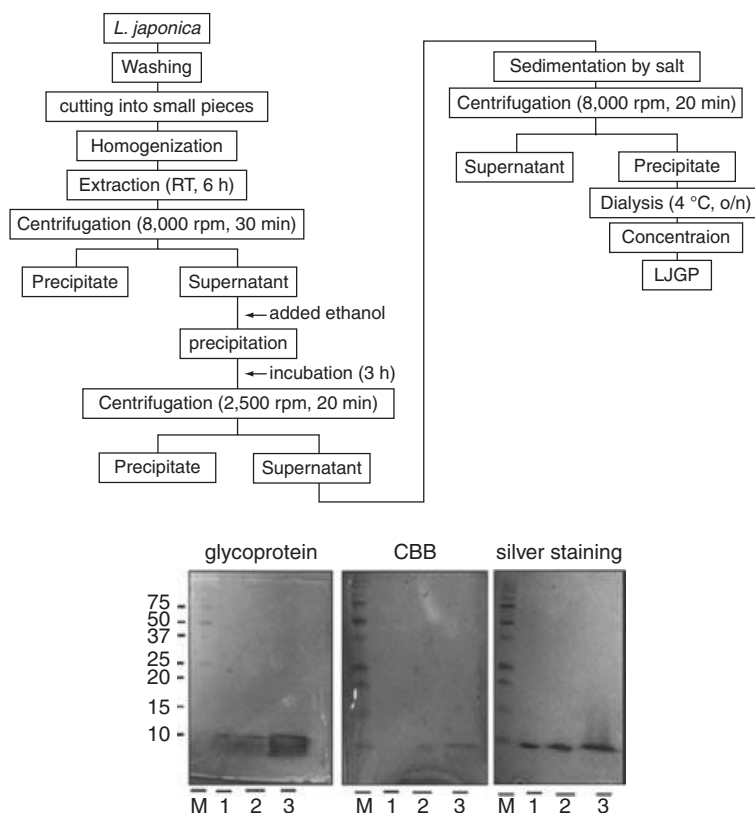
### 22.1 Introduction

Recently, seaweeds have received much attention from scientific researchers. Moreover, their consumption has been promoted heavily for children and pregnant women as well-balanced, harmless, natural sources of highly bioavailable trace elements (Booth, 1964). Marine algae contain bioactive compounds that are not found commonly in terrestrial plants, and a number of investigators have found that these traditional food sources can provide not only nutritional benefits, but also help fight disease and contribute to the maintenance of good health. For example, some researchers have reported that extracts of a variety of edible seaweeds showed antioxidative and antiproliferative activities (Yuan and Walsh, 2006), and that a methanol extract of the seaweed *Gloiopeltis furcata* induced G2/M arrest in hepatocytes (Bae and Choi, 2007). Furthermore, ethanol extracts from *Callophyllis japonica* and *Spatoglossum asperum* have been reported to have various biological activities (Ara *et al.*, 2005; Kang *et al.*, 2005). A polysaccharide from *Fucus evanescens* exhibited antitumor and antimetastatic activities in C57BI/6 mice with transplanted Lewis lung adenocarcinoma (Alekseyenko *et al.*, 2007). Additionally, by downregulating tissue factor expression, *Grateloupia longifolia* polysaccharides inhibited angiogenesis in HMEC-1 endothelial cells (Zhang *et al.*, 2006). However, as noted above, most research on seaweeds has focused on their polysaccharides, and few studies have investigated their proteins.

Seaweeds are composed primarily of carbohydrates, proteins, and other minor components. Thus, we hypothesized that the medicinal effects of seaweeds would be due to carbohydrates or proteins. In this chapter, we will deal with the biological effects of marine algae, using *Hizikia fusiformis* (Hwang *et al.*, 2008a; Choi *et al.*, 2009, 2010), *Porphyra yezoensis* (Hwang *et al.*, 2008b), *Capsosiphon fulvescens* (Hwang *et al.*, 2008c), and *Laminaria japonica* as examples (Go *et al.*, 2009).

### 22.2 Stimulatory effect of a glycoprotein from *LAMINARIA Japonica* on cell proliferation

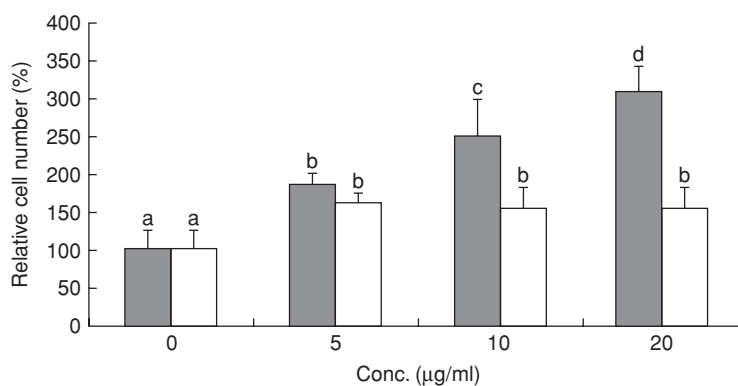
Marine algae, such as seaweeds, are abundant in seawater. Seaweeds contain many useful components and physiologically active substances that remain unidentified (Wang and Yang, 1997; Shin *et al.*, 2006). Carrageenan and alginic acid, which are isolated from red and brown algae, are known to lower cholesterol levels in the blood and liver (Wang and Yang, 1997). We separated an extract from *L. japonica* and used sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to identify a glycoprotein, which we designated LJGP (Figure 22.1). The results from the MTS assay demonstrated that LJGP stimulated IEC-6 cell growth in a dose-dependent manner (Figure 22.2).



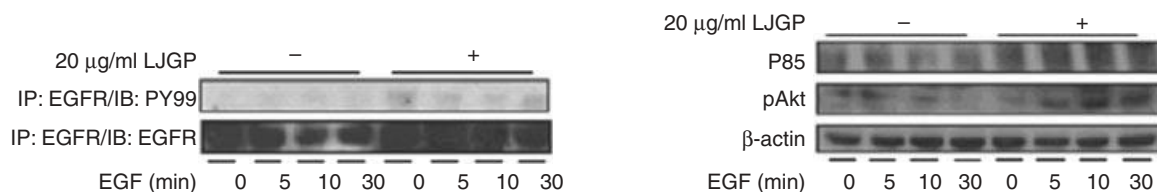
**Figure 22.1** Schematic diagram for obtaining glycoproteins from *L. japonica* and the electrophoresis profiles of LJGP.

Stimulation of cell proliferation is dependent on multiple signaling pathways. In this study, we focused on the epidermal growth factor receptor (EGFR) signaling pathway. EGFR expression was detected in various cell types, including epithelial, mesenchymal, and nerve cells. Its activation

leads to cell differentiation and proliferation. Our results demonstrated that LJGP induced EGFR phosphorylation (Figure 22.3). Activated EGFR leads to the phosphorylation of specific tyrosine residues within the EGFR cytoplasmic domain that acts as a docking site for effector molecules,



**Figure 22.2** Effect of LJGP treatment on the growth of IEC-6 small intestine epithelial cells. Cells were treated with the indicated concentrations of LJGP (black bars) or bovine serum albumin (BSA) (white bars), and the relative cell number was determined using the MTS assay. Values represent the mean  $\pm$  SD;  $n = 6$  ( $p < 0.01$ ).



**Figure 22.3** Tyrosine phosphorylation of EGFR in response to LJGP treatment. Cells were treated with or without LJGP and the intracellular proteins were collected using lysis buffer. One representative gel from three separate experiments is shown.

triggering downstream signaling pathways (Chiu *et al.*, 2004).<sup>16</sup> These findings indicated that EGFR activation also contributes to LJGP-induced proliferation through activation of the phosphoinositol-3-kinase (PI3K)/Akt pathway. The PI3K/Akt pathway has been identified as a key player in cell survival (Parrizas *et al.*, 1997; Kandel and Hay, 1999). Akt also functions in normal growth, as demonstrated in Akt-knockout mice, which show retarded growth (Carrie *et al.*, 2005). PI3K is activated by EGFR through heterodimerization with ErbB3, which contains a docking site for the p85 subunit of PI3K (Morgan and Grandis, 2008). Once the p85 subunit is positioned, the p110 subunit of PI3K generates phosphatidylinositol 3,4,5-triphosphate (PIP3), which activates Akt (Morgan and Grandis, 2008).<sup>20</sup> Consistent with this model, we detected the phosphorylation of Akt (Figure 22.3).

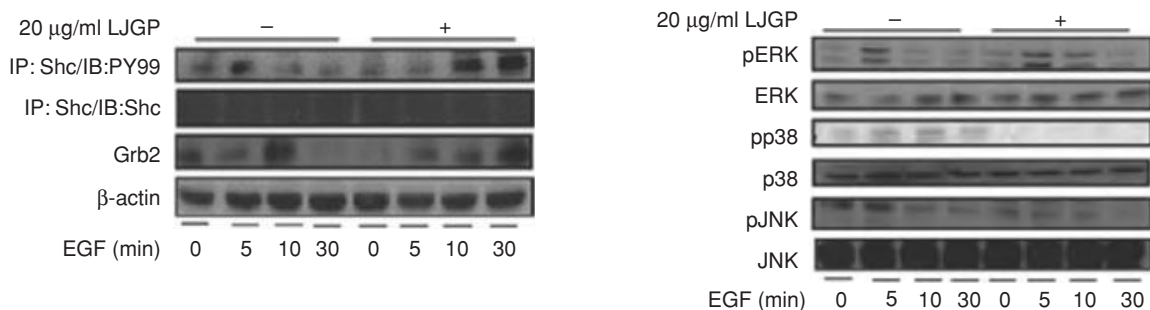
One of the downstream signaling pathways is the Ras/Raf/ mitogen-activated protein kinase (MAPK) pathway. The MAPK family in mammalian cells includes extracellular signal-regulated kinase-1 and kinase-2 (ERK-1/2), the c-Jun NH<sub>2</sub> terminal kinase (JNK), and p38 (Paruchuri *et al.*, 2002). In accordance with the LJGP-induced cell proliferation, ERK 1/2 was activated after exposure to LJGP, an important mediator that regulates cell growth and differentiation (Figure 22.4). In contrast, decreased phosphorylation of JNK and p38 was observed, phenomena that are associated with cell death and oxidative stress. For most cell types, EGFR has been proposed to mediate Ras/Raf/ERK

activation and the ERK pathway has been implicated in mitogenic signal transduction in response to several stimuli. Moreover, we confirmed the interaction of the adaptor protein Shc and Grb2. The interaction of Shc and Grb2 is an essential step following EGFR activation in MAPK signal translocation.

In the present study, we extracted a glycoprotein from *L. japonica* that not only possessed anticancer activity (data not shown), but also enhanced cell growth in normal intestinal cells. Furthermore, we studied the intracellular mechanisms involved in cell proliferation through the EGFR signaling pathway. The results showed that LJGP induced Akt/ERK activation and downregulated JNK/p38 (Figure 22.4). Although further studies are needed to define its interaction on the cell membrane surface, we suggest that LJGP may be a potentially useful agent for understanding intestinal function.

## 22.3 Chemoprotective effect of marine algae extracts against acetaminophen toxicity

Acetaminophen (AAP) is a safe and effective analgesic when used at therapeutic levels. However, an overdose of AAP can induce severe hepatotoxicity in both experimental animals and humans (Thomas, 1993). AAP overdose is the leading



**Figure 22.4** The effect of LJGP on the MAPK signaling pathway. Cells were treated with or without LJGP and proteins were assayed. One representative gel from three separate experiments is shown.

cause of drug-induced liver failure requiring transplantation in the United States (Lee, 2004). Thus, numerous studies have sought to identify the mechanism of AAP-induced liver injury to prevent or repair it. Grape seed extract can protect against AAP-induced cell death (Ray *et al.*, 1999), and treatment with *Protium heptaphyllum* extract can protect against AAP-induced liver injury (Oliveira *et al.*, 2005). Furthermore, an extract of the marine alga *Sargassum polycystum* inhibits AAP toxicity (Raghavendran *et al.*, 2005).

Although many mechanisms are involved with AAP toxicity, such as covalent binding of the highly reactive metabolite of AAP (NAPQI: *N*-acetyl-*p*-enzoquinoneimine) to cellular macromolecules, lipid peroxidation, the oxidation of critical sulfhydryl groups, and the alteration of calcium homeostasis, increasing evidence suggests that enhanced oxidative stress contributes to AAP-induced liver injury. An accumulation of oxidative damage may activate a cascade that induces cell death. Oxidative damage may activate several different signaling pathways that are capable of inducing a variety of responses, including stress adaptation or cell death (McCubrey *et al.*, 2006); among them, MAPK signaling is involved in the regulation of oxidative stress (Matsukawa *et al.*, 2004).

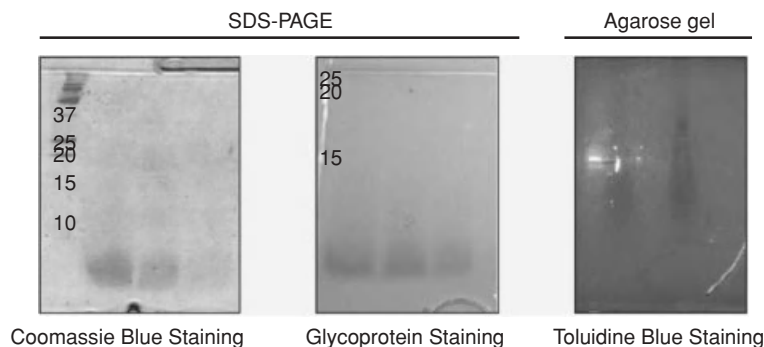
The MAPK family includes three kinases: ERK, JNK, and p38 kinase. Previously, we demonstrated that ERK is activated by AAP in Chang liver cells (Hwang *et al.*, 2007). Although ERK activation plays a protective role against apoptosis (Takeda and Ichijo, 2002), it is activated by other stimuli, such as ethanol treatment.<sup>28</sup> Poly(ADP-ribose) polymerase (PARP) activation is regulated by ERK1/2 via direct phosphorylation and there are Erk-2 phosphorylation sites on PARP-1 (Kauppinen *et al.*, 2006). Moreover, AAP-induced hepatotoxicity has been shown to be triggered by oxidative stress, and the main stress signaling pathways in response to oxidative stress are the Erk, p38, and MAPK signaling cascades and the Akt pathway (Nair *et al.*, 2004).

### 22.3.1 Effect of a glycoprotein from *Hizikia fusiformis* on acetaminophen-induced liver injury

*Hizikia fusiformis*, a brown alga, grows primarily in temperate seaside areas of the northwest Pacific, including China, Korea, and Japan, where it is consumed as a vegetable. This alga possesses a number of beneficial compounds, including antioxidants (Siriwardhana *et al.*, 2004) and anticoagulants (Kim *et al.*, 1998); however, it also contains inorganic arsenic, which is a carcinogen in humans (Watanabe *et al.*, 1979; Nakamura *et al.*, 2008). In this study, we extracted a bioactive glycoprotein from *H. fusiformis* and demonstrated its chemoprotective effect against AAP toxicity. We performed SDS-PAGE and agarose gel electrophoresis to determine whether the *H. fusiformis* extract contained a polysaccharide or a protein. We used Coomassie blue staining to assay for the presence of protein, and toluidine blue and glycoprotein staining were used to assay for polysaccharides and glycoproteins, respectively.

As shown in Figure 22.5, we identified HFPG as a glycoprotein. We analyzed the changes in body weight and liver weight among the rats (Table 22.1) and found that pretreatment with AAP or AAP + HFPG had no significant effect, suggesting that 100 mg/kg/day HFPG was non-toxic.

We induced liver injury in rats by treatment with AAP; AAP is used commonly for the screening of hepatoprotective drugs (Davis *et al.*, 1974). To examine the protective effects of HFPG against AAP toxicity, we measured the levels of glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GPT) in serum, as indicators of liver injury. As illustrated in Figure 22.6A, the serum GOT level following AAP treatment was the same as that in the control group. However, the GPT level increased to  $27.34 \pm 6.0$  karmen by AAP treatment, as compared to  $20.23 \pm 3.9$  karmen



**Figure 22.5** Electrophoretic analyses of HFPG. HFPG was separated by SDS-PAGE and agarose gel electrophoresis and then analyzed by Coomassie blue staining to detect the presence of protein, toluidine blue staining to detect the presence of polysaccharides, and glycoprotein staining.

**Table 22.1** Effect of pretreatment with AAP or AAP + HFPG on body weight and liver weight

	Control	AAP	AAP + HFPG
Body weight gain (g)	85.7 ± 9.4	77.2 ± 14.3	84.2 ± 6.0
Liver weight (g)	9.7 ± 1.0	10.2 ± 1.2	9.9 ± 0.5

The AAP + HFPG group was pretreated with HFPG (100 mg/kg, [oral intake] o.i.) once a day for 2 weeks, while the control and AAP groups were given saline. After the final treatment, the control group was treated with 1 mL of saline, while the AAP and HFPG co-treatment groups were given 700 mg/kg AAP (i.p.); body and liver weight was measured 24 h later. Each value represents the mean ± SD for ten rats. Different superscripts in the same column indicate significant differences ( $p < 0.05$ ) among the groups.

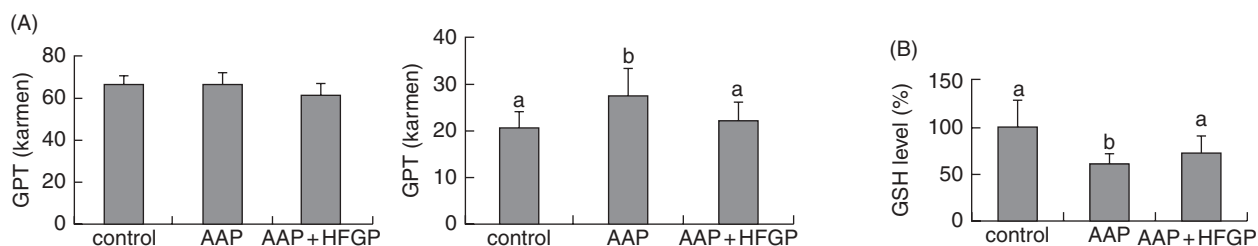
in the control group, whereas it decreased to  $22.17 \pm 3.7$  karmen in the AAP + HFPG group.

Recent studies have suggested that reactive metabolite formation, glutathione-S-transferase (GSH) depletion, and protein alkylation are important initiating events in AAP-related toxicity. Early investigations into the mechanism of oxidative stress-induced cell death identified GSH as a critical factor in the detoxification of the reactive metabolite of AAP (Mitchell *et al.*, 1973). Studies using various experimental models have established that severe hepatocellular injury can lead to intracellular, mitochondria-derived oxidative stress (Jaeschke and Mitchell, 1989). Elevated hepatic and mitochondrial GSSG levels are indicators of mitochondrial reactive oxygen species (ROS) formation, and AAP-induced cell death may be a result of oxidative cytotoxicity (Jaeschke, 1990; Knight *et al.*, 2001). To determine whether HFPG can inhibit AAP-induced GSH depletion, we measured the GSH levels in serum. Our results revealed

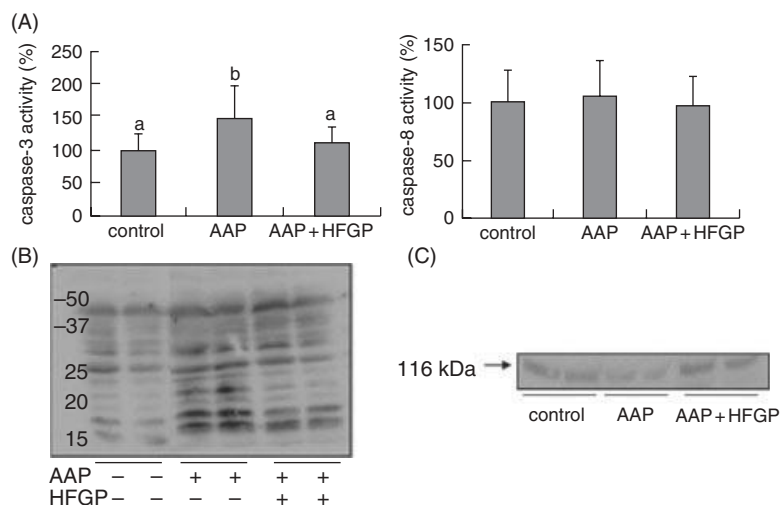
that co-treatment with HFPG and AAP inhibited AAP-induced GSH depletion (Figure 22.6B). AAP is believed to kill rats by inducing apoptosis, based on the observation that AAP treatment results in the activation of caspase-3 and -9 (Figure 22.7). In contrast, no caspase-8 activation was detected in the AAP or AAP + HFPG groups. Caspase-9, but not caspase-8, was activated by AAP, suggesting that AAP-induced damage to the mitochondria was prevented by cotreatment with HFPG. Activated caspase-9 in turn induces caspase-3 to cleave the 116 kDa PARP into 89- and 24 kDa peptides, rendering PARP unable to carry out the poly-ADP-ribosylation of various proteins involved in DNA repair. In this study, the amount of intact PARP decreased with AAP treatment, whereas co-treatment with AAP and HFPG inhibited caspase-3/-9 activation (Figure 22.7) and PARP cleavage (data not shown).

Based on the results of the *in vivo* assay, we examined the effect of HFPG on AAP-treated HepG2 cells. The MTS assay and Hoechst 33342 staining results confirmed that HFPG protected against AAP-induced cell death (Figure 22.8).

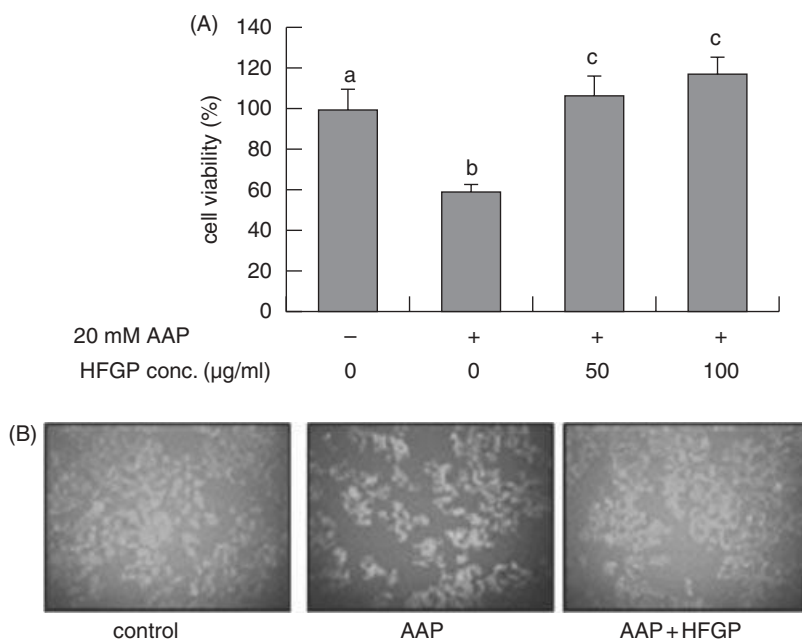
As mentioned, there are many mechanisms involved with AAP toxicity. In this study, we focused on AAP-induced oxidative injury among the various mechanisms. AAP, a well-known model compound for producing chemical hepatic injury, requires biotransformation by the hepatic microsomal cytochrome P450 to produce its hepatotoxic metabolite, NAPQI, which can react with sulfhydryl groups, such as those of GSH and protein thiols. The covalent binding of trichloromethyl free radicals to cell proteins is considered to be the initial step in the events leading to cell death. Furthermore, the MAPK signaling pathway is directly related to this oxidative stress. The MAPK family, which includes the ERK, JNK, and p38 subgroups, plays important roles in cellular proliferation, apoptosis, and differentiation. Signaling through JNK and p38 is a proximal component in the induction of apoptosis (Xia *et al.*, 1995; Takeda and Ichijo,



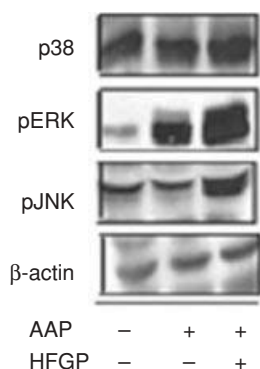
**Figure 22.6** Effect of HFPG on AAP-induced liver injury. The animals were pretreated with HFPG (100 mg/kg, o.i.) once per day for 2 weeks, while the control and AAP groups were given saline. After the final treatment, the control group was treated with 1 mL saline, while the AAP and HFPG co-treatment groups were given 700 mg/kg AAP (i.p.). Hepatotoxicity was measured 24 h later by quantifying the serum levels of GOT/GPT (A) and GSH (B). Each value represents the mean ± SD ( $n = 10$ ). Letters next to the values indicate significant differences among the groups (Duncan's multiple-range test).



**Figure 22.7** Effect of HFGP on AAP-induced liver injury. Animals were pretreated with HFGP (100 mg/kg, o.i.) once per day for 2 weeks, while the control and AAP groups were given saline. After the final treatment, the control group was treated with 1 ml saline, while the AAP and HFGP co-treatment groups were given 700 mg/kg AAP (i.p.). The animals were sacrificed 24 h later and their liver was removed, washed in saline, and analyzed for the effects of HFGP on AAP toxicity. (A), caspase-3/-8 activity in liver tissue; (B), Western blotting for caspase-9 and PARP.

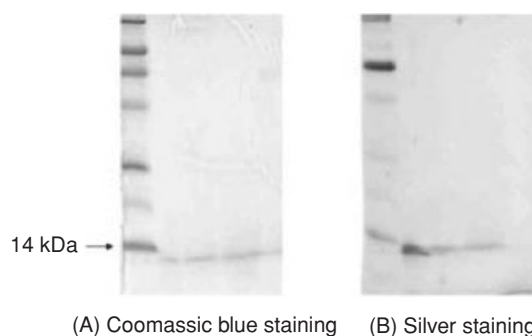


**Figure 22.8** Protective effect of HFGP against AAP-induced cell death. Cells were cultured with AAP or AAP + HFGP. (A) Cell viability as measured by the MTS assay. Values represent the mean  $\pm$  SD;  $n = 6$  ( $p < 0.01$ ). Letters next to the values indicate significant differences among the groups (Duncan's multiple-range test). (B) Morphological changes in the cells. Cells stained with Hoechst 33342 and PI were observed by fluorescence microscopy ( $\times 200$ ).



**Figure 22.9** Analysis of AAP-induced cell death in HFGP-treated cell extracts. Whole-cell extracts were prepared and analyzed by Western blotting using anti-phospho-JNK, -pp38, -phospho-ERK 1/2, and - $\beta$ -actin antibodies. One representative gel from three separate experiments is shown. The amount of protein in each band was quantified by densitometry.

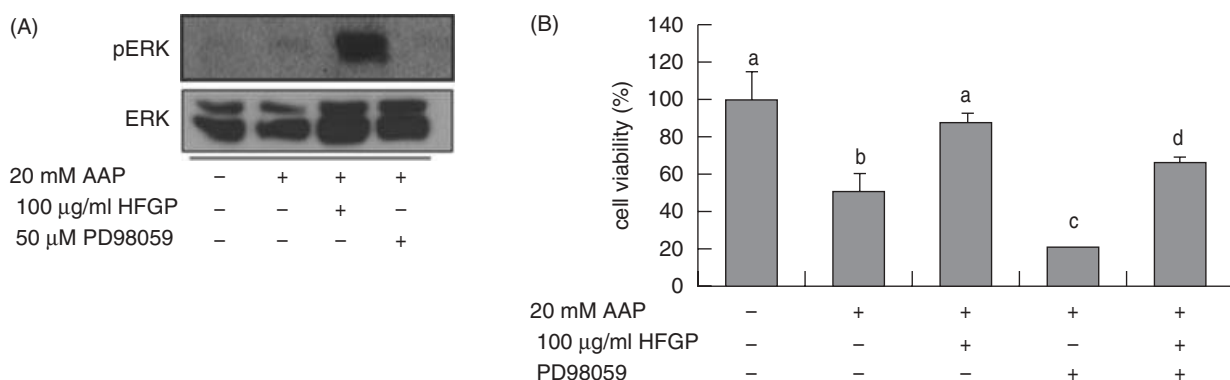
2002); however, oxidative stress also activates JNK signaling. Metamphetamine-induced oxidative stress in SHSY5Y cells leads to JNK activation (Wang *et al.*, 2008). In contrast, ERK has been shown to protect cells against apoptosis in response to oxidative stress (Wang *et al.*, 2004),<sup>44</sup> growth factor deprivation (Xia *et al.*, 1995), and proapoptotic drugs (Stadheim and Kucera, 1998). Moreover, oxidative stress-induced ERK activation has been reported to function as an antiapoptotic signal in homeostasis (Bae and Choi, 2007).



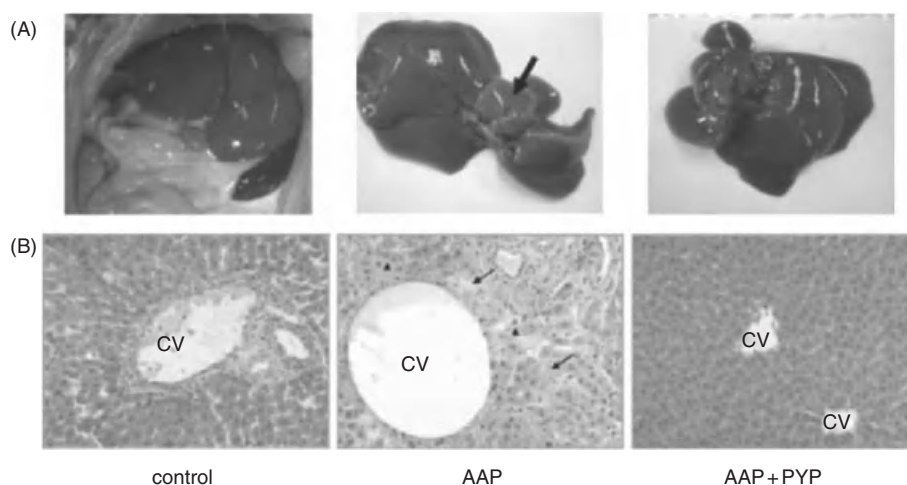
**Figure 22.11** SDS-PAGE bands of PYP. The extract was separated by SDS-PAGE, and then visualized with (A) Coomassie blue and (B) silver staining. M, marker; lanes 1 and 2 show the *P. yezeensis* protein band.

In the present study, treatment with AAP clearly induced ERK1/2 activation, and the addition of HFGP to AAP-treated cells resulted in enhanced ERK1/2 activation (Figure 22.9). Nevertheless, HFGP protected cells against AAP-induced cell death, as shown by the MTS assay and Hoechst 33342 staining (Figure 22.8). This further supports the idea that ERK phosphorylation is involved in the effect of HFGP on cell survival.

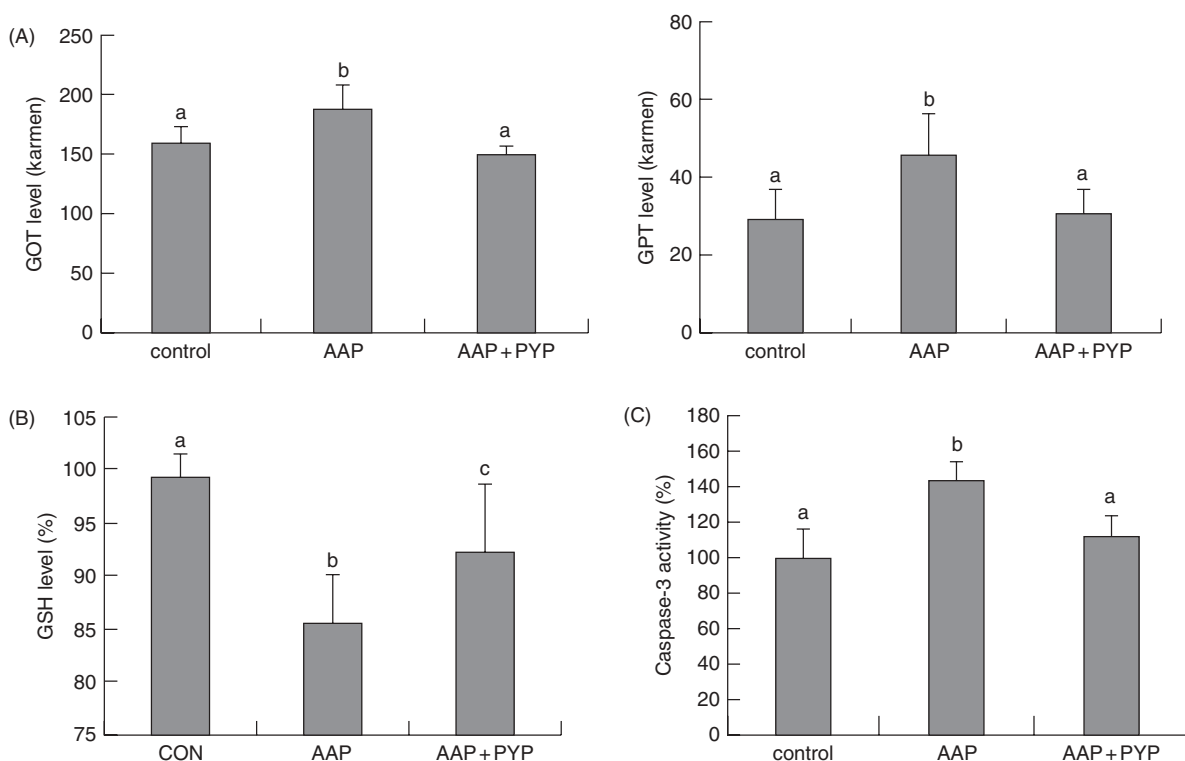
Next, we used the specific MAPK-1 inhibitor, PD98059, to determine whether ERK1/2 signaling is involved in AAP-induced HepG2 cell death. As expected, treatment with AAP and PD98059 inhibited ERK1/2 phosphorylation, compared with treatment with AAP alone (Figure 22.10A).



**Figure 22.10** Protective effect of HFGP against AAP-induced cell death via ERK 1/2 signaling. (A) Monolayers of HepG2 cells were untreated (control) or treated with 20 mM AAP, AAP + 100  $\mu$ g/mL HFGP, or AAP + 50 mM PD98059. Whole-cell extracts were then prepared and analyzed by Western blotting using anti-PARP, anti-phospho-ERK1/2, and anti-pan ERK antibodies. One representative gel from three separate experiments is shown. The amount of protein in each band was quantified by densitometry. (B) Cell viability measured using the MTS assay. Values represent the mean  $\pm$  SD;  $n = 6$  ( $p < 0.01$ ). Letters next to the values indicate significant differences among the groups (Duncan's multiple-range test).



**Figure 22.12** Photographs and H&E staining of liver after treatment with AAP alone or with AAP + PYP. (A) Liver photographs. The sites of liver damage are indicated by arrows. Note the color change to light brown compared with the control. In contrast, AAP + PYP treatment did not produce a color change. (B) Representative H&E-stained liver sections of the controls and animals treated with 700 mg/kg AAP or with AAP + PYP ( $\times 200$ ). Control: the liver was histologically normal with no change in lobular architecture. AAP treatment: the hepatocyte nuclei showed either chromatin condensation (arrowhead) or were completely absent (arrow). AAP + PYP co-treatment: the liver tissue was similar in appearance to that of the control.



**Figure 22.13** Effect of PYP on GOT/GPT levels in rats ( $n = 10$ ) exposed to AAP. The animals were fed 100 mg/kg PYP for 2 weeks and then administered 700 mg/kg AAP. After 24 h, blood and liver tissue were collected and analyzed for serum levels of (A) GOT/GPT, (B) GSH and (C) caspase-3 activity.

Moreover, combined treatment with AAP and PD98059 attenuated AAP-induced PARP cleavage (data not shown). To verify the connection with ERK, we analyzed the level of proliferation in cells treated with AAP and PD98059. Treatment with AAP and PD98059 ( $21.4 \pm 0.6\%$ ) inhibited cellular proliferation, compared with treatment with AAP alone ( $50.0 \pm 11.8\%$ , Figure 22.10B). Furthermore, treatment with AAP, HFPG, and PD98059 ( $61.49 \pm 1.8\%$ ) increased cellular proliferation compared with treatment with AAP alone or AAP + PD98059 (Figure 22.10B). Taken together, these results indicate that although ERK signaling is associated with AAP-induced cell death, it is insufficient to mediate the cytotoxicity of AAP. Thus, we propose that another signaling pathway may be linked to AAP-induced cytotoxicity, and this unknown pathway plays a more important role than ERK signaling. The results of this study demonstrate that HFPG has a potent hepatoprotective effect on AAP-induced hepatic damage in Sprague–Dawley rats and HepG2 cells.

Our results indicate that the hepatoprotective effects of HFPG may be due to its ability to block AAP toxicity via inhibition of caspase-3/-9 activation, which results in reduced serum GOT/GPT levels and increased GSH. Furthermore, the hepatoprotective effects of HFPG are most likely due to the activation of ERK, which is directly related to AAP-induced oxidative stress. Thus, we propose that HFPG may be used to protect against AAP-induced liver injury, and that ERK activation is pivotal in inducing the protective effect of HFPG; however, additional studies are required to clarify the mechanism.

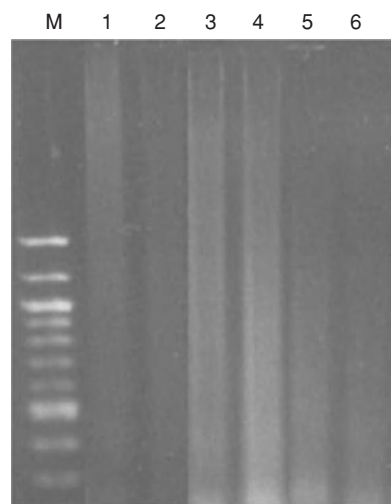
### 22.3.2 Chemoprotective effects of a protein from the red algae *Porphyra yezoensis* in drug-induced liver injury

Red algae belonging to the genus *Porphyra* are found from polar to tropical seas, and they have long been consumed in Pacific and Asian cultures. Chemoprotective effects, which are among the many bioactive effects of marine algae, have also been reported. For example, there are several reports of the protective effect of *Sargassum polycystum* against AAP-induced lipid peroxidation in rats (Raghavendran *et al.*, 2005), as well as the protective effects of seaweeds against liver injury caused by CCl<sub>4</sub> in rats (Gujral *et al.*, 2002).

In this study, a 14 kDa protein, termed PYP, was extracted from the marine alga *P. yezoensis* and we investigated whether PYP could protect against AAP-induced injury. As shown in Figure 22.11, a 14 kDa band was obtained by SDS-PAGE; this was used as the starting material in the subsequent experiments. Although no difference was observed in body or liver weight between the AAP and AAP + PYP

groups (data not shown), AAP treatment produced a change in color of the liver tissue to light brown, whereas the AAP + PYP and control groups did not exhibit any color change (Figure 22.12A). Hematoxylin and eosin (H&E) staining of the AAP-treated liver tissues (Figure 22.12B) revealed evidence of chromatin condensation or loss of nuclei in some cases; however, the sections from the AAP + PYP group showed no evidence of AAP-induced liver injury.

In order to examine the toxic effects of AAP on the liver, the levels of GOT and GPT, indicators of liver injury, were measured. As shown in Figure 22.13A, the serum GOT level following AAP treatment increased, to  $187.95 \pm 20.7$  karmen, compared with  $158.97 \pm 13.4$  karmen in the control group, whereas treatment with AAP + PYP reduced it, to  $148.41 \pm 6.7$  karmen. Similarly, the GPT level increased to  $44.83 \pm 11.7$  karmen by AAP treatment, compared with  $28.83 \pm 7.9$  karmen in the control group, whereas AAP + PYP decreased it, to  $30.22 \pm 6.6$  karmen. In view of these results, it is suggested that PYP may inhibit AAP-induced liver injury. To determine whether PYP could inhibit AAP-induced GSH depletion, the GSH levels were measured in rat liver tissues (Figure 22.13B). The results showed that co-treatment with PYP and AAP inhibited AAP-induced GSH depletion. Furthermore, AAP increased the activity of caspase-3, which is activated during apoptosis, as well as the amount of DNA fragmentation (Figure 22.14) and



**Figure 22.14** DNA fragmentation pattern after treatment with AAP alone or with AAP + PYP. The animals were treated with saline or with 100 mg/kg PYP for 2 weeks, and then administered 70 mg/kg AAP. After 24 h, liver tissue was collected and DNA fragmentation was analyzed by agarose gel electrophoresis. M, marker; lanes 1 and 2, control tissue; lanes 3 and 4, tissue treated with 700 mg/kg AAP; lanes 5 and 6, tissue treated with AAP + PYP.

the serum GOT/GPT levels, which are indicators of liver injury. PYP and AAP co-treatment led to caspase-3 activity, DNA fragmentation, GSH and GOT/GPT levels that matched those observed in the control group. Although further studies are needed, it was concluded that PYP may inhibit AAP-induced liver injury.

## References

- Alekseyenko, T.V., Zhanayeva, S.Y., Venediktova, A.A. *et al.* (2007) Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk Sea *Fucus evanescens* brown alga. *Bull. Exp. Biol. Med.*, **143**(6), 730–732.
- Ara, J., Sultana, V., Qasim, R., Ehteshamu-Haque, S., and Ahmad, V.U. (2005) Biological activity of *Spatoglossum asperum*: a brown alga. *Phytother. Res.*, **19**(7), 618–623.
- Bae, S.J. and Choi, Y.H. (2007) Methanol extract of the seaweed *Gloiopeltis furcata* induces G2/M arrest and inhibits cyclooxygenase-2 activity in human hepatocarcinoma HepG2 cells. *Phytotherapy Research*. **21**(1), 52–57.
- Booth, E. (1964) Trace elements and seaweeds. In: *Proceedings of the 4th International Seaweed Symposium* (eds A.D. De Virville and J. Feldmann). Macmillan, London, pp. 385–393.
- Carrie, E., McCurdy, C.E. and Cartee, G.D. (2005) Akt2 is essential for the full effect of calorie restriction on insulin-stimulated glucose uptake in skeletal muscle. *Diabetes*, **54**, 1349–1356.
- Chiu, T., Santiskulvong, C. and Rozengurt, E. (2004) EGF receptor transactivation mediates ANG-II-stimulated mitogenesis in intestinal epithelial cells through the PI3-kinase/Akt/mTOR/p70S6K1 signaling pathway. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **288**, 182–194.
- Choi E.Y., Hwang H.J. and Nam T.J. (2010) Protective effect of a polysaccharide from *Hizikia fusiformis* against ethanol-induced cytotoxicity in IEC-6 cells. *Toxicol. In Vitro*, **24**(1), 79–84.
- Choi E.Y., Hwang H.J., Kim I.H. and Nam T.J. (2009) Protective effects of a polysaccharide from *Hizikia fusiformis* against ethanol toxicity in rats. *Food Chem. Toxicol.*, **47**(1), 134–139.
- Davis, D.C., Potter, W.Z., Jollow, D.J. and Mitchell, J.R. (1974) Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. *Life Sci.*, **14**, 2099–2109.
- Go H., Hwang H.J. and Nam T.J. (2009) Glycoprotein extraction from *Laminaria japonica* promotes IEC-6 cell proliferation. *Int. J. Mol. Med.*, **24**(6), 819–824.
- Gujral, J.S., Knight, T.R., Farhood, A., Bajt, M.L. and Jaeschke, H. (2002) Mode of cell death after acetaminophen overdose in mice: apoptosis or oncotic necrosis? *Toxicol. Sci.*, **67**(2), 322–328.
- Hwang, H.J., Kwon, M.J. and Nam, T.J. (2007) Chemoprotective effect of insulin-like growth factor 1 against acetaminophen-induced cell death in Chang liver cells via ERK1/2 activation. *Toxicology*, **230**(1), 76–82.
- Hwang, H.J., Kim, I.H. and Nam, T.J. (2008a) Effect of a glycoprotein from *Hizikia fusiformis* on acetaminophen-induced liver injury. *Food Chem. Toxicol.*, **46**(11), 3475–3481.
- Hwang H.J., Kwon M.J., Kim I.H. and Nam T.J. (2008b) Chemoprotective effects of a protein from the red alga *Porphyra yezoensis* on acetaminophen-induced liver injury in rats. *Phytother. Res.*, **22**(9), 1149–53.
- Hwang H.J., Kwon M.J., Kim I.H. and Nam T.J. (2008c) The effect of polysaccharide extracted from the marine alga *Capsosiphon fulvescens* on ethanol administration. *Food Chem. Toxicol.*, **46**(8), 2653–2657.
- Jaeschke, H. and Mitchell, J.R. (1989) Mitochondria and xanthine oxidase both generate reactive oxygen species after hypoxic damage in isolated perfused rat liver. *Biochem. Biophys. Res. Commun.*, **160**, 140–147.
- Jaeschke, H. (1990) Glutathione disulfide formation and oxidant stress during acetaminophen-induced hepatotoxicity in mice in vivo: the protective effect of allopurinol. *J. Pharmacol. Exp. Ther.*, **255**, 935–941.
- Kandel, E.S. and Hav, N. (1999) The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. *Exp. Cell Res.*, **253**, 210–229.
- Kang, K.A., Bu, H.D., Park, D.S. *et al.* (2005) Antioxidant activity of ethanol extract of *Callophyllis japonica*. *Phytother. Res.*, **19**(6), 506–510.
- Kauppinen, T.M., Chan, W.Y., Suh, S.W., Wiggins, A.K., Huang, E.J. and Swanson, R.A. (2006) Direct phosphorylation and regulation of poly(ADP-ribose) polymerase-1 by extracellular signal-regulated kinases 1/2. *Proc. Natl. Acad. Sci. USA*, **103**, 7136–7141.
- Kim, J.S., Kim, W.Y., Rho, H.W., *et al.* (1998) Purification and characterization of adenosine diphosphate ribose pyrophosphatase from human erythrocytes. *Int. J. Biochem. Cell Biol.*, **30**(5), 629–638.
- Knight, T.R., Kurtz, A., Bajt, M.L., Hinson, J.A. and Jaeschke, H. (2001) Vascular and hepatocellular peroxynitrite formation during acetaminophen toxicity: role of mitochondrial oxidant stress. *Toxicol. Sci.*, **62**, 212–220.
- Lee, W.M. (2004) Acetaminophen and the US Acute Liver Failure Study Group: lowering the risks of hepatic failure. *Hepatology*, **40**, 6–9.

- Matsukawa, J., Matsuzawa, A., Takeda, K. and Ichijo, H. (2004) The ASK1-MAP kinase cascades in mammalian stress response. *J. Biochem.*, **136**(3), 261–265.
- McCubrey, J.A., Lahair, M.M. and Franklin, R.A. (2006) Reactive oxygen species-induced activation of the MAP kinase signaling pathways. *Antioxidants and Redox Signaling*, **8**(9–10), 1775–1789.
- Mitchell, J.R., Jollow, D.J., Potter, W.Z., Gillete, J.R. and Brodie, B.B. (1973) Acetaminophen-induced hepatic necrosis. *J. Pharmacol. Exp. Ther.*, **187**, 211–217.
- Morgan, S. and Grandis, J.R. (2008) ErbB receptors in the biology and pathology of the aerodigestive tract. *Exp. Cell Res.*, **315**, 572–582.
- Nair, V.D., Yuen, T., Olanow, C.W. and Sealfon, S.C. (2004) Early single cell bifurcation of pro- and antiapoptotic states during oxidative stress. *J. Biol. Chem.*, **279**, 27494–27501.
- Nakamura, Y., Narukawa, T. and Yosinaga, J. (2008) Cancer risk to Japanese population from the consumption of inorganic arsenic in cooked Hijiki. *J. Agric. Food Chem.*, **56**, 2536–2540.
- Oliveira, F.A., Chaves, M.H., Almeida, F.R. *et al.* (2005) Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. Trunk wood resin, against acetaminophen-induced liver injury in mice. *J. Ethnopharmacol.*, **98**(1–2), 103–108.
- Parrizas, M., Saltiel, A.R. and LeRoith, D. (1997) Insulin-like growth factor 1 inhibits apoptosis using phosphatidylinositol 3'-kinase and mitogen-activated protein kinase pathways. *J. Biol. Chem.*, **272**, 154–161.
- Paruchuri, S., Hallberg, B., Juhas, M., Larsson, C. and Sjolander, A. (2002) Leukotriene D(4) activates MAPK through a Ras-independent but PKCepsilon-dependent pathway in intestinal epithelial cells. *J. Cell Sci.*, **115**, 1883–1893.
- Raghavendran, H.R.B., Sathivel, A. and Devaki, T. (2005) Effect of *Sargassum polycystum* (Phaeophyceae)-sulfated polysaccharide extract against acetaminophen-induced hyperlipidemia during toxic hepatitis in experimental rats. *Molec. Cell. Biochem.*, **276**, 89–96.
- Ray, S.D., Kumar, M.A. and Bagchi, D. (1999) A novel proanthocyanidin IH636 grape seed extract increases in vivo Bcl-XL expression and prevents acetaminophen-induced programmed and unprogrammed cell death in mouse liver. *Arch. Biochem. Biophys.*, **369**, 42–58.
- Shin, H.C., Hwang H.J., Kang K.J. and Lee B.H. (2006) An antioxidative and anti-inflammatory agent for potential treatment of osteoarthritis from *Ecklonia cava*. *Arch. Pharm. Res.*, **29**, 165–171.
- Siriwardhana, N., Lee, K.W., Kim, S.H., Ha, W.J. and Jeon, Y.J. (2004) Enzymatic hydrolysis for effective extraction of antioxidative compounds from *Hizikia fusiformis*. *Algae*, **19**, 59–68.
- Stadheim, T.A. and Kucera, G.L. (1998) Extracellular signal-regulated kinase (ERK) activity is required for TPA-mediated inhibition of drug-induced apoptosis. *Biochem. Biophys. Res. Commun.*, **245**(1), 266–271.
- Takeda, K. and Ichijo, H. (2002) Neuronal p38 MAPK signaling: an emerging regulator of cell fate and function in the nervous system. *Genes Cells*, **7**, 1099–1111.
- Thomas, S.H.L. (1993) Paracetamol (acetaminophen) poisoning. *Pharmacol. Ther.*, **60**, 91–20.
- Wang, C. and Yang, G. (1997) Comparison of effects of two kinds of soluble algae polysaccharide on blood lipid, liver lipid, platelet aggregation and growth in rats. *Zhonghua Yu Fang Yi Xue Za Zhi*, **31**, 342–345.
- Wang, S.F., Yen, J.C., Yin, P.H., Chi, C.W. and Lee, H.C. (2008) Involvement of oxidative stress-activated JNK signaling in the methamphetamine-induced cell death of human SH-SY5Y cells. *Toxicology*, **246**(2–3), 234–241.
- Wang, X., Grammatikakis, N., Sigano, A., Stevenson, M.A., Calderwood, S.K. (2004) Interactions between extracellular signal-regulated protein kinase 1, 14–3-3 epsilon, and heat shock factor 1 during stress. *J. Biol. Chem.*, **279**(47), 49460–49469.
- Watanabe, T., Hirayama, T., Takahashi, T., Kokubo, T. and Ikeda, M. (1979) Toxicological evaluation of arsenic in edible seaweed, *Hizikia* species. *Toxicology*, **14**, 1–22.
- Xia, Z., Dicken, M., Raingeaud, J., Davis, R.J. and Greenberg, M.E. (1995) Opposing effects of ERK and JNK-p38 MAP kinase on apoptosis. *Science*, **270**, 1326–1331.
- Yuan, Y.V. and Walsh, N.A. (2006) Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food Chem. Toxicol.*, **44**(7), 1144–1150.
- Zhang, C., Yang, F., Zhang, H. *et al.* (2006) *Grateloupia longifolia* polysaccharide inhibits angiogenesis by down-regulating tissue factor expression in HMEC-1 endothelial cells. *Br. J. Pharmacol.*, **148**, 741–751.

# 23

## Functional Ingredients from Marine Algae as Potential Antioxidants in the Food Industry

Isuru Wijesekara<sup>1</sup>, Mahinda Senevirathne<sup>2</sup>, Yong-Xin Li<sup>1</sup> and Se-Kwon Kim<sup>1,2</sup>

<sup>1</sup>*Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan, Republic of Korea*

<sup>2</sup>*Marine Bioprocess Research Center, Pukyong National University, Busan, Republic of Korea*

### 23.1 Introduction

Since more than 70% of the world's surface is covered by oceans, the wide diversity of marine organisms offer a rich source of natural products with valuable nutraceutical, pharmaceutical, and cosmeceutical potential. Among marine organisms, edible seaweeds have been identified as an under-exploited plant resource and a source of functional foods. In addition, they have long been used in food diets as well as traditional remedies in Asian countries mainly China, Japan, and Korea. Recently, their importance as a source of novel bioactive substances is growing rapidly and researchers have revealed that marine algal originated compounds exhibit various biological activities (Borrow and Shahidi, 2008; Wijesekara *et al.*, 2010, 2011). Edible marine algae, sometimes referred to seaweeds, have attracted a special interest as potential sources of nutrients and one particular interesting feature is their richness in phlorotannins, sulfated polysaccharides (SPs), carotenoid pigments, and bioactive peptides.

The deterioration of some foods has been identified due to oxidation of lipids or rancidity and formation of undesirable secondary lipid peroxidation products. Lipid oxidation by reactive oxygen species (ROS) such as superoxide anion, hydroxyl radicals and H<sub>2</sub>O<sub>2</sub> also causes a decrease in nutritional value of lipid foods, and affect their safety and appearance. In the food and pharmaceutical industries, many synthetic commercial antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) have been used to retard the oxidation and peroxidation processes. However, the use of these synthetic antioxidants must be under strict regulation due to potential health hazards (Hettiarachchy *et al.*, 1996; Park *et al.*, 2001). Hence, the search for natural antioxidants as safe alternatives from natural resources such as marine algae is important in the food industry. This chapter focuses on potential application of marine algae-derived novel antioxidants such as phlorotannins, sulfated polysaccharides, and carotenoids in the food industry.

## 23.2 Marine algae-derived functional ingredients and their antioxidant effect

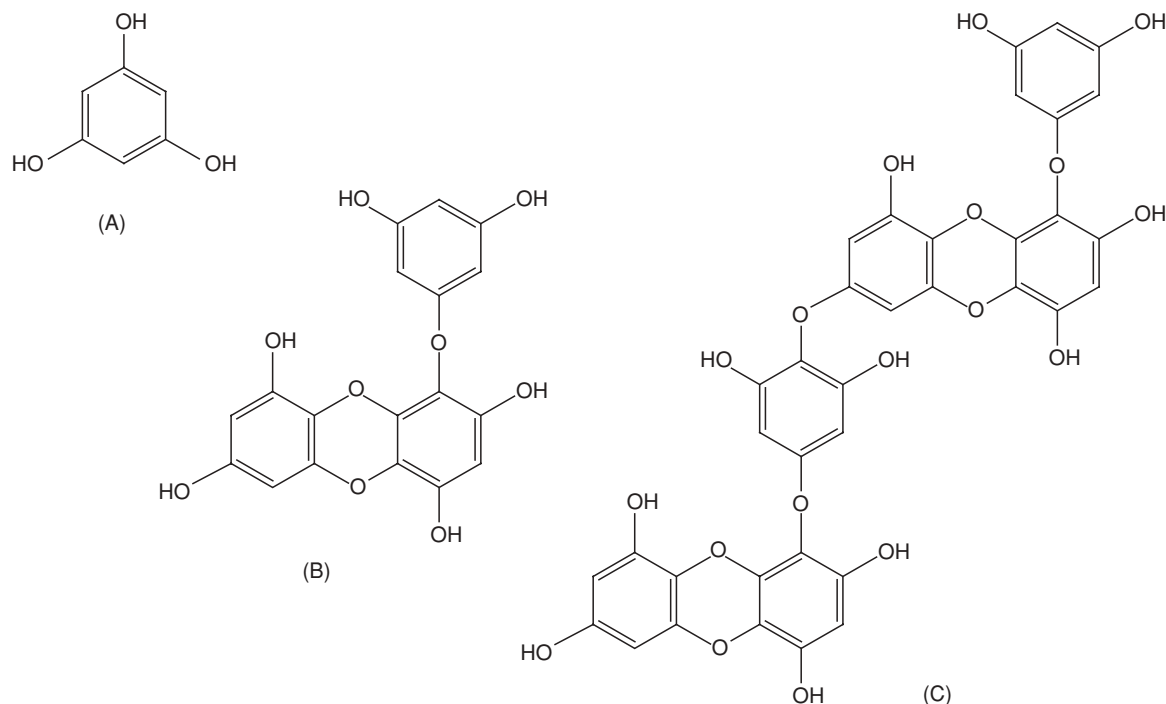
### 23.2.1 Phlorotannins

Marine brown algae accumulate a variety of phloroglucinol-based polyphenols, as phlorotannins. These phlorotannins consist of phloroglucinol units linked to each other in various ways, and are of wide occurrence amongst marine brown algae (Singh and Bharate, 2006). Many researchers have shown that phlorotannins (Figure 23.1) derived from marine brown algae have strong antioxidant activities on free radicals (Heo *et al.*, 2005; Kang *et al.*, 2003, 2004; Shibata *et al.*, 2008). The antioxidant activity can be the result of specific scavenging of radicals formed during peroxidation or of oxygen-containing compounds as well as chelating metal ions. According to the results of total antioxidant activity in the linoleic acid model system, phlorotannins showed potential activity against 1,1-diphenyl 1,2-picrylhydrazyl (DPPH), hydroxyl, superoxide, and peroxy radicals scavenging *in vitro*, using electron spin resonance (ESR) technique (Li *et al.*, 2009). Various phlorotannins have been reported to overcome the sensitivity problem inherent in the detection of endogenous radicals in biological systems. Furthermore, several phlorotannins that have been purified

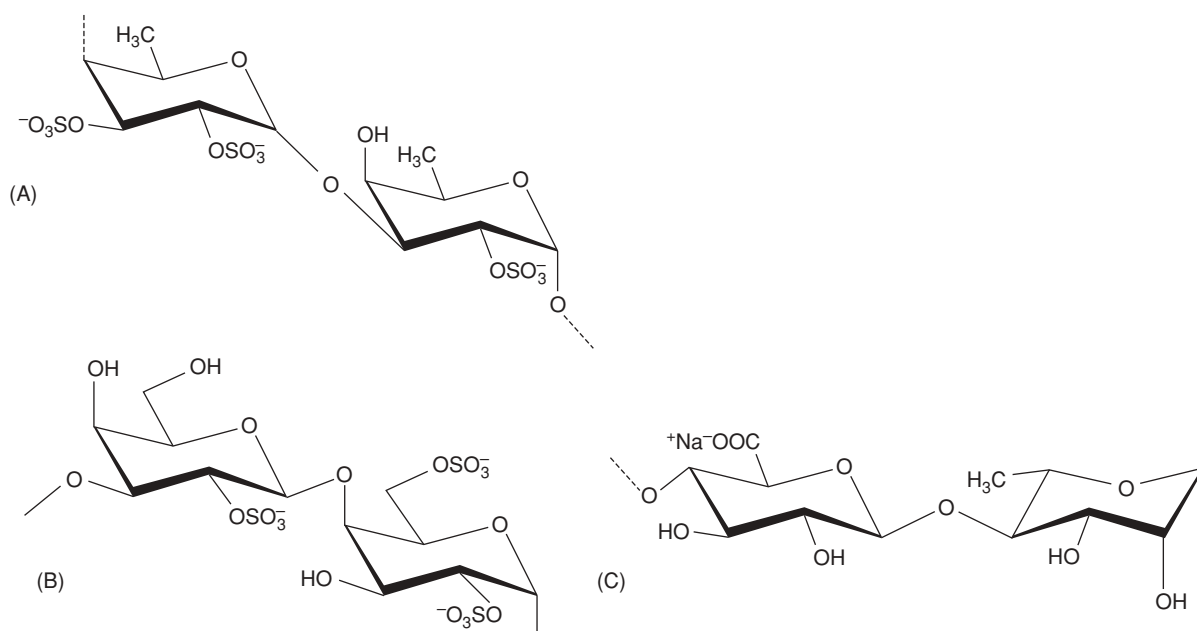
from brown seaweeds such as *Ecklonia cava*, *E. kurome*, *Eisenia bicyclis*, and *Hizikia fusiformis* are responsible for antioxidant activities and shown protective effects against hydrogen peroxide-induced cell damage (Kang *et al.*, 2005, 2006). In addition, eckol, phlorofucofuroeckol A, dieckol, and 8,8'-bieckol have shown a potent inhibition of phospholipid peroxidation at the concentration of 1  $\mu$ M in a liposome system (Shibata *et al.*, 2008). Further, these phlorotannins have shown significant radical scavenging activities against superoxide and DPPH radicals compare to those of ascorbic acid and  $\alpha$ -tocopherol. Hence, phlorotannins can be used as potential antioxidants in the food industry.

### 23.2.2 Sulfated polysaccharides

Edible marine algae have attracted a special interest as good sources of nutrients and one particular interesting feature is their richness in sulfated polysaccharides (SPs), the uses of which span from food, cosmetic and pharmaceutical industries to microbiology and biotechnology (Ren, 1997). These chemically anionic SPs polymers are widespread not only in marine algae but also occur in animals such as mammals and invertebrates (Mourao and Pereira, 1999; Mourao, 2007). Marine algae are the most important source of non-animal SPs and the chemical structure of these polymers varies according to the algal species. The amount of



**Figure 23.1** Some antioxidative phlorotannins derived from marine algae: (A) phloroglucinol, (B) eckol, and (C) dieckol.



**Figure 23.2** Antioxidative sulfated polysaccharides derived from marine algae: (A) fucoidan, (B) carrageenan, and (C) ulvan.

SPs present is found to be different according to the three major divisions of marine algae, Chlorophyceae (green algae), Rhodophyceae (red algae), and Phaeophyceae (brown algae). The major SPs found in marine algae (Figure 23.2) include fucoidan and laminarans of brown algae, carrageenan of red algae and ulvan of green algae.

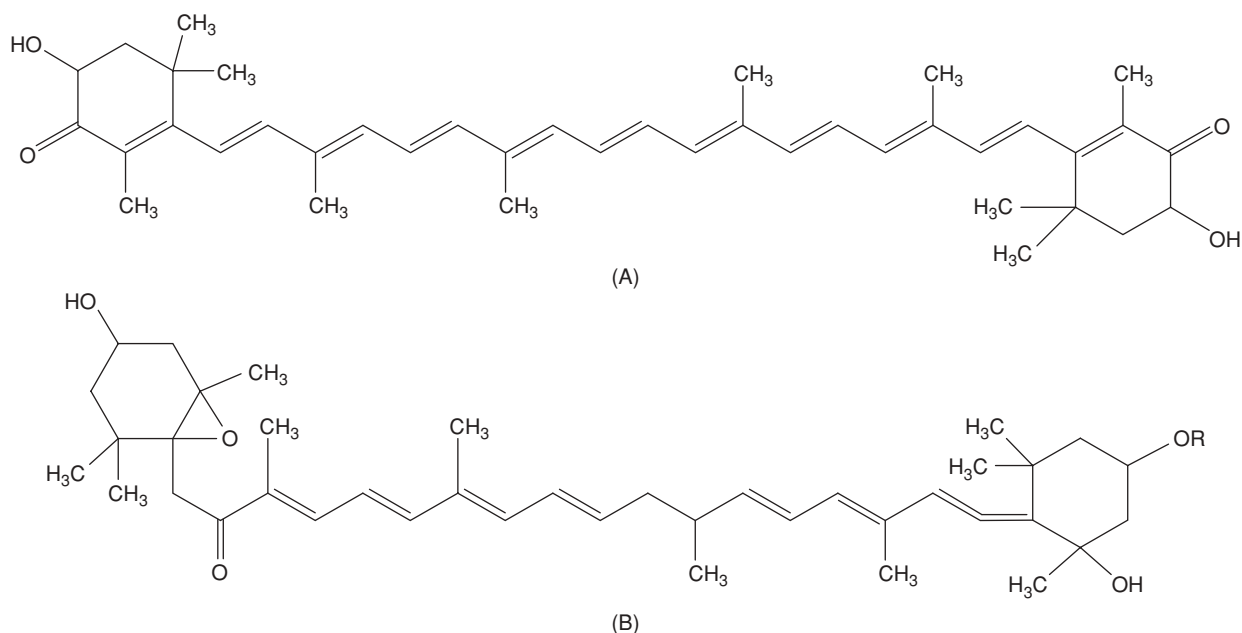
The antioxidant activity of SPs depends on their structural features such as degree of sulfation, molecular weight, type of major sugar, and glycosidic branching (Qi *et al.*, 2005; Zhang *et al.*, 2003). For example, low molecular weight SPs have shown potent antioxidant activity than that of high molecular weight SPs (Sun *et al.*, 2009). The rationale for this is low molecular weight SPs may incorporate into the cells more efficiently and donate proton effectively compared to high molecular weight SPs. Furthermore, SPs from marine algae are known to be important free-radical scavengers and antioxidants for the prevention of oxidative damage which is an important contributor in carcinogenesis. Seaweed-derived SPs prove to be one of the useful candidates in the search of effective, non-toxic substances with potential antioxidant activity. Moreover, SPs are by-products in the preparation of alginates from edible brown seaweeds and could be used as a rich source of natural antioxidants with potential application in the food industry.

### 23.2.3 Carotenoids

Carotenoids are a family of pigmented compounds that are synthesized by plants, algae, fungi and microorganisms, but

not animals. These carotenoid pigments are thought to be responsible for the beneficial properties of preventing human diseases including cardiovascular diseases, cancer, and other chronic diseases. The beneficial effects of carotenoids are thought to be due to their role as antioxidants. The antioxidant actions of carotenoids are based on their singlet oxygen quenching properties and their ability to trap free radicals, which mainly depends on the number of conjugated double bonds of the molecule and carotenoid end groups or the nature of constituents in carotenoids containing cyclic end groups (Stahl and Sies, 1996). In this regard, fucoxanthin and astaxanthin were known to be major ingredients of marine algal carotenoids (Figure 23.3), which show strong antioxidant activity due to quenching of singlet oxygen and scavenging of free radicals.

Furthermore, the cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* has been investigated against  $H_2O_2$ -induced cell damage (Heo *et al.*, 2008). The results obtained in their study showed that fucoxanthin effectively inhibited intracellular ROS formation, DNA damage, and apoptosis induced by  $H_2O_2$ . Noticeably, fucoxanthin also strongly exhibited the cell viability against  $H_2O_2$  induced oxidative damage. Moreover, the protective effect of fucoxanthin was investigated against UV-B induced cell injury in human fibroblast and showed significantly decreased intracellular ROS formation and increased cell survival rate at dose-dependent manner (Heo and Jeon, 2009). In addition to fucoxanthin, astaxanthin has also known for its versatile antioxidant property. The higher antioxidant



**Figure 23.3** Antioxidative carotenoids derived from marine algae: (A) fucoxanthin, and (B) astaxanthin.

activity of astaxanthin than other carotenoids is related to the presence of hydroxyl and keto endings on each ionone ring in its structure. Furthermore, astaxanthin is effective as  $\alpha$ -tocopherol in inhibiting free radical-initiated lipid peroxidation in rat liver microsomes (Palozza and Krinsky, 1992), and is 100 times higher than  $\alpha$ -tocopherol in protecting rat mitochondria against  $\text{Fe}^{2+}$ -catalyzed lipid peroxidation *in vivo* and *in vitro*. Overall, these results indicate that fucoxanthin and astaxanthin can be used as a source of natural antioxidants and ingredients in functional food related to the prevention and control oxidative stress.

### 23.3 Conclusion

Marine algae-derived functional ingredients play a vital role in human health and nutrition. Furthermore, increasing consumer knowledge of the link between diet and health has raised the awareness and demand for novel functional food ingredients and nutraceuticals. Hence, radical scavenging compounds such as phlorotannins, SPs and carotenoid pigments including fucoxanthin and astaxanthin from marine algae and their by-products can be used indirectly as functional ingredients to reduce most of chronic diseases in human body. Collectively, the wide range of biological activities associated with the antioxidative ingredients derived from marine algae has potential to expand its health beneficial value not only in the food industry but also in the pharmaceutical and cosmeceutical industries.

### References

- Barrow, C., and Shahidi, F. (2008) *Marine Nutraceuticals and Functional Foods*. CRC Press, New York, USA.
- Heo, S. J., and Jeon, Y. J. (2009) Protective effect of fucoxanthin isolated from *Sargassum siliquastrum* on UV-B induced cell damage. *J. Photochem. Photobiol. B: Biol.*, **95**, 101–107.
- Heo, S. J., Ko, S. C., Kang, S. M., *et al.* (2008) Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against  $\text{H}_2\text{O}_2$ -induced cell damage. *Eur. Food Res. Technol.*, **228**, 145–151.
- Heo, S. J., Park, P. J., Park, E. J., Kim, S. K., and Jeon, Y. J. (2005) Antioxidant activity of enzymatic extracts from a brown seaweed *Ecklonia cava* by electron spin resonance spectrometry and comet assay. *Eur. Food Res. Technol.*, **221**, 41–47.
- Hettiarachchy, N. S., Glenn, K. C., Gnanasambandan, R., and Johnson, M. G. (1996) Natural antioxidant extract from fenugreek (*Trigonella foenumgraecum*) for ground beef patties. *J. Food Sci.*, **61**, 516–519.
- Kang, H. S., Chung, H. Y., Jung, J. H., Son, B. W., and Choi, J. S. (2003) A new phlorotannin from the brown alga *Ecklonia stolonifera*. *Chem. Pharm. Bull.*, **51**, 1012–1014.
- Kang, H. S., Chung, H. Y., Kim, J. Y., Son, B. W., Jung, H. A., and Choi, J. S. (2004) Inhibitory phlorotannins from the edible brown alga *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation. *Arch. Pharm. Res.*, **27**, 194–198.

- Kang, K. A., Lee, K. H., Chae, S., *et al.* (2005) Eckol isolated from *Ecklonia cava* attenuates oxidative stress induced cell damage in lung fibroblast cells. *FEBS Lett.*, **579**, 6295–6304.
- Kang, K. A., Lee, K. H., Chae, S., *et al.* (2006) Cytoprotective effect of phloroglucinol on oxidative stress induced cell damage via catalase activation. *J. Cell. Biochem.*, **97**, 609–620.
- Li, Y., Qian, Z. J., Ryu, B. M., Lee, S. H., Kim, M. M., and Kim, S. K. (2009) Chemical components and its antioxidant properties in vitro: An edible marine brown alga, *Ecklonia cava*. *Bioorg. Med. Chem.*, **17**, 1963–1973.
- Mourao, P. A. (2007) A carbohydrate-based mechanism of species recognition in sea urchin fertilization. *Brazilian J. Med. Biol. Res.*, **40**, 5–17.
- Mourao, P. A. S., and Pereira, M. S. (1999) Searching for alternatives for to heparin: Sulfated fucans from marine invertebrates. *Trends Cardiovasc. Med.*, **9**, 225–232.
- Palozza, P., and Krinsky, N. (1992) Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. *Arch. Biochem. Biophys.*, **297**, 291–295.
- Park, P. J., Jung, W. K., Nam, K. D., Shahidi, F., and Kim, S. K. (2001) Purification and characterization of antioxidative peptides from protein hydrolysate of lecithin-free egg yolk. *J. Am. Oil Chem. Soc.*, **78**, 651–656.
- Qi, H., Zhang, Q., Zhao, T., *et al.* (2005) Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) in vitro. *Int. J. Biol. Macromol.*, **37**, 195–199.
- Ren, D. (1997) Biotechnology and the red seaweed polysaccharide industry: Status, needs and prospects. *Trends Biotechnol.*, **15**, 9–14.
- Shibata, T., Ishimaru, K., Kawaguchi, S., Yoshikawa, H., and Hama, Y. (2008) Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *J. Appl. Phycol.*, **20**, 705–711.
- Singh, I. P., and Bharate, S. B. (2006) Phloroglucinol compounds of natural origin. *Nat. Prod. Rep.*, **23**, 558–591.
- Stahl, W., and Sies, H. (1996) Lycopene: a biologically important carotenoid for humans? *Arch. Biochem. Biophys.*, **336**, 1–9.
- Sun, L., Wang, C., Shi, Q., and Ma, C. (2009) Preparation of different molecular weight polysaccharides from *Porphyridium cruentum* and their antioxidant activities. *Int. J. Biol. Macromol.*, **45**, 42–47.
- Wijesekara, I., Pangestuti, R., and Kim, S. K. (2011) Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydr. Polym.*, **84**, 14–21.
- Wijesekara, I., Yoon, N. Y., and Kim, S. K. (2010) Phlorotannins from *Ecklonia cava* (Phaeophyceae): Biological activities and potential health benefits. *Biofactors*, **36**, 408–414.
- Zhang, Q., Li, N., Zhou, G., Lu, X., Xu, Z., and Li, Z. (2003) In vivo antioxidant activity of polysaccharide fraction from *Porphyra haitanensis* (Rhodophyta) in aging mice. *Pharmacol. Res.*, **48**, 151–155.

# 24

## Algal Carotenoids as Potent Antioxidants

Kazuo Miyashita<sup>1</sup>, M. Airanthi K. Widjaja-Adhi<sup>1</sup>, Masayuki Abe<sup>1,2</sup>, and Masashi Hosokawa<sup>1</sup>

<sup>1</sup>Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan

<sup>2</sup>Kaneka Co., Nakanoshima, Kita-ku, Osaka, Japan

### 24.1 Introduction

The unique and phenomenal biodiversity of the marine environment provides a large pool of novel and bioactive molecules, “marine nutraceuticals”. Algae are one of the potential marine nutraceutical sources. Marine algae can be divided into the two groups of macroalgae (seaweed) and microalgae. Seaweeds are photosynthesizing plants that form the basic biomass in the intertidal zone. As seaweeds lack many distinct organs, as found in terrestrial plants, the whole plants are available for biomass resource. There are about 6000 species of seaweeds divided into three main classes: green (chlorophytes), red (rhodophytes), and brown (phaeophytes). Seaweeds have been used since ancient times as food, fodder fertilizer, and as sources of medicinal drugs. Today seaweeds are used as raw material for industrial production of agar, carrageenan, and alginates, while they continue to be widely consumed as food in Asian countries. They are nutritionally valuable, in both fresh as well as dried forms, as ingredients in a wide variety of prepared foods.

Microalgae have an advantage over many other organisms in that they can be appropriately grown for the production of desirable bioactive compounds such as docosahexaenoic acid (DHA). Extensive scientific research in the recent past has documented the numerous health benefits

of omega-3 polyunsaturated fatty acids (PUFA) from marine origin (Lands, 2005; Li *et al.*, 2003; Narayan *et al.*, 2006; Shahidi and Miraliakbari, 2006; Sinclair *et al.*, 2005). Considerable quantities of PUFA can be obtained by culturing microalgae from the classes of dinoflagellates and thraustochytrids, and subjecting the cultures to extraction with an organic solvent and subsequent purification (Kyle, 1996). *Cryptocodinium cohnii* is a unique heterotrophic marine dinoflagellate, in that DHA is almost exclusively the only PUFA present in its lipid. *Schizochytrium* spp. is also a heterotrophic microalga belonging to the order Thrausochytriales with the phylum Heterokonta, which can yield about 40% of DHA from its total fatty acid production.

Of late, algae have been explored for some of antioxidants such as polyphenols and carotenoids. In comparison with their terrestrial counterparts, plants, algae are potentially good sources of antioxidants and have an advantage over many other organisms in that they can appropriately produce a large amount of desirable and specific bioactive compounds. Carotenoids in general, especially those of terrestrial origin, have been thoroughly reviewed with respect to their occurrence, biological functions and possible health benefits; however, there has been relatively little information on those from marine origin. Hence, an effort has been done to review the published literature with respect to occurrence of algal carotenoids.

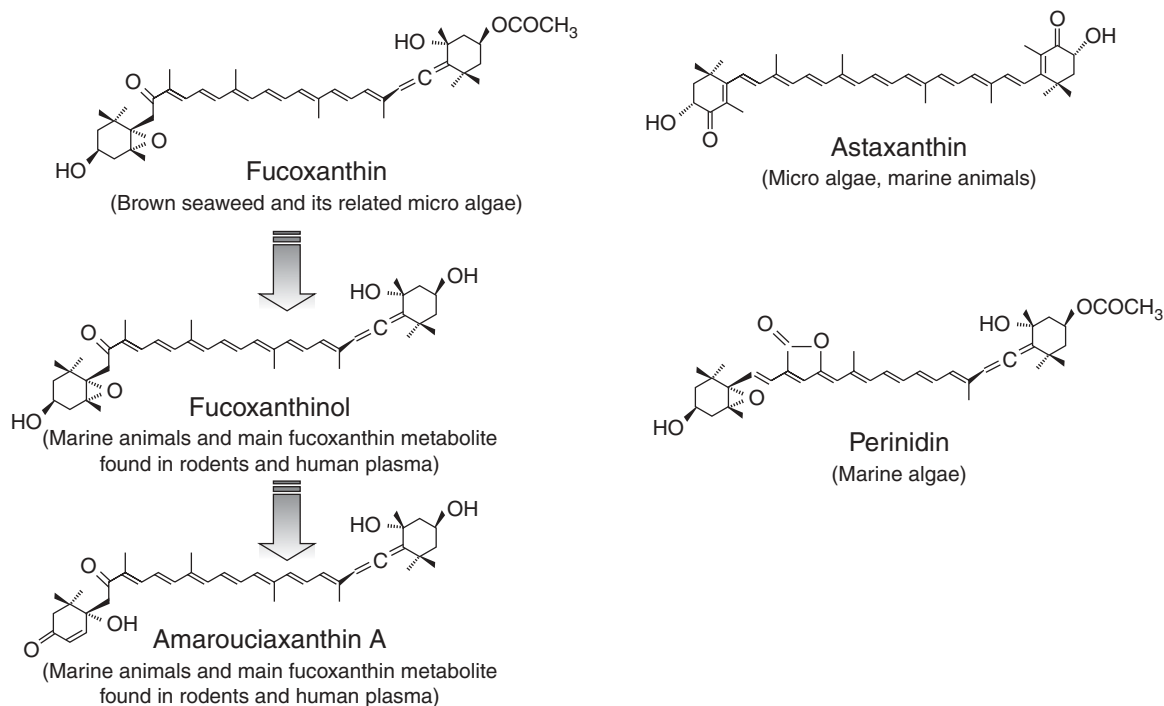
## 24.2 Algal carotenoids

Carotenoids belong to the tetraterpene family found principally in plants, algae, photosynthetic bacteria, and animals. They are among the most important pigments occurring in nature, which are responsible for various colors. Animals, including humans, are incapable of synthesizing carotenoids and many are colored by carotenoids derived from their diets. The distribution of carotenoids in animal sources is primarily the result of specific dietary habits, absorption, and metabolic transformation. The number of naturally occurring carotenoids reported continues to rise and has now reached more than 700. In the marine environments carotenoids are also widely present in both algae and animals. Marine photosynthetic organisms such as micro-/macroalgae synthesize  $\beta$ -carotene *de novo* from isoprenyl diphosphate via phytoene and lycopene, and then alter it to produce other derivatives. Marine animals do not synthesize carotenoids *de novo* and those found in the animal bodies are either result of the direct accumulation of carotenoids from food or are partly modified through metabolic reactions. The origin of most of these carotenoids is from micro-/macroalgae.

Palermo *et al.* (1991) reported the presence of  $\beta$ -carotene, zeaxanthin, fucoxanthin, and fucoxanthinol in the red seaweeds. Antheraxanthin, lutein, and violax-

anthin are also identified as major carotenoids of red seaweeds (Marquardt and Hanelt, 2004; Schubert *et al.*, 2006). In brown seaweeds fucoxanthin is the dominant carotenoid (Dembisky and Maoka, 2007). Fucoxanthin is the most abundant carotenoid contributing more than 10% of the estimated total production of carotenoids in nature (Matsuno, 2001). Fucoxanthin has a unique structure including an unusual allenic bond and 5,6-monoepoxide in its molecule (Figure 24.1). Of approximately 700 naturally occurring carotenoids, about 40 carotenoids contain the allenic bond. The principal allenic carotenoids are fucoxanthin from brown seaweeds, perinidin from microalgae (Figure 24.1), neoxanthin in higher plants, and fucoxanthin metabolites, fucoxanthinol and amarouciaxanthin A (Figure 24.1). Among these allenic carotenoids much attention has been paid to fucoxanthin due to its novel functionalities based on the specific molecular mechanisms (Miyashita, 2009; Miyashita *et al.*, 2010). This unique biofunctional carotenoid is known to be specific to its occurrence in brown algae such as *Hizikia fusiforme*, *Laminaria japonica* and *Undaria pinnatifida* (Mori *et al.*, 2004; Kanazawa *et al.*, 2008). In addition, fucoxanthin is one of the major carotenoids in several diatoms (Dembitsky and Maoka, 2007; Lohr and Wilhelm, 2001).

Microalgae are a major natural source of many kinds of bioactive compounds, including carotenoids. Yellow,



**Figure 24.1** Structures of major algal carotenoids and metabolites of fucoxanthin.

orange, and red carotenoids have an industrial use in food products and cosmetics as vitamin supplements and health food products and as feed additives for poultry, livestock, fish, and crustaceans. The biotechnology of microalgae has gained considerable progress and relevance in recent decades, with carotenoid production representing one of its most successful domains. The halophilic green biflagellate microalga, *Dunaliella salina* has since long been recognized as an efficient biological source of  $\beta$ -carotene (Ben-Amotz and Avron, 1990).  $\beta$ -Carotene accumulation in the oil globules is found in the interthylakoid spaces of the chloroplast of this microalga and it depends on high salinity, a stress temperature, high light intensity, and nitrogen limitation. Under these conditions, up to 12% of the algal dry weight is  $\beta$ -carotene (Del Campo *et al.*, 2007).

Lutein is present in dark, leafy green vegetables, such as spinach and kale, as well as in corn, egg yolk, and some other foods with yellow color. Lutein has been studied widely and proven to show diverse beneficial effects on human health, particularly on optimizing eye health. A major source of lutein for the market is represented by the petals from marigold flowers (Piccaglia *et al.*, 1998). More than 95% of the lutein in these plant sources is esterified form. Thus, the conventional plant pigment workup process comprises chemical saponification. Microalgae such as *Muriellopsis* sp. can produce lutein, where lutein usually appears in the free non-esterified form. Some other microalgae (*Scenedesmus almeriensis*, *Chlorella protothecoides*, and *Chlorella zofingiensis*) also accumulate lutein (Del Campo *et al.*, 2000, 2004).

Astaxanthin (Figure 24.1) is another carotenoid that is produced on an industrial scale using microalgae (Ernst, 2002). Only two sources of microbial origin compete with synthetic astaxanthin, which presently dominates the market: the heterobasidious yeast *Phaffia rhodozyma* and the microalga, *Hematococcus pluvialis*, have been widely studied for more than two decades from the viewpoint of astaxanthin production. Unicellular micro-algae, *H. pluvialis*, produce configurational isomers of astaxanthin (Kobayashi *et al.*, 1997). The main astaxanthin isomer (3S,3S') produced by the this microalga is identical to that present in wild salmon.

## 24.3 Carotenoids as dietary antioxidants

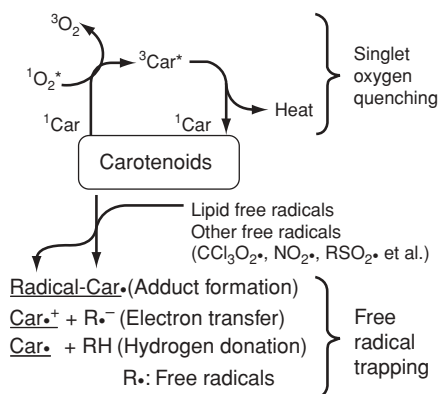
The best known biological function of carotenoids is their established role as provitamin A. Carotenoids such as  $\alpha$ - and  $\beta$ -carotene and  $\beta$ -cryptoxanthin can be converted to retinoic acid. Retinoic acid in their all-*trans* or 9-*cis* configuration is highly-potent activities of the retinoic acid

receptors (RAR) and the retinoid-X receptors (RXR). By activation of these nuclear receptors retinoic acids can influence the transcription of various retinoid-response genes (De Luca, 1991). In addition, dietary carotenoids, including non-provitamin A carotenoids, are considered to play a role in the prevention of common chronic diseases such as cardiovascular disease, age related macular degeneration, and cancers (Cooper *et al.*, 1999a, b). Further, epidemiological studies established a positive correlation between carotenoid consumption and a reduced risk of cancer (Willett, 2001; Riboli and Norat, 2003).

Reactive oxygen species (ROS) and oxidative damage to biomolecules have been widely postulated to be involved in the cause and progression of several chronic diseases, including cancer and cardiovascular diseases. Carotenoids have been implicated as important dietary nutrients having antioxidant potential, being involved in the scavenging of ROS, singlet oxygen and peroxy radicals generated in the process of peroxidation (Edge *et al.*, 1997). The antioxidant properties of carotenoids have been suggested as being the main mechanism by which they afford their beneficial health effects (Giovannucci, 1999; Hadley *et al.*, 2002; Tapiero *et al.*, 2004). Much of the research has focused on the potential role of the carotenoids as dietary antioxidants (Evans and Halliwell 2001; Halliwell 1996).

Free radicals and ROS such as  $\bullet\text{O}_2^-$  (superoxide anion),  $\text{H}_2\text{O}_2$  (hydrogen peroxide),  $\bullet\text{OH}$  (hydroxyl radical),  $^1\text{O}_2$  (singlet oxygen) are produced in the body by the normal aerobic metabolism. These molecules are highly reactive oxidizers of polyunsaturated lipids, proteins, and DNA in biological systems. It is believed that healthy cell function requires a fine balance between ROS and endogenous antioxidants. Any disturbance of this balance in favor of ROS may cause an increase in oxidative stress and initiate subcellular changes that can lead to pathological conditions (Lau *et al.*, 2008; Nakamura and Lipiton, 2009; Paravicini and Touyz, 2008).

Carotenoids serve a protective role by effectively dissipating excess energy, preventing the formation of ROS, and by deactivating  $^1\text{O}_2$  generated during the photosynthetic process (Figure 24.2). The quenching of singlet oxygen by carotenoids has been attributed mainly to physical mechanism, where the excess energy of  $^1\text{O}_2$  is transferred to carotenoid. The carotenoid with added energy is excited to triplet state and upon losing the energy as heat relaxes to singlet state without change in the structure. In addition, dietary carotenoids react with a wide range of free radicals such as lipid free radicals,  $\text{CCl}_3\text{O}_2\bullet$ ,  $\text{RSO}_2\bullet$ ,  $\text{NO}_2\bullet$  and various arylperoxy radicals via adduct formation of carotenoids and free radicals, electron transfer producing the radical cation of the carotenoid, and donation of hydrogen radical from carotenoids (Figure 24.2).



**Figure 24.2** Antioxidant activity of carotenoids.

The singlet oxygen quenching rates of carotenoids is characterized by the rate constant  $k_q$ , with larger the  $k_q$  values the faster being the quenching reaction. The efficacy of carotenoids for the physical quenching is related to the number of conjugated double bonds present in the molecule, which determines their lowest triplet energy level. As the number of conjugated double bonds increases the energies of the excited states decrease and this is reflected in the dependence of the singlet oxygen quenching rate constant on carotenoid chain length (Stahl and Sies, 2003). Thus, the ability to quench  $^1\text{O}_2$  increases with increasing number of conjugated double bonds (Edge *et al.*, 1997). Determination of the quenching rate constant ( $k_q/\text{M}^{-1} \text{s}^{-1}$ ) of carotenoids showed that the efficacy of deactivation of singlet oxygen by carotenoids basically increased with the number of conjugated double bonds, although the quenching ability varied with chain structure, functional groups, and solvent viscosity (Conn *et al.*, 1991; Hirayama *et al.*, 1994; Mascio *et al.*, 1991). Mascio *et al.* (1991) reported that the quenching rate constant ( $k_q/\text{M}^{-1} \text{s}^{-1}$ ) of carotenoids in ethanol/chloroform/water were  $3.1 \times 10^{10}$  for lycopene,  $2.4 \times 10^{10}$  for astaxanthin, and  $1.4 \times 10^{10}$  for  $\beta$ -carotene.

Fukuzawa *et al.* (1998) observed that  $^1\text{O}_2$  quenching of carotenoids is 40–80 times higher than that of  $\alpha$ -tocopherol in ethanol, but only six times higher in liposomes. On the other hand, Cantrell *et al.* (2003) reported that lycopene and  $\beta$ -carotene showed the fastest  $^1\text{O}_2$  quenching rate constants ( $2.3\text{--}2.5 \times 10^{10}/\text{M}^{-1} \text{s}^{-1}$ ) with lutein the least efficient ( $1.1 \times 10^{10}/\text{M}^{-1} \text{s}^{-1}$ ), when the ability of six kinds of dietary carotenoids to quench  $^1\text{O}_2$  was compared in a model membrane system (unilamellar liposomes). Thus, the  $^1\text{O}_2$  quenching rates of carotenoids in biological systems will depend on the factors such as concentration of carotenoids in membranes, membrane localization of active groups, solubility of generation site of singlet oxygen in membranes and the mobility of carotenoids in membranes.

## 24.4 Brown seaweeds as rich source of antioxidants

Algal extracts have been reported to show antioxidant activity (Athukorala *et al.*, 2006; Cho *et al.* 2007; Fayaz *et al.*, 2005; Ganesan *et al.*, 2008; Takamatsu *et al.*, 2003; Yuan and Walsh 2006). Antioxidant substances found in algae are chemicals that are structurally related to terrestrial plant-derived antioxidants. The main antioxidant activity of algal extracts arises from tocopherols (Miyashita and Takagi, 1987), carotenoids (Hosokawa *et al.*, 2009) and polyphenols (Parys *et al.*, 2007). Tocopherols and polyphenols are free radical acceptors, thus acting as chain-breaking antioxidants, while carotenoids can act as antioxidants by quenching singlet oxygen and/or trapping free radicals.

In addition, tetraprenyltoluquinols (Seo *et al.*, 2006), fucosterol (Lee *et al.*, 2003), and sulfated polysaccharides (Rupérez *et al.*, 2002; Zhao *et al.*, 2004, 2008; Zhou *et al.*, 2008) were also identified as antioxidant compounds from brown algae. Besides some unknown compounds present, marine algae may also act as active constituents in inhibiting lipid oxidation.

Various extraction methods have been used to release these identified and unidentified antioxidant substances from marine algae. Solvent extraction methods employ different solvent systems depending on the solubility of the desired bioactive materials in certain solvents. The lipophilic extracts from 16 species of seaweeds showed potential antioxidant activities proportional to the content of unsaturated fatty acids (Huang and Wang, 2004). Enzyme assisted extraction has been proposed to prepare potential natural water soluble antioxidants from marine algae. Enzymes such as carbohydrases and proteases are used to macerate the tissues of the seaweeds, break down the cell walls to release interior compounds (Heo *et al.*, 2005a,b).

Much concern about algal antioxidants has been given to polyphenols, especially, phlorotannins, which are the largest group of polyphenols in brown seaweeds. Antioxidant activity of seaweed polyphenols have been reported by many researchers (Kang *et al.*, 2004; Nakai *et al.*, 2006; Shibata *et al.*, 2008; Zou *et al.*, 2008). Nakai *et al.* (2006) screened the antioxidant activities of 50% ethanol extracts of 25 common Japanese seaweeds. The highest radical scavenging effect was obtained for *Sargassum ringgoldianum*. The chemical structure of the main active fraction contained kinds of phlorotannins. The partially purified phlorotannin-rich fraction exhibited significant scavenging potencies on superoxide anion radicals, which were around five times higher than that of catechin. Shibata *et al.* (2008) identified several phlorotannins including eckol, phlorofucofuroeckol A, dieckol, and 8,8'-bieckol from the Japanese laminariaceous brown seaweeds (*Ecklonia cava*, *Eisenia*

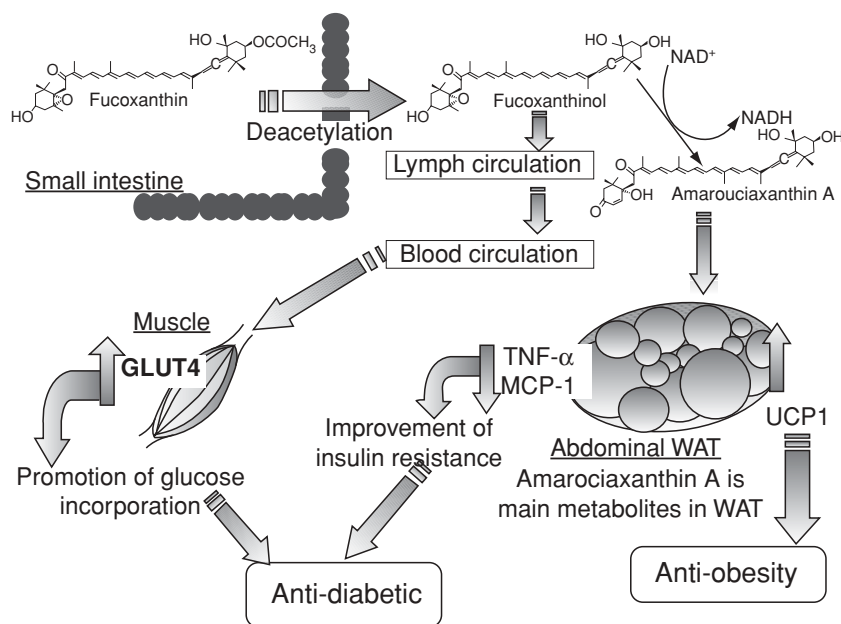
*bicyclis*, and *Ecklonia kurome*). With the exception of eckol, all the phlorotannins exhibited extraordinary superoxide anion radical scavenging abilities, which was around 2–10 times more effective than ascorbic acid and  $\alpha$ -tocopherol.

Studies have revealed that algal polyphenols possess many biological activities: including anti-inflammatory (Shin *et al.*, 2006), hepatoprotective (Kim *et al.*, 2005; Zhao *et al.*, 2004), antitumor (Kim *et al.*, 2006; Yuan and Walsh, 2006), antihypertensive (Jung *et al.*, 2006), and human immunodeficiency virus-1 reverse transcriptase (Ahn *et al.*, 2004) activities as well as antidiabetic activity on the basis of inhibition of  $\alpha$ -glucosidase (Iwai, 2008). Their multiple physiological activities, thus, may offer many advantages for potential applications in nutraceutical, pharmaceutical, and cosmetic industry. However, the evaluation of the effects exerted by algal polyphenols gives several problems when moving from simple experimental systems to the complexity of human body. The major problem is their bioavailability and the difficulties in unraveling the complex mechanisms of absorption and metabolism. Up to date most of the studies on the biological activities of algal polyphenols have been done *in vitro* systems using cultivated cell lines. A basic research will be needed to define the absorption rate of algal polyphenols, identification of their metabolites, and the real molecular events which underline the biological effects of algal polyphenols.

On the other hand, absorption mechanism of brown seaweed carotenoid, fucoxanthin, has been well documented (Asai *et al.*, 2004, 2008; Hashimoto *et al.*, 2009;

Matsumoto *et al.*, 2010; Sangeetha *et al.*, 2010; Tsukui *et al.*, 2009). Fucoxanthin converts to fucoxanthinol, and then, some of the fucoxanthinol further converts to amarouci-axanthin A (Figure 24.3). Moreover, strong antioxidant activity of fucoxanthin has been reported (Hosokawa *et al.*, 2009; Murakami *et al.*, 2000; Nishino, 1998; Nomura *et al.*, 1997; Sachindra *et al.*, 2007; Yan *et al.*, 1999). In brown seaweeds fucoxanthin is the dominant carotenoid (Dembisky and Maoka, 2007) and it showed 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS) and hydroxyl radical scavenging activities as measured by chemiluminescence technique (Sachindra *et al.*, 2007). The scavenging activity by fucoxanthin was 13.5 times higher than that of  $\alpha$ -tocopherol. Similar trend was observed when the hydroxyl radical scavenging was assessed by electron spin resonance (ESR). ESR analysis of superoxide radical scavenging activity also showed the superiority of fucoxanthin. Thus, radical scavenging activity of brown seaweed extracts would be strongly correlated with fucoxanthin content as well as amount of phenolics.

Comparative study on methanol extracts from five kinds of brown seaweeds has shown the higher antioxidant activities, DPPH, peroxy and ABTS radical scavenging activities of *Sargassum horneri* and *Cystoseira hakodaten-sis* (Widjaja-Adhi *et al.*, 2011). These activities were attributed to higher contents of both total phenolics (202.4 and 377.0 mg/100 g dry sample, respectively) and fucoxanthin (109.3 and 152.9 mg/100 g dry sample, respectively).



**Figure 24.3** Antiobesity and antidiabetic effect of fucoxanthin.

The higher peroxy radical scavenging activities of methanol extracts from *S. horneri* and *C. hakodatensis* than those of the other three brown seaweeds could be explained by their higher levels of total phenolics and fucoxanthin. In the soybean phosphatidylcholine (PC) liposome system, there was little difference in the antioxidant activity of phenolics from *C. hakodatensis* and purified fucoxanthin (Widjaja-Adhi *et al.*, 2011). However, when both antioxidants were combined, the induction period of the oxidation was much longer than that calculated from the single data of the phenolics or fucoxanthin. This result is in accordance with that by Milde *et al.* (2007), which showed the synergistic antioxidant effect of combination of phenolic compound (rutin) and carotenoids (lutein or lycopene) on low-density lipoprotein (LDL) oxidation. They demonstrated that the synergistic effect is most likely due to different allocation of the antioxidant in the LDL-molecule and to different mechanisms of antioxidant action. In the liposome, fucoxanthins are oriented parallel to the hydrocarbon chains of PC in liposomes and this orientation is favorable for the carotenoid to trap the free radical attack at the PC bilayer surface (Matshushita *et al.*, 2000), while most of the phenolics will be located in the water layer and scavenge the AAPH radicals in this phase. This different location would be due to the synergistic effect of brown seaweed phenolics and fucoxanthin.

## 24.5 Antioxidant activity of algal carotenoids

Representative algal carotenoids, astaxanthin and fucoxanthin, have been reported to be effective antioxidants. Astaxanthin has been found to be more effective than  $\beta$ -carotene in preventing fatty acid preoxidation in chemical solutions (Terao, 1989) and delaying lipid peroxidation in membrane model (Lim *et al.*, 1992). Goto *et al.* (2001) demonstrated that the higher antioxidant activity of astaxanthin compared to  $\beta$ -carotene is due to trapping of radicals at the surface and inside the phospholipid membrane and the unique structure of the terminal ring moiety. The antioxidant activity of astaxanthin and astaxanthin- $\beta$ -glucoside in liposomes was also reported to be higher than those of  $\beta$ -carotene and zeaxanthin (Matsushita *et al.*, 2000). Further studies have confirmed that astaxanthin is a better agent to destroy free radicals than other carotenoids (Lawlor and O'Brien, 1995).

ERS analysis showed the quenching ability of fucoxanthin against both organic radicals DPPH and 12-doxylstercic acid (12DS) (Nishino, 1998). Yan *et al.* (1999) demonstrated the strong DPPH radical scavenging activity of fucoxanthin. The structure of fucoxanthin will affect its antioxidant activity. The ability of carotenoid to quench

$^1\text{O}_2$  increase with increasing number of conjugated double bonds (Conn *et al.*, 1991), whereas antioxidant activity of carotenoids increases with the presence of functional group in terminal rings as seen in astaxanthin and fucoxanthin (Miller *et al.*, 1996). Murakami *et al.* (2000) screened 19 natural carotenoids for their structure–function relationship with respect to radical scavenging activity using human promyelocytic HL-60 cells. They found that the presence of an allenic bond, as seen in fucocoxanthin, is an increasing factor for inhibition of superoxide and NO generation, while the presence of 4-oxo- $\beta$ -end group in the structures of astaxanthin and canthaxanthin enhances NO generation.

It has been recognized that oxidative damage plays a central role in the development of degenerative diseases, including inflammatory diseases, cardiovascular disease, and cancer. Antioxidant activity of astaxanthin has been reported to be several fold higher than those of other carotenoids and tocopherols (Olaizola, 2008); thus, this powerful antioxidant activity may be related to health beneficial effects of astaxanthin such as photo-protection of eye and skin, anti-inflammatory properties, enhancement of heart health, and cancer prevention.

There have been several studies on the relationship between the antioxidant activity of astaxanthin and the prevention of cardiovascular disease (CVD) (Fassett and Coombes, 2009; Fredric *et al.*, 2008; Higuera-Ciapara, 2006; Hussein *et al.*, 2006; Riccioni, 2009). CVD is one of the leading causes of death in many countries. Atherosclerosis is a main condition inducing the coronary arteries. In the development of atherosclerosis gradual uptake of oxidized LDL by the endothelium occurs and results in an inflammatory response leading to deposition of plaques in the arterial walls. Oxidized LDL also stimulates endothelial cells to produce chemokines and other factors that have direct chemotactic activity for monocytes to adhere to the endothelium. In addition, oxidized LDL is preferentially taken up by macrophage cells via scavenger receptors, and they consequently become loaded with lipids and convert into “foam cells”. These foam cells tend to accumulate in fatty streaks, by which the inside diameter of the vein reduced. Thus, blood flow is restricted, which can aggravate or produce hypertension and eventually cause irreparable damage to the heart. Prevention of atherosclerosis by astaxanthin intake can be explained by their protective effects on LDL and vein endothelial cells against oxidative injury and dysfunction.

Astaxanthin has undergone investigation in a large number of experimental studies related to the reduction of disease risk. Human studies of astaxanthin have been performed in healthy volunteers to assess dosing (Iwamoto *et al.*, 2000), bioavailability (Rufer *et al.*, 2008; Coral-Hinostroza *et al.*, 2004), safety (Spiller and Dewell, 2003),

and oxidative stress and inflammation (Iwamoto *et al.*, 2000; Karppe *et al.*, 2007). Though no studies have yet been published specifically assessing cardiovascular parameters in humans, antioxidant activity of astaxanthin has been considered to be related to its protection against cardiovascular diseases (Fassett and Coombes, 2009; Fredric *et al.*, 2008; Graziano, 2009; Hussein *et al.*, 2006).

## 24.6 Antiobesity and antidiabetic effect of fucoxanthin

In algae and plants, carotenoids play multiple and essential roles in photosynthesis. They contribute to light harvesting, maintain structure and function of photosynthetic complexes, quench chlorophyll triplet states, scavenge ROS, and dissipate excess energy. The demonstrated antioxidant activity of carotenoids is dependent on the protection against oxidative stress in many organisms and situations of algae and plants. The antioxidant properties of carotenoids have also been suggested as being the main mechanism by which they afford their beneficial effects on human health (Astley *et al.*, 2004; Evans and Halliwell, 2001; Giovannucci, 1999; Hadley *et al.*, 2002; Halliwell, 1996; Krinsky and Johnson, 2005; Miller *et al.*, 1996; Nomura *et al.*, 1997; Seifried *et al.*, 2007; Tapiero *et al.*, 2004).

There is little doubt that, under the right conditions, the antioxidant activity of carotenoids can protect cells, tissues, and other structures such as lipoproteins against oxidative damage. On the other hand, we can understand that not all of the biological activities ascribed to carotenoids must be necessarily linked to their ability to scavenge free radicals and ROS. Other mechanisms of action that are independent of the antioxidant activity are likely to be more important. Although there are several reports published on the antioxidant activity of fucoxanthin (Nomura *et al.*, 1997; Nishino, 1998; Yan *et al.*, 1999; Murakami *et al.*, 2000; Sachindra *et al.*, 2007), the main mechanism for the physiological importance of fucoxanthin is its modulation effect on specific gene and protein expression in biological systems. In this case the nutritional activities of fucoxanthin depend on its specific chemical structures.

Much attention has been paid to the antiobesity effect of fucoxanthin, as this effect is based on the induction of a specific protein, uncoupling protein 1 (UCP1), in abdominal white adipose tissues (WAT). By feeding fucoxanthin to the obese model mice and normal mice fed a high-fat diet, body weight gain was significantly reduced compared with that of the control, although there was no difference in the amount of food intake (Hosokawa *et al.*, 2010; Maeda *et al.*, 2005, 2007, 2009). This reduction was consistent with the decrease in the weight of total abdominal WAT. On

the other hand, no effect was found on normal mice fed normal diet (Hosokawa *et al.*, 2010), suggesting that suppressive effect of fucoxanthin on the WAT weight gain is specific for adiposity in the development of obesity in mice. The molecular mechanism for the antiobesity effect of fucoxanthin has been made clear on the basis of the unique and ideal pathway, which results in UCP1 induction in WAT by fucoxanthin metabolites. UCP1 is the inner-membrane mitochondrial protein only found in brown adipose tissue, where the gene expression is increased by cold, adrenergic stimulation,  $\beta_3$ -agonists, retinoids and thyroid hormone (Miyashita, 2007). A great deal of interest has focused on adaptive thermogenesis by UCP1 as a physiological defense against obesity, hyperlipidemia, and diabetes.

Obese model mice, KK- $A^y$  mice, used in the above study not only developed obesity but also hyperleptinemia and hyperinsulinemia along with insulin resistance. Therefore, glucose levels of mice fed the control diet reached levels higher than 400 mg/dl. On the other hand, mice fed the 0.1% and 0.2% purified fucoxanthin diets had significantly lower blood glucose concentrations of around 220 mg/dl and 170 mg/dl, respectively (Maeda *et al.*, 2007). The antidiabetic effects of fucoxanthin were also found in high-fat (HF) diet-induced obesity in mice (Maeda *et al.*, 2009). This antidiabetic effect of fucoxanthin is partly due to the regulation of cytokine secretions from the WAT. WAT plays an important role as an energy storage organ, as well as an endocrine organ producing adipocytokines such as membrane chemoattractant protein-1, tumor necrosis factor- $\alpha$ , interleukin-6, and adiponectin (Flier, 2004). In obesity, dysregulation of the adipocytokine production in WAT is induced, which promotes glucose intolerance, dyslipidemia, and high blood pressure (Friedman, 2003).

Regulatory effect of fucoxanthin on glucose transporter 4 (GLUT4) expression found in muscle of normal mice fed high fat diet (Maeda *et al.*, 2009) is also related to the antidiabetic activity of fucoxanthin. Skeletal muscle accounts for nearly 40% of body mass, and its role in the insulin-induced stimulation of glucose uptake is well documented (Abel *et al.*, 2001; Wellen and Hotamisligil, 2005). GLUT4 translocation is linked to reduced glucose utilization in insulin-resistant muscle, and a significant reduction in GLUT4 protein and mRNA levels in skeletal muscle has been reported in HF diet cases. In addition, transgenic animals with over-expressed or knocked-out GLUT4 have provided insights into the role of GLUT4 in glucose homeostasis. The administration of a fucoxanthin-containing diet promoted the recovery of blood glucose uptake to muscle by regulating GLUT4 mRNA expression (Maeda *et al.*, 2009). In this study, mice were fed HF or normal fat (NF) diets for 10 weeks. Then the NF group or half of HF group continued to receive the NF diet or HF diet for another

5 weeks, respectively, while another HF diet-fed group was administered HF diet containing fucoxanthin for a further 5 weeks. After 15 weeks, GLUT4 mRNA levels in skeletal muscle tissue were markedly lower in the HF group compared to the NF group. However, GLUT4 mRNA levels in the HF group fed fucoxanthin were restored to levels observed in the NF group. Some thiazolidinedione family drugs increase GLUT4 mRNA expression in the muscle tissue of type 2 diabetes (Armoni *et al.*, 2007; Olefsky and Saltiel, 2000), similar to the effect of fucoxanthin found in this study.

## 24.7 Conclusion

Epidemiological studies have established a positive correlation between antioxidant consumption and a reduced risk of common chronic diseases such as cardiovascular disease and cancers. Decrease in the oxidative stress by antioxidants is one of the major mechanisms of their action. In addition, the health beneficial effects of some antioxidants have been made clear on the basis of nutritional and nutrigenomic studies, showing that the mechanisms in which the actions of antioxidants go beyond the modulation of oxidative stress. Dietary guidelines recommended increased consumption of fruits and vegetables to combat the incidence of human diseases such as cancer, cardiovascular disease, and diabetes, as fruits and vegetables are good sources of antioxidant phytochemicals. Algal carotenoids such as astaxanthin and fucoxanthin are also regarded as playing an important role in the prevention of human disease and maintaining good health.

Basic mechanisms for the antioxidant activity of these marine phytochemicals are same as those from their terrestrial counterparts. Antioxidant activity of carotenoids is based on both quenching singlet oxygen and scavenging free radicals. The reactivity of each antioxidant differs in oxidation system; *in vitro*, *ex vivo*, and *in vivo*. Further effort is needed to clarify the relationship between the antioxidant activity in biological systems and its physiological significance.

Biological activities of antioxidants are closely linked to their absorption and metabolism. There are several problems to evaluate the true effects exerted by marine antioxidants. The major problems are the many kinds of antioxidants found in food, their large differences in bioavailability and the difficulties in unraveling the complex mechanisms of absorption and metabolism. Thus, to understand the health beneficial effects of marine antioxidants, it is important to identify and determine quantitatively the antioxidant metabolites and to evaluate the mechanisms with special reference to their regulations on relative gene and protein expressions.

## References

- Abel, E.D., Peroni, O., Kim, J.K., *et al.* (2001) Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature*, **409**, 672–673.
- Ahn, M.-J., Yoon, K.-D., Min, S.-Y., *et al.* (2004) Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga *Ecklonia cava*. *Biol. Pharm. Bull.*, **27**, 544–547.
- Armoni, M., Harel, C. and Karnieli, E. (2007) Transcriptional regulation of the GLUT4 gene: from PPAR- $\gamma$  and FOXO1 to FFA and inflammation. *Trends Endocr. Metabol.*, **18**, 100–107.
- Asai, A., Sugawara, T., Ono, H. and Nagao, A. (2004) Bio-transformation of fucoxanthinol into amarouciaxanthin A in mice and HepG2 cells: formation and cytotoxicity of fucoxanthin metabolites. *Drug Metab. Dispos.*, **32**, 205–211.
- Asai, A., Yonekura, L. and Nagao, A. (2008) Low bioavailability of dietary epoxyxanthophylls in humans. *Br. J. Nutr.*, **100**, 273–277.
- Astley, S.B., Hughes D.A., Wright A.J.A., Elliott, R.M. and Southon, S. (2004) DNA damage and susceptibility to oxidative damage in lymphocytes: effects of carotenoids *in vitro* and *in vivo*. *Br. J. Nutr.*, **91**, 53–61.
- Athukorala, Y., Kim, K.-N. and Jeon, Y.-J. (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food Chem. Toxicol.*, **44**, 1065–1074.
- Ben-Amotz, A. and Avron, M. 1990. The biotechnology of cultivating the halotolerant alga *Dunaliella*. *Trends Biotechnol.* **8**: 121–125.
- Cantrell, A., McGarvey, D.J., Truscott, G.T., Rancan, F. and Böhm, F. (2003) Singlet oxygen quenching by dietary carotenoids in a model membrane environment. *Arch. Biochem. Biophys.*, **412**, 47–54.
- Cho, S.-H., Kang, S.-E., Cho, J.-Y., *et al.* (2007) The antioxidant properties of brown seaweed (*Sargassum siliculosum*) extracts. *J. Med. Food*, **10**, 479–485.
- Conn, P.F., Schalch, W. and Truscott, G.T. (1991) The singlet oxygen and carotenoid interaction. *J. Photochem. Photobiol. B: Biology*, **11**, 41–47.
- Cooper, D.A., Eldridge, A.L. and Peters, J.C. (1999a) Dietary carotenoids and lung cancer : a review of recent research. *Nutr. Rev.*, **57**, 133–145.
- Cooper, D.A., Eldridge, A.L. and Peters, J.C. (1999b) Dietary carotenoids and certain cancers, heart disease and age related macular degeneration: a review of recent research. *Nutr. Rev.*, **57**, 201–214.
- Coral-Hinostroza, G.N., Ytreostoyl, T., Ruyter, B. and Bjerkeng B. (2004) Plasma appearance of unesterified astaxanthin geometrical E/Z and optical R/S isomers in

- men given single doses of a mixture of optical 3 and 3'R/S isomers of astaxanthin fatty acyl diesters. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **139**, 99–110.
- Del Campo, J.A., Moreno, J., Rodríguez, H., Vargas, M.A., Rivas, J. and Guerrero, M.G. (2000) Carotenoid content of chlorophycean microalgae. Factors determining lutein accumulation in *Muriellopsis* sp. (Chlorophyta). *J. Biotechnol.*, **76**, 51–59.
- Del Campo, J.A., Rodríguez, H., Moreno, J., Vargas, M.A., Rivas, J. and Guerrero, M.G. (2004) Accumulation of astaxanthin and lutein in *Chlorella zofingiensis* (Chlorophyta). *Appl. Microbiol. Biotechnol.*, **64**, 848–854.
- Del Campo, J.A., García-González, M. and Guerrero, M.G. (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Appl. Microbiol. Biotechnol.*, **74**, 1163–1174.
- De Luca, L.M. (1991) Retinoids and their receptors in differentiation, embryogenesis, and neoplasia. *FASEB J.*, **5**, 2924–2933.
- Dembitsky, V.M. and Maoka, T. (2007) Allenic and cumulenyl lipids. *Prog. Lipid Res.*, **46**, 328–375.
- Edge R., McGarvey D.J. and Truscott T.G. (1997) The carotenoids as anti-oxidants – a review. *J. Photochem. Photobiol. B: Biology*, **41**, 189–200.
- Ernst, H. 2002. Recent advances in industrial carotenoid synthesis. *Pure Appl. Chem.*, **74**, 2213–2226.
- Evans, P. and Halliwell, B. (2001) Micronutrients: oxidant/antioxidant status. *Br. J. Nutr.*, **85**, S67–S74.
- Fassett, R.G. and Coombes, J.S. (2009) Astaxanthin, oxidative stress, inflammation and cardiovascular disease. *Future Card.*, **5**, 333–342.
- Fayaz, M., Namitha, K.K., Murthy K.N.C., *et al.* (2005) Chemical composition, iron bioavailability, and antioxidant activity of *Kappaphycus alvarezzi* (Doty). *J. Agric. Food Chem.*, **53**, 792–797.
- Flier, J.S. (2004) Obesity wars: molecular progress confronts an expanding epidemic. *Cell*, **116**, 337–350.
- Fredric, J.P., David, G.W. and Charles, L.C. (2008) Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease. *Am. J. Cardiol.*, **101**, 58D–68D.
- Friedman, J.M. (2003) A war on obesity, not the obese. *Science*, **299**, 856–858.
- Fukuzawa, K., Inokami, Y., Tokumura, A., Terao, J. and Suzuki, A. (1998) Rate constants for quenching singlet oxygen and activities for inhibiting lipid peroxidation of carotenoids and  $\alpha$ -tocopherol in liposomes. *Lipids*, **33**, 751–756.
- Ganesan, P., Kumar, C.S. and Bhaskar, N. (2008) Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Biores. Technol.*, **99**, 2717–2723.
- Giovannucci, E. (1999) Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J. Natl Cancer Inst.*, **91**, 317–331.
- Goto, S., Kogure, K., Abe, K., *et al.* (2001) Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin. *Biochim. Biophys. Acta*, **1512**, 251–258.
- Graziano, R. (2009) Carotenoids and cardiovascular disease. *Curr. Atherosclerosis Rep.*, **11**, 434–439.
- Hadley, C.W., Miller, E.C., Schwartz, S.J. and Clinton, S.K. (2002) Tomatoes, lycopene, and prostate cancer: progress and promise. *Exp. Biol. Med.*, **227**, 869–880.
- Halliwell, B. (1996) Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free. Rad. Res.*, **25**, 57–74.
- Hashimoto, T., Ozaki, Y., Taminato, M., *et al.* (2009) The distribution and accumulation of fucoxanthin and its metabolites after oral administration in mice. *Br. J. Nutr.*, **102**, 242–248.
- Heo, S.-J., Park, E.-J., Lee, K.-W. and Jeon, Y.-J. (2005a) Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresource Tech.*, **96**, 1613–1623.
- Heo, S.-J., Park, P.-J., Park, E.-J., Kim, S.-K. and Jeon, Y.-J. (2005b) Antioxidant activity of enzymatic extracts from a brown seaweed *Ecklonia cava* by electron spin resonance spectrometry and comet assay. *Eur. Food Res. Technol.*, **221**, 41–47.
- Higuera-Ciapara, I., Félix-Valenzuela, L. and Goycoulea, F.M. (2006) Astaxanthin: a review of chemistry and applications. *Crit. Rev. Food Sci. Nutr.*, **46**, 185–196.
- Hirayama, O., Nakamura, K., Hamada, S. and Kobayasi, Y. (1994) Singlet oxygen quenching ability of naturally occurring carotenoids. *Lipids*, **29**, 149–150.
- Hosokawa, M., Okada, T., Mikami, N., Konishi, I. and Miyashita, K. (2009) Bio-functions of marine carotenoids. *Food Sci. Biotechnol.*, **18**(1), 1–11.
- Hosokawa, M., Miyashita, T., Nishikawa, S., *et al.* (2010) Fucoxanthin regulates adipocytokine mRNA expression in white adipose tissue of diabetic/obese KK-Ay mice. *Arch. Biochem. Biophys.*, **504**, 17–25.
- Huang, H.-L. and Wang, B.-G. (2004) Antioxidant capacity and lipophilic content of seaweeds collected from the Qingdao coastline. *J. Agric. Food Chem.*, **52**, 4993–4997.
- Hussein, G., Sankawa, U., Goto, H., Matsumoto, K. and Watanabe, H. (2006) Astaxanthin, a carotenoid with potential in human health and nutrition. *J. Nat. Prod.*, **69**, 443–449.
- Iwamoto, T., Hosoda, K. and Hirano, R. (2000) Inhibition of low-density lipoprotein oxidation by astaxanthin. *J. Atheroscler. Thromb.*, **7**, 216–222.

- Iwai, K. (2008) Antidiabetic and antioxidant effects of polyphenols in brown alga *Ecklonia stolonifera* in genetically diabetic KK-Ay mice. *Plant Foods Human Nutr.*, **63**, 163–169.
- Jung, A., Hyun, S.K., Kim, H.R. and Choi, J.S. (2006) Angiotensin-converting enzyme I inhibitory activity of phlorotannins from *Ecklonia stolonifera*. *Fish. Sci.*, **72**, 1292–1299.
- Kanazawa, K., Ozaki, Y., Hashimoto, T., *et al.* (2008) Commercial-scale preparation of biofunctional fucoxanthin from waste parts of brown sea algae *Laminaria japonica*. *Food Sci. Technol. Res.*, **14**, 573–582.
- Kang, H.S., Chung, H.Y., Kim, J.Y., Son, B.W., Jung, H.A. and Choi, J.S. (2004) Inhibitory phlorotannins from the edible brown alga *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation. *Arch. Pharm. Res.*, **27**, 194–198.
- Karppi, J., Rissanen, T.H. and Nyyssonen, K. (2007) Effects of astaxanthin supplementation on lipid peroxidation. *Int. J. Vitam. Nutr. Res.*, **77**, 3–11.
- Kim, M.-M., Ta, Q.V., Mendis, E., *et al.* (2006) Phlorotannins in *Ecklonia cava* extract inhibit matrix metalloproteinase activity. *Life Sci.*, **79**, 1436–1443.
- Kim, Y.C., An, B., Yoon, N.Y., Nam, T.J. and Choi, J.S. (2005) Hepatoprotective constituents of the edible brown alga *Ecklonia stolonifera* on tacrine-induced cytotoxicity in Hep G2 cells. *Arch. Pharm. Res.*, **28**, 1376–1380.
- Kobayashi, M., Kakizono, T., Nishio, N., Nagai, S., Kurimura, Y. and Tsuji, Y. (1997) Antioxidant role of astaxanthin in the green alga *Haematococcus pluvialis*. *Appl. Microbiol. Biotechnol.*, **48**, 351–356.
- Krinsky, N.I. and Johnson, E.J. (2005) Carotenoid actions and their relation to health and disease. *Mol. Aspects Med.*, **26**, 459–516.
- Kyle, D.J. (1996) Production and use of a single cell oil which is highly enriched in docosahexaenoic acid. *Lipid Technol.*, **9**, 107–110.
- Lands, W.E.M. (2005) *Fish, Omega-3 and Human Health*, 2nd edn. AOCS Press, Champaign, IL, pp. 3–160.
- Lau, A.T.Y., Wang, Y. and Chiu, J.-F. (2008) Reactive oxygen species: current knowledge and applications in cancer research and therapeutic. *J. Cell. Biochem.*, **104**, 657–667.
- Lawlor, S.M. and O'Brien, N.M. (1995) Astaxanthin: antioxidant effects in chicken embryo fibroblasts. *Nutr. Res.*, **15**, 1695–1704.
- Lee, S., Lee, Y.S., Jung, S.H., Kang, S.S. and Shin, K.H. (2003) Anti-oxidant activities of fucosterol from the marine algae *Pelvetia siliquosa*. *Arch. Pharm. Res.*, **26**, 719–722.
- Li, D. and Bode, O., Drummond, H. and Sinclair, A.J. (2003) Omega-3 (n-3) fatty acids. In: *Lipids for Functional Foods and Nutraceuticals* (ed. F.D. Gunstone). The Oily Press, Bridgwater, England, pp. 225–262.
- Lim, B.P., Nagao, A., Terao, J., Tanaka, K., Suzuki, T. and Takama, K. (1992) Antioxidant activity of xanthophylls on peroxy radical-mediated phospholipid peroxidation. *Biochim. Biophys. Acta*, **1126**, 178–184.
- Lohr, M. and Wilhelm, C. (2001) Xanthophyll synthesis in diatoms: quantification of putative intermediates and comparison of pigment conversion kinetics with rate constants derived from a model. *Planta*, **212**, 382–391.
- Maeda, H., Hosokawa, M., Sashima, T., Funayama, K. and Miyashita, K. (2005) Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commun.*, **332**, 392–397.
- Maeda, H., Hosokawa, M., Sashima, T. and Miyashita, K. (2007) Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decrease blood glucose in obese/diabetic KK-A<sup>y</sup> mice. *J. Agric. Food Chem.*, **55**, 7701–7706.
- Maeda, H., Hosokawa, M., Sashima, T., Murakami-Funayama, K. and Miyashita, K. (2009) Anti-obesity and anti-diabetic effects of fucoxanthin on diet-induced obesity conditions in a murine model. *Mol. Med. Rep.*, **2**, 897–902.
- Marquardt, J. and Hanelt, D. (2004) Carotenoid composition of *Delesseria lancifolia* and other marine red algae from polar and temperate habitats. *Eur. J. Phycol.*, **39**, 285–292.
- Mascio, P.D., Murhy, M.E. and Sies, H. (1991) Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. *Am. J. Clin. Nutr.*, **53**, 194S–200S.
- Matsumoto, M., Hosokawa, M., Matsukawa, N., *et al.* (2010) Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. *Eur. J. Nutr.*, **49**, 243–249.
- Matsuno, T. (2001) Aquatic animal carotenoids. *Fish. Sci.*, **67**, 771–783.
- Matsushita, Y., Suzuki, R., Nara, E., Yokoyama, A. and Miyashita, K. (2000) Antioxidant activity of polar carotenoids including astaxanthin- $\beta$ -glucoside from marine bacterium on PC liposomes. *Fish. Sci.*, **66**, 980–985.
- Milde, J., Elstner, E.F. and Graßmann, J. (2007) Synergistic effects of phenolics and carotenoids on human low-density lipoprotein oxidation. *Mol. Nutr. Food Res.*, **51**, 956–961.
- Miller, N.J., Sampson, J., Candeias, L.P., Bramley, P.M. and Rice-Evans, C.A. (1996) Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.*, **384**, 240–242.
- Miyashita, K. (2009) The carotenoid fucoxanthin from brown seaweed affects obesity. *Lipid Technol.*, **21**, 186–190.

- Miyashita, K. and Takagi, T. (1987) Tocopherol content of Japanese algae and its seasonal variation. *Agric. Biol. Chem.*, **51**, 3115–3118.
- Miyashita, K., Maeda, H., Okada, T., Abe, M. and Hosokawa, M. (2010) Anti-obesity and anti-diabetic effects of allenic carotenoid, fucoxanthin. *AgroFOOD*, **21**, 24–27.
- Mori, K., Ooi, T., Hiraoka, M., *et al.* (2004) Fucoxanthin and its metabolites in edible brown algae cultivated in deep seawater. *Mar. Drugs*, **2**, 63–72.
- Murakami, A., Nakashima, M., Koshib, T., *et al.* (2000) Modifying effects of carotenoids on superoxide and nitric oxide generation from stimulated leukocytes. *Cancer Lett.*, **149**, 115–123.
- Nakai M., Kageyama, N., Nakahara, K. and Miki, W. (2006) Phlorotannins as radical scavengers from the extract of *Sargassum ringgoldianum*. *Mar. Biotechnol.*, **8**, 409–414.
- Nakamura, T. and Lipiton, S.A. (2009) Cell death: protein misfolding and neurodegenerative diseases. *Apoptosis*, **14**, 455–468.
- Narayan, B., Miyashita, K. and Hosokawa, M. (2006) Physiological effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)-a review. *Food Rev. Int.*, **22**, 291–307.
- Nishino, H. (1998) Cancer prevention by carotenoids. *Mutat. Res.*, **402**, 159–163.
- Nomura, T., Kikuchi, M., Kubodera, A. and Kawakami, Y. (1997) Proton-donative antioxidant activity of fucoxanthin with 1,1-diphenyl-2-picrylhydrazyl (DPPH). *Biochem. Mol. Biol. Int.*, **42**, 361–370.
- Olaizola, M. (2008) The production and health benefits of Astaxanthin. In: *Marine Nutraceuticals and Functional Foods* (eds C. Barrow and F. Shahidi). CRC Press, New York, pp. 321–343.
- Olefsky, J.M. and Saltiel, A.R. (2000) PPARgamma and the treatment of insulin resistance. *Trends Endocr. Metabol.*, **11**, 362–368.
- Palermo, J.A., Gros, E.G. and Seldes, A.M. (1991) Carotenoids from tree red algae of the corallinaceae. *Phytochemistry*, **30**, 2983–2986.
- Paravicini, T.M. and Touyz, R.M. (2008) NADPH oxidases, reactive oxygen species, and hypertension. *Diabetes Care*, **31**, S170–S180.
- Parys, S., Rosenbaum, A., Kehraus, S., Reher, G., Glombitza, K.-W. and König, G.M. (2007) Evaluation of quantitative methods for the determination of polyphenols in algal extracts. *J. Nat. Prod.*, **70**, 1865–1870.
- Piccaglia, R., Marotti, M. and Grandi, S. (1998) Lutein and lutein ester content in different types of *Tagetes patula* and *T. erecta*. *Ind. Crops Prod.*, **8**, 45–51.
- Riboli, E. and Norat, T. (2003) Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am. J. Clin. Nutr.*, **78**, 559S–569S.
- Riccioni, G. (2009) Carotenoids and cardiovascular disease. *Curr. Atherosclerosis Rep.*, **11**, 434–439.
- Rüfer, C.E., Moeseneder, J., Briviba, K., Rechkemmer, G. and Bub, A. (2008) Bioavailability of astaxanthin stereoisomers from wild (*Oncorhynchus* spp.) and aquacultured (*Salmo salar*) salmon in healthy men: a randomised, double-blind study. *Br. J. Nutr.*, **99**, 1048–1054.
- Rupérez, P., Ahrazem, O. and Leal, A.J. (2002) Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *J. Agric. Food Chem.*, **50**, 840–845.
- Sachindra, N.M., Sato, E., Maeda, H., *et al.* 2007. Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *J. Agric. Food Chem.*, **55**, 8516–8522.
- Sangeetha, R.K., Bhaskar, N., Divakar, S. and Baskaran, V. (2010) Bioavailability and metabolism of fucoxanthin in rats: structural characterization of metabolites by LC-MS (APCI). *Mol. Cell Biochem.*, **333**, 299–310.
- Schubert, N., García-Mendoza, E. and Pacheco-Ruiz, I. (2006) Carotenoid composition of marine red algae. *J. Phycol.*, **42**, 1208–1216.
- Seifried, H.E., Anderson, D.E., Fisher, E.I. and Milner, J.A. (2007) A review of the interaction among dietary antioxidants and reactive oxygen species. *J. Nutr. Biochem.*, **18**, 567–579.
- Seo, Y., Park, K.E., Kim, Y.A., *et al.* (2006) Isolation of tetraprenyltoluquinols from the brown alga *Sargassum thunbergii*. *Chem. Pharm. Bull.*, **54**, 1730–1733.
- Shahidi, F. and Miraliakbari, H. (2006) Marine oils: compositional characteristics and health effects. In: *Nutraceutical and Specialty Lipids and Their Co-Products* (ed. F. Shahidi). RC Press, New York, pp. 227–250.
- Shibata, T., Ishimaru, K., Kawaguchi, S., Yoshikawa, H. and Hama, Y. (2008) Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *J. Appl. Phycol.*, **20**, 705–711.
- Shin, H.-C., Hwang, H.J., Kang, K.J. and Lee, B.H. (2006) An antioxidative and antiinflammatory agent for potential treatment of osteoarthritis from *Ecklonia cava*. *Arch. Pharm. Res.*, **29**, 165–171.
- Sinclair, A., Wallace, J., Martin, M., Attar-Bashi, N., Weisinger, R. and Li, D. (2005) The effects of eicosapentaenoic acid in various clinical conditions. In: *Healthful Lipids* (eds C.C. Akoh and O.-M. Lai). AOCS Press, Champaign, IL, pp. 361–394.
- Spiller, G.A. and Dewell, A. (2003) Safety of an astaxanthin-rich *Haematococcus pluvialis* algal extract: a randomized clinical trial. *J. Med. Food*, **6**, 51–56.
- Stahl, W. and Sies, H. (2007) Carotenoids and flavonoids contribute to nutritional protection against skin damage from sunlight. *Mol. Biotechnol.*, **37**, 26–30.

- Takamatsu, S., Hodges, T.W., Rajbhandari, I., Gerwick, W.H., Hamann, M.T. and Nagle, D.G. (2003) Marine natural products as novel antioxidant prototypes. *J. Nat. Prod.*, **66**, 605–608.
- Tapiero, H., Townsend, D.M. and Tew, K.D. (2004) The role of carotenoids in the prevention of human pathologies. *Biomed. Pharm.*, **58**, 100–110.
- Terao, J. (1989) Antioxidant activity of  $\beta$ -carotene-related carotenoids in solution. *Lipids*, **24**, 659–661.
- Tsukui, T., Baba, T., Hosokawa, M., Sashima, T. and Miyashita, K. (2009) Enhancement of hepatic docosahexaenoic acid and arachidonic acid contents in C57BL/6J mice by dietary fucoxanthin. *Fish. Sci.*, **75**, 261–263.
- Wellen, K.E. and Hotamisligil, G.S. (2005) Inflammation, stress, and diabetes. *J. Clin. Invest.*, **115**, 1111–1119.
- Willett, W.C. (2001) Diet and cancer: One view at the start of the millennium. *Cancer Epidemiol. Biomark. Prev.*, **10**, 3–8.
- Widjaja-Adhi, M.K.A., Hosokawa, M. and Miyashita, K. (2011) Comparative study of antioxidant activity of edible Japanese brown seaweeds. *J. Food Sci.* **76**, C104–C111.
- Yan, X., Chuda, Y., Suzuki, M. and Nagata, T. (1999) Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Biosci. Biotech. Biochem.*, **63**, 605–607.
- Yuan, Y.V. and Walsh, N.A. (2006) Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food Chem. Toxicol.*, **44**, 1144–1150.
- Zhao, X., Xue, C.-H. and Li, B.-F. (2008) Study of antioxidant activities of sulfated polysaccharides from *Laminaria japonica*. *J. Appl. Phycol.*, **20**, 431–436.
- Zhao, X., Xue, C.-H., Li, Z.-J., Cai, Y.-P., Liu, H.-Y. and Qi H.-T. (2004) Antioxidant and hepatoprotective activities of low molecular weight sulfated polysaccharide from *Laminaria japonica*. *J. Appl. Phycol.*, **16**, 111–115.
- Zhou, J., Hu, C., Wu, Y.-L., Pan, Y.J. and Sun, C.-R. (2008) Preliminary studies on the chemical characterization and antioxidant properties of acidic polysaccharides from *Sargassum fusiforme*. *J. Zhejiang Univ. Sci. B*, **9**, 1673–1581.
- Zou, Y., Qian, J., Li, Y., Kim, M.-M., Lee, S.-H. and Kim, S.-K. (2008) Antioxidant effects of phlorotannins isolated from *Ishige okamurae* in free radical mediated oxidative systems. *J. Agric. Food Chem.*, **56**, 7001–7009.

# **PART IV**

## **Biotechnology of Seaweeds**

# 25

## Anti-HIV Activities of Marine Macroalgae

**Thanh-Sang Vo<sup>1</sup>, Dai-Hung Ngo<sup>1</sup> and Se-Kwon Kim<sup>1,2</sup>**

<sup>1</sup>*Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan, Republic of Korea*

<sup>2</sup>*Marine Bioprocess Research Center, Pukyong National University, Busan, Republic of Korea*

### 25.1 Introduction

Human immunodeficiency virus type-1 (HIV-1) is identified as the causative agent of acquired immune deficiency syndrome (AIDS), which is one of the most important diseases with about 33.2 million people infected worldwide up to now (Ojewole *et al.*, 2008; Govender *et al.*, 2008). Thus, the generation of anti-HIV drugs has been developed to treat AIDS patients after the introduction of AIDS in early 1980 (De Clercq, 2000; Lipsky, 1996). However, failure of anti-AIDS treatment is observed in more than 50% of the patients infected with HIV as a result of drug-resistant strains of virus (Clavel and Hance, 2004; Tantillo *et al.*, 1994). Therefore, the search for potential drug candidates with higher inhibitory activity against various HIV strains is increasing in the pharmaceutical industry. In this regard, natural bioactive compounds and their derivatives are great sources for the development of new generation anti-HIV therapeutics, which are more effective with less side effects (Tzileleka *et al.*, 2003; Singh *et al.*, 2005; Schaeffer and Krylov, 2000).

With marine species comprising approximately one half of the total global biodiversity, the sea offers an enormous resource for novel compounds (Aneiros and Garateix, 2004). Moreover, very different kinds of substances have been procured from marine organisms because they live

in very exigent, competitive, and aggressive surroundings, very different in many aspects from the terrestrial environment; this is a situation that demands the production of quite specific and potent active molecules. Marine environment serves as a source of functional materials, including polyunsaturated fatty acids (PUFA), polysaccharides, minerals and vitamins, antioxidants, enzymes, and bioactive peptides (Wijesekara and Kim, 2010; Pomponi, 2001). This chapter focuses on anti-HIV therapeutic agents derived from marine macroalgae and their potential applications in pharmaceutical industry as novel functional ingredients in anti-HIV therapy.

### 25.2 Potential anti-HIV agents from marine macroalgae

#### 25.2.1 Sulfated polysaccharides

Sulfated polysaccharides (SPs) comprise a complex group of macromolecules with a wide range of important biological activities. These polymers are chemically anionic and distributed widely not only in marine algae but also in animals such as mammals and invertebrates (Vijayavel *et al.*, 2006; Alban and Franz, 2001). Marine algae are the most important source of non-animal SPs and the chemical

structure of the polymers varies according to the algal species (Costa *et al.*, 2010). In recent years, various SPs isolated from marine algae have attracted much attention in the fields of biochemistry and pharmacology. They exhibit beneficial biological activities such as anti-HIV-1 (Schaeffer and Krylov, 2000), antiadhesive (Ley *et al.*, 1991), anticoagulant (Majdoub *et al.*, 2009), anticancer (Synytsya *et al.*, 2010) and anti-inflammatory (Na *et al.*, 2010).

Many species of marine algae contain significant quantities of complex structural SPs that have been shown to inhibit the replication of enveloped viruses including members of the flavivirus, togavirus, arenavirus, rhabdovirus, orthopoxvirus, and herpesvirus families (Witvrouw and De Clercq, 1997). Therefore, SPs are preferred candidates for antiviral drug development. In this sense, marine macroalgae-derived SPs are great sources for the development of a new generation of anti-HIV therapeutics as reported by several studies. Indeed, a number of SPs from red algae have exhibited appreciable HIV-1 inhibitory activity. The sulfated glucuronogalactan from the red alga *Schizymenia dubyi* was reported to possess anti-HIV activity (Bourgougnon *et al.*, 1996). This study determined the antiviral activity with HIV-1 by measuring of the protective effect of sulfated glucuronogalactan against the virus-induced cytopathogenicity in MT4 cells over 8 days. As shown in their study, the syncytial formation was completely suppressed with 5 µg/ml of this polysaccharide. Furthermore, HIV-1 reverse transcriptase was inhibited at concentrations as low as 5 µg/ml, without cytotoxicity to MT4 cells. They suggested that the mechanism of action of this polysaccharide *in vitro* can be been mainly attributed to the inhibition of virus–host cell attachment or an early step of HIV infection.

In addition, sulfated galactans, GFP extracted from the red alga *Grateloupia flicina* and GLPE obtained from *Grateloupia longifolia*, also have antiretroviral activity *in vitro*. The sulfated galactan GFP has sulfate ester groups at carbon 2 and at carbons 2 and 6 for GLPE. Wang *et al.* (2007) investigated the antiretroviral activity of these sulfated galactans in a model based on a primary isolate of HIV-1 and human peripheral blood mononuclear cells. These results showed that both GFP and GLPE had potent anti-HIV-1 activity when added at the time of infection and 2 h post-infection ( $EC_{50}$ s, 0.010–0.003 µM and  $EC_{90}$ s, 0.87–0.33 µM, respectively) with low cytotoxicity.

Moreover, brown algae are also known to produce a variety of interesting SPs, which have been found to exhibit anti-HIV activity with different mechanisms of action. Sulfated polymannuroguluronate (SPMG), a new form of sulfated polysaccharide extracted from brown algae with an average molecular mass of 8.0 kDa, is rich in 1,4-linked β-D-mannuronate, with an average of 1.5 sulfates and 1.0

carboxyl groups per sugar residue (Miao *et al.*, 2005). The involution of marine SPMG in inhibition of HIV-1 entry was reported by Meiyu *et al.* (2003). They indicated that binding of SPMG either to soluble oligomeric rgp120 or to complexed rgp120–sCD4 mainly resided in the V3 loop region. The V3 loop of gp120, considered as a positively charged region, was targeted by negatively charged polysaccharides. Additionally, the preincubation of SPMG with rgp120 triggered partial suppression of rgp120 binding to sCD4. Thus, they suggested that SPMG either shares common binding sites on gp120 with sCD4 or masks the docking sites of gp120 for sCD4. Finally, SPMG was shown to be less accessible for sCD4 when sCD4 was precombined with rgp120, though SPMG multivalently bound to sCD4 with relatively low affinity. However, SPMG may suppress the multivalent binding of rgp120 to the sCD4 receptor when SPMG is added either prior to or after the interaction of rgp120 with sCD4. These effective suppressions indicate that SPMG endows both preventive and therapeutic potential on HIV-1 entry.

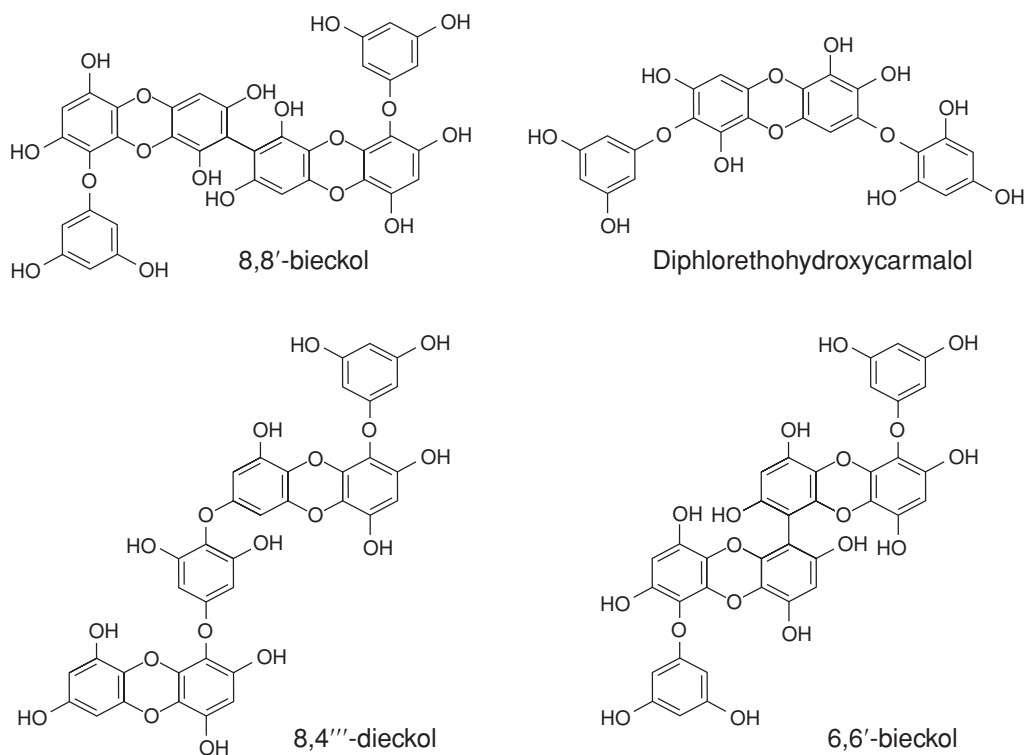
Fucans are a class of high molecular weight SPs. They are widely distributed in several species of brown algae and composed of a mainly repeating chain of fucose. The sulfated fucans from the seaweed species *Dictyota merten-sii*, *Lobophora variegata*, *Spatoglossum schroederi*, and *Fucus vesiculosus* were reported to inhibit HIV reverse transcriptase (RT) by Queiroz *et al.* (2008). They have indicated that the galactofucan fraction from *L. variegata*, which is rich in galactose, fucose and glucose with a lower sulfate content, had a marked inhibitory effect on reverse transcriptase, with 94% inhibition for synthetic polynucleotides at a concentration of 1.0 µg/ml. On the other hand, fucan A from *S. schroederi* and *D. merten-sii*, which contains mainly fucose with a lower sulfate level, showed a high inhibitory effect on RT at 1.0 mg/ml, with 99.03 and 99.3% inhibition, respectively. Meanwhile, fucan B from *S. schroederi*, which contains galactose, fucose and high sulfate level, showed a lower inhibitory activity (53.9%) at the same concentration. Taking another approach, the authors purified a fucan fraction from *F. vesiculosus*, a homofucan containing only sulfated fucose with high sulfate content, which exhibited high inhibitory activity of HIV on RT. This fraction inhibited 98.1% of the reaction with poly(rA)-oligo(dT) at a concentration of 0.5 mg/ml. Furthermore, they modified SPs by carboxy-reduction and desulfation to determine the structure–activity relationship. These modified conditions reduced the inhibitory activities of these polysaccharides for RT approximately fourfold. From these results, they have suggested that fucan activity is dependent on both the ionic changes and the sugar rings that act to spatially orientate the charges in a configuration and recognizes the enzyme, thus determining the specificity of the binding.

In a recent study, Trincherro *et al.* (2009) have shown that galactofucan fractions from the brown algae *Adenocystis utricularis* exhibited anti-HIV-1 activity *in vitro*. Among five fractions, EA1–20 and EC2–20 had a strong inhibitory effect on HIV-1 replication *in vitro* with low  $IC_{50}$  values (0.6 and 0.9  $\mu\text{g/ml}$ , respectively). Additionally, EA1–20 and EC2–20 displayed this capacity against wild type and drug-resistant HIV-1 strains. For active fractions, it was also shown that the inhibitory effect was not due to an inactivating effect on the viral particles but rather to a blockade of early events of viral replication. Based on these results, marine macroalgae-derived SPs are regarded as promising candidates for further studies on prevention of HIV-1 infection.

Recent studies demonstrated that SPs could be used as a vaginal antiviral formulation without disturbing essential functions of the vaginal epithelial cells and normal bacterial flora. It will be a continuous challenge to select the most promising drug candidates among the wide array of available polysaccharide compounds. The numerous advantages over other classes of antiviral drugs, such as relatively low production costs, broad spectrum of antiviral properties, low cytotoxicity, safety, wide acceptability and novel modes of action, suggest that SPs are promising drug candidates in the near future.

### 25.2.2 Phlorotannins

Phlorotannins are formed by the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) monomer units and biosynthesized through the acetate–malonate pathway (Targett and Arnold, 1998; Shibata *et al.*, 2004). The phlorotannins are highly hydrophilic components with a wide range of molecular sizes ranging between 126 Da and 650 kDa (Ahn *et al.*, 2007). Marine brown algae accumulate a variety of phloroglucinol-based polyphenols, as phlorotannins of low, intermediate and high molecular weight containing both phenyl and phenoxy units (Singh and Bharate, 2006; Glombitza and Li, 1991). Furthermore, phlorotannins consist of phloroglucinol units linked to each other in various ways, and are of wide occurrence amongst marine organisms, especially brown and red algae (Singh and Bharate, 2006). Based on the means of linkage, phlorotannins can be classified into four subclasses: fuhals and phlorethols (phlorotannins with an ether linkage), fucols (with a phenyl linkage), fucophloroethols (with an ether and phenyl linkage), and eckols (with a dibenzodioxin linkage) (La Barre *et al.*, 2010). Brown algae, which high produce of phlorotannins (Figure 25.1), have been reported to possess anti-HIV activity.



**Figure 25.1** Chemical structure of phlorotannins.

For the first time, Ahn *et al.* (2004) reported that 8,8'-bieckol and 8,4'''-dieckol, which they isolated from the brown algae *Ecklonia cava* Kjellman, show an inhibitory effect on HIV-1 RT and protease. The inhibition against RT of 8,8'-bieckol with a biaryl linkage ( $IC_{50}$ , 0.5  $\mu$ M) was 10-fold higher than that of 8,4'''-dieckol with a diphenyl ether linkage ( $IC_{50}$ , 5.3  $\mu$ M), although these two phlorotannins are dimers of eckol. The authors suggested that the steric hindrance of the hydroxyl and aryl groups near the biaryl linkage of 8,8'-bieckol caused to the potent inhibitory activity. Moreover, 8,8'-bieckol selectively inhibited reverse transcriptase over protease, and the inhibitory effect was comparable to the positive control nevirapine ( $IC_{50}$ , 0.28  $\mu$ M). It is clear that the 8,8'-bieckol possessed higher inhibitory activity than 8,4'''-dieckol compound. Therefore, they evaluated the molecular mechanisms of this compound against HIV-1 RT using a homopolymeric template/primer under steady-state condition. Kinetic study showed that 8,8'-bieckol inhibited the RNA-dependent DNA synthesis activity of HIV-1 RT non-competitively against dUTP/dTTP with a  $K_i$  value of 0.78  $\mu$ M. Meanwhile, this compound also exhibited a non-competitive inhibition ( $K_i$ , 0.23  $\mu$ M) with respect to a homopolymeric template/primer, (rA)<sub>n</sub>(dT)<sub>15</sub>. A possible suggestion for this phenomenon is that 8,8'-bieckol binds to HIV-1 RT only after the template/primer initially binds to the enzyme. The inhibitory effects of this compound shown in this kinetic model are consistent with non-nucleoside RT inhibitors, such as pyridinones (Carroll *et al.*, 1993), trovirdine (Zhang *et al.*, 1995). As a result, 8,8'-bieckol might be considered as a new non-nucleoside HIV-1 RT inhibitor.

Furthermore, 6,6'-bieckol, one of the main phloroglucinol derivative naturally occurring in *Ecklonia cava*, has a potent wild inhibition against HIV-1-induced syncytia formation, lytic effects, and viral p24 antigen production (Artan *et al.*, 2008). Moreover, 6,6'-bieckol has selectively inhibited the activity of HIV-1 RT with an  $IC_{50}$  of 1.07  $\mu$ M, as well as the inhibition of HIV-1 entry. In addition, it exhibited no cytotoxicity at a concentration whereas it inhibited HIV-1 replication almost completely. Therefore, 6,6'-bieckol can be employed for the development of new generation therapeutic agents against HIV.

### 25.2.3 Diterpenes

Diterpenes are natural compounds largely distributed in the plant kingdom and also found in marine algae. Notably, the compounds isolated from *Dictyota menstrualis*, identified as 6-hydroxydichotoma-3,14-dien-1,17-dial and its acetate derivative 6-acetoxydichotoma-3,14-dieno-1,17-dial, exhibit inhibitory activities against HIV-1 replication

since they act in the recombinant HIV-1 RT activity in a dose-dependent manner (Pereira *et al.*, 2004, 2005). Moreover, the dolabellane diterpene isolated from *Dictyota paffii* exhibited the inhibitory effect on HIV-1 infection and the activity of a purified HIV-1 enzyme RT in a dose-dependent manner (Barbosa *et al.*, 2004; Cirne-Santos *et al.*, 2006, 2008). Recently, the diterpenes isolated from *D. paffii*, *D. menstrualis*, and *Canistrocarpus cervicornis* were shown to inhibit herpes simplex virus-1 infection in Vero cells (Abrantes *et al.*, 2009; Vallim *et al.*, 2010). Collectively, marine macroalgae-derived diterpenes could be suggested as potential inhibitors against HIV-1 infection and replication.

### 25.2.4 Lectins

Lectins (or carbohydrate-binding proteins) are found in a variety of different species, ranging from prokaryotes to corals, algae, fungi, plants, invertebrates, and vertebrates. They are involved in many biological processes, including host-pathogen interactions, cell-cell communication, induction of apoptosis, cancer metastasis and differentiation, targeting of cells, as well as recognizing and binding carbohydrates. Interestingly, lectins have the potential to block the binding of HIV to target cells, preventing HIV infection and dissemination (Sato and Hori, 2009). In particular, Griffithsin (GRFT), a lectin isolated from the red algae *Griffithsia* sp., displayed potent anti-HIV activity. GRFT is a completely novel protein with a molecular weight of 12.7 kDa. It consists of 120 usual amino acids including one unusual amino acid at position 31 (151 Da). There are no cysteine residues among its 121 amino acids and no homology to any of the proteins or translated nucleotide sequences. Most likely, GRFT molecule is formed as a domain-swapped dimer in solution (Mori *et al.*, 2005). It was determined that both native and recombinant GRFT potently inhibited the cytopathic effects of laboratory strains and clinical primary isolates of HIV-1 on T-lymphoblastic cells at concentrations as low as 0.043  $\mu$ M. It was also shown to be active against both T-cell tropic and macrophage-tropic strains of HIV-1 at the same concentrations. Furthermore, GRFT blocked cell-cell fusion between chronically infected and uninfected cells at sub-nanomolar concentrations. In addition, it aborted the binding of CD4-dependent glycoprotein gp120 to receptor-expressing cells in a glycosylation-dependent manner, and prevent gp120 binding to 2G12 and 48d monoclonal antibody. Interestingly, soluble gp120 binding to GRFT was inhibited by the monosaccharides glucose, mannose and Glc-NAC but not by galactose, xylose, fucose, GalNAc or sialic acid-containing glycoproteins. Also, this study indicated that GRFT may present four carbohydrate-binding domains, separated by three linker sequences Gly-Gly-Ser-Gly-Gly. This

organization for this lectin could explain its unusually potent activity due to the possibility of the formation of multivalent bonds between GRFT and oligosaccharides on gp120. These properties make GRFT a promising potential candidate for development as a future pharmaceutical agent.

### 25.2.5 Bioactive peptides

Marine-derived bioactive peptides have been isolated widely by enzymatic hydrolysis of marine organisms and have been known to possess many physiological functions (Kim and Wijesekara, 2010). Among various marine bioactive peptides, Kahalalide F has been noted for its effectiveness in some AIDS study cases (Smit, 2004). Kahalalide F is a cyclic depsipeptide isolated from the sacoglossan mollusk, *Elysia rufescens*, but is most probably derived from *Bryopsis* sp., its green algal diet. Due to its promising anti-HIV property, Kahalalide F is being further studied in clinical trials.

## 25.3 Conclusion

Recent studies have provided evidence that marine macroalgae-derived anti-HIV agents play a vital role against HIV. The possibilities of designing new drug candidates and pharmaceuticals to support reducing or regulating the HIV infection related chronic malfunctions are promising. Moreover, these evidences suggest that due to valuable biological functions with health beneficial effects, marine macroalgae-derived anti-HIV agents have potential as active ingredients for preparation of novel pharmaceutical products. Until now, most of studies on anti-HIV activity of marine macroalgae-derived HIV inhibitors have been observed *in vitro* or in mouse model systems. Therefore, further research studies are needed in order to investigate their activities in human subjects.

## References

- Abrantes JL, Barbosa J, Cavalcanti D, *et al.* (2009) The effects of the diterpenes isolated from the Brazilian brown algae *Dictyota paffii* and *Dictyota menstrualis* against the herpes simplex type-1 replicative cycle. *Planta Med.*, **75**, 1–6.
- Ahn GN, Kim KN, Cha SH, *et al.* (2007) Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H<sub>2</sub>O<sub>2</sub>-mediated DNA damage. *Eur. Food Res. Technol.*, **226**, 71–79.
- Ahn MJ, Yoon KD, Min SY, *et al.* (2004) Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga *Ecklonia cava*. *Biol. Pharm. Bull.*, **27**, 544–547.
- Alban S and Franz G (2001) Partial synthetic glucan sulfates as potential new antithrombotics: a review. *Biomacromolecules*, **2**, 354–361.
- Aneiros A and Garateix A (2004) Bioactive peptides from marine sources: pharmacological properties and isolation procedures. *J. Chromatogr. B*, **803**, 41–53.
- Artan M, Li Y, Karadeniz F, Lee SH, Kim MM and Kim SK (2008) Anti-HIV-1 activity of phloroglucinol derivative, 6,6'-bieckol, from *Ecklonia cava*. *Bioorg. Med. Chem.*, **16**, 7921–7926.
- Barbosa JP, Pereira RC, Abrantes JL, *et al.* (2004) In vitro antiviral diterpenes from the Brazilian brown alga *Dictyota paffii*. *Planta Med.*, **70**, 856–860.
- Bourgougnon N, Lahaye M, Quemener B, *et al.* (1996) Annual variation in composition and in vitro anti-HIV-1 activity of the sulfated glucuronogalactan from *Schizymenia dubyi* (Rhodophyta Gigartinales). *J. Appl. Phycol.*, **8**, 155–161.
- Carroll SS, Olsen DB, Bennett CD, *et al.* (1993) Inhibition of HIV-I reverse transcriptase by pyridinone derivatives. *The J. Biol. Chem.*, **268**, 276–281.
- Cirne-Santos CC, Souza TM, Teixeira VL, *et al.* (2008) The dolabellane diterpene dolabelladienetriol is a typical noncompetitive inhibitor of HIV-1 reverse transcriptase enzyme. *Antiviral Res.*, **77**, 64–71.
- Cirne-Santos CC, Teixeira VL, Castello-Branco LR, Frugulhetti ICPP and Bou-Habib DC (2006) Inhibition of HIV-1 replication in human primary cells by a dolabellane diterpene isolated from the Brazilian algae *Dictyota paffii*. *Planta Med.*, **72**, 295–299.
- Clavel F and Hance AJ (2004) HIV drug resistance. *N. Engl. J. Med.*, **350**, 1023–1035.
- Costa LS, Fidelis GP, Cordeiro SL, *et al.* (2010) Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomed. Pharmacother.*, **64**, 21–28.
- De Clercq E (2000) Current lead natural products for the chemotherapy of human immuno deficiency virus infection. *Med. Res. Rev.*, **20**, 323–349.
- Glombitza KW and Li SM (1991) Hydroxyphlorethols from the brown alga *Carpophyllum maschalocarpum*. *Phytochemistry*, **30**, 2741–2745.
- Govender T, Ojewole E and Naidoo P (2008) Polymeric nanoparticles for enhancing antiretroviral drug therapy. *Drug Delivery*, **15**, 493–501.
- Kim SK and Wijesekara I (2010) Development and biological activities of marine-derived bioactive peptides: A review. *J. Funct. Foods*, **2**, 1–9.
- La Barre S, Potin P, Leblanc C and Delage L (2010) The halogenated metabolism of brown algae (Phaeophyta)

- its biological importance and its environmental significance. *Mar. Drugs*, **8**, 988–1010.
- Ley K, Cerrito M and Arfors KE (1991) Sulfated polysaccharides inhibit leukocyte rolling in rabbit mesentery venules. *Am. J. Physiol.*, **260**, H1667–H1673.
- Lipsky JJ (1996) Antiretroviral drugs for AIDS. *Lancet*, **348**, 800–803.
- Majdoub H, Ben Mansour M, Chaubet F, Roudesli MS and Maaroufi RM (2009) Anticoagulant activity of a sulfated polysaccharide from the green alga *Arthrospira platensis*. *Biochim. Biophys. Acta*, **1790**, 1377–1381.
- Meiyu G, Fuchuan L, Xianliang X, Jing L, Zuwei Y and Huashi G (2003) The potential molecular targets of marine sulfated polymannuroguluronate interfering with HIV-1 entry. Interaction between SPMG and HIV-1 rgp120 and CD4 molecule. *Antiviral Res.*, **59**, 127–135.
- Miao B, Li J, Fu X, Gan L, Xin X and Geng M (2005) Sulfated polymannuroguluronate a novel anti-AIDS drug candidate inhibits T cell apoptosis by combating oxidative damage of mitochondria. *Mol. Pharmacol.*, **68**, 1716–1727.
- Mori T, O'Keefe BR, Sowder II RC, *et al.* (2005) Isolation and characterization of Griffithsin a novel HIV-inactivating protein from the red alga *Griffithsia* sp. *J. Biol. Chem.*, **280**, 9345–9353.
- Na YS, Kim WJ, Kim SM, *et al.* (2010) Purification, characterization and immunostimulating activity of water-soluble polysaccharide isolated from *Capsosiphon fulvescens*. *Int. Immunopharmacol.*, **10**, 364–370.
- Ojewole E, Mackraj I, Naidoo P and Govender T (2008) Exploring the use of novel drug delivery systems for antiretroviral drugs. *Eur. J. Pharm. Biopharm.*, **70**, 697–710.
- Pereira HS, Leão-Ferreira LR, Moussatché N, *et al.* (2004) Antiviral activity of diterpenes isolated from the Brazilian marine alga *Dictyota menstrualis* against human immunodeficiency virus type 1 (HIV-1). *Antiviral Res.*, **64**, 69–76.
- Pereira HS, Leão-Ferreira LR, Moussatché N, *et al.* (2005) Effects of diterpenes isolated from the Brazilian marine alga *Dictyota menstrualis* on HIV-1 reverse transcriptase. *Planta Med.*, **71**, 1019–1024.
- Pomponi SA (2001) The oceans and human health: the discovery and development of marine-derived drugs. *Oceanography*, **14**, 78–87.
- Queiroz KCS, Medeiros VP, Queiroz LS, *et al.* (2008) Inhibition of reverse transcriptase activity of HIV by polysaccharides of brown algae. *Biomed. Pharmacother.*, **62**, 303–307.
- Sato T and Hori K (2009) Cloning expression and characterization of a novel anti-HIV lectin from the cultured cyanobacterium *Oscillatoria agardhii*. *Fish. Sci.*, **75**, 743–753.
- Schaeffer DJ and Krylov VS (2000) Anti-HIV activity of extracts and compounds from algae and cyanobacteria. *Ecotoxicol. Env. Safety*, **45**, 208–227.
- Shibata T, Kawaguchi S, Hama Y, Inagaki M, Yamaguchi K and Nakamura T (2004) Local and chemical distribution of phlorotannins in brown algae. *J. Appl. Phycol.*, **16**, 291–296.
- Singh IP and Bharate SB (2006) Phloroglucinol compounds of natural origin. *Nat. Prod. Rep.*, **23**, 558–591.
- Singh IP, Bharate SB and Bhutani KK (2005) Anti-HIV natural products. *Curr. Sci.*, **89**, 269–290.
- Smit AJ (2004) Medicinal and pharmaceutical uses of seaweed nature products: a review. *J. Appl. Phycol.*, **16**, 245–262.
- Synytysa A, Kim WJ, Kim SM, *et al.* (2010) Structure and antitumor activity of fucoidan isolated from sporophyll of Korean seaweed *Undaria pinnatifida*. *Carbohydr. Polym.*, **81**, 41–48.
- Tantillo C, Ding J, Jacobo-Molina A, *et al.* (1994) Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. Implications for mechanisms of drug inhibition and resistance. *J. Mol. Biol.*, **243**, 369–387.
- Targett NM and Arnold TM (1998) Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. *J. Phycol.*, **34**, 195–205.
- Trinchero J, Ponce NMA, Cordoba OL, *et al.* (2009) Antiretroviral activity of fucoidans extracted from the brown seaweed *Adenocystis utricularis*. *Phytother. Res.*, **23**, 707–712.
- Tzileleka LA, Vagias C and Roussis V (2003) Natural products with anti-HIV activity from marine organisms. *Curr. Topics Med. Chem.*, **3**, 1512–1535.
- Vallim MA, Barbosa JE, Cavalcanti DN, *et al.* (2010) In vitro antiviral activity of diterpenes isolated from the Brazilian brown alga *Canistrocarpus cervicornis*. *J. Med. Plants Res.*, **4**, 2379–2382.
- Vijayavel K, Anbuselvam C and Balasubramanian MP (2006) Free radical scavenging activity of the marine mangrove *Rhizophora apiculata* bark extract with reference to naphthalene induced mitochondrial dysfunction. *Chem.-Biol. Interact.*, **163**, 170–175.
- Wang SC, Bligh SWA, Shi SS, *et al.* (2007) Structural features and anti-HIV-1 activity of novel polysaccharides from red algae *Grateloupia longifolia* and *Grateloupia fllicina*. *Int. J. Biol. Macromol.*, **41**, 369–375.
- Wijesekera I and Kim SK (2010) Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources:

- prospects in the pharmaceutical industry. *Marine Drugs*, **8**, 1080–1093.
- Witvrouw M and De Clercq E (1997) Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *Gen. Pharmacol.*, **29**, 497–511.
- Zhang H, Vrang L, Backbro K, *et al.* (1995) Inhibition of human immunodeficiency virus type 1 wild-type and mutant reverse transcriptases by the phenyl ethyl thiazolyl thiourea derivatives trovirdine and MSC-127. *Antiviral Res.*, **28**, 331–342.

# 26

## Biotechnology of Seaweeds: Facing the Coming Decade

Lin Hanzhi<sup>1</sup>, Qin Song<sup>1,2</sup> and Jiang Peng<sup>1</sup>

<sup>1</sup>Key Laboratory of Experimental Marine Biology, Chinese Academy of Sciences at Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

<sup>2</sup>Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China

### 26.1 Introduction

The discovery and usage of seaweeds can be traced back 2500 years ago, and a worldwide large-scale seafood cultivation industry has been successfully established. Seaweed, an essential resource of fine chemicals, proteins and other health care products plays an increasingly significant role in improving the quality of life. With the rapid development of systems biology and integrative biotechnology, the seaweed biotechnology has entered a new era in the 21st century. While facing such severe issues as exhausted resources, energy crisis and environmental pollution, it is urgent to further exploit seaweed resources. In this review, we summarize the main progress of seaweed biotechnologies on agriculture, industry, medicine, environment, etc.

### 26.2 Biotechnology of seaweeds in 'blue farming'

The key problem of seaweed biotechnology is the 'seed' problem. Some species of seaweeds have been very successfully cultivated for commercial exploitations, such as *Laminaria*, *Undaria*, *Porphyra*, and *Gracilaria*. The marvelous success of seaweed farming was partly due to the application of biotechnological approaches of seed selection and

breeding techniques. Beginning with the cytogenetic technology applied to the genetic improvements of seaweed in the middle of the 20th century, many excellent strains were cultivated for agricultural production with the characteristics of fast growth, high yield, and stress resistance.

Tissue culture, transgenic technology and molecular markers are the main three biotechnologies applied in modern seaweed breeding (Table 26.1). Breeding by cell hybridization overcomes the disadvantage of traditional cross-breeding that sexual reproduction was necessary in breeding. The nurse cell in thallus of *Porphyra* is haploid, whose phenotype is the same as its genotype. This character makes *Porphyra* a good candidate for seaweed breeding (Saito and Fujita, 1991). Many attempts (Araki *et al.*, 1994; Baweja *et al.*, 2009; Chen and Chiang, 1994) were made to breed seaweed via cell hybridization; however, researchers have not yet got a good strain for further agricultural cultivation. The factors affecting the success of cell hybridization exist in every step. The first one is the preparation of the protoplast, the second is the protoplast fusion, and the last but not the least is the selection and identification of the hybridized cell.

Transgenic technology has been applied to the seaweed breeding since the 1990s. Research on transgenic technology mainly focuses on the economic seaweeds, including Phaeophyta (*Laminaria japonica*, etc.) (Jiang *et al.*, 2003;

**Table 26.1** Molecular markers applied in seaweed biotechnology

Seaweed	Molecular markers
Red Algae	
<i>Gracilaria changii</i>	Random Amplification of Polymorphic DNA (RAPD) (Sim <i>et al.</i> 2007)
<i>Gracilaria lemaneiformis</i>	Inter-simple sequence repeat (ISSR), RAPD (Li <i>et al.</i> 1998; Sun <i>et al.</i> 2006)
<i>Gracilaria chilensis</i>	Microsatellite markers (Guillemin <i>et al.</i> 2005)
<i>Gracilaria gracilis</i>	Microsatellite markers (Luo <i>et al.</i> 1999)
<i>Porphyra lines</i>	RAPD, SCAR, Sequence tagged site (STS), Amplified fragment length polymorphism( AFLP) (Jia <i>et al.</i> 2000; Sun <i>et al.</i> 2005; Weng <i>et al.</i> 2005)
<i>Porphyra haitanensis</i>	AFLP, Sequence-characterized amplified region (SCAR) (Zuo <i>et al.</i> 2006)
<i>Porphyra yezoensis</i>	Microsatellite markers (Kong <i>et al.</i> 2009)
<i>Ectocarpus siliculosus</i>	Microsatellite markers (Heesch <i>et al.</i> 2010)
Brown Algae	
<i>Fucus</i>	Simple sequence repeat (SSR) (Coyer <i>et al.</i> 2009)
<i>Laminaria lines</i>	RAPD, SCAR (Wang <i>et al.</i> 2004)
<i>Laminaria japonica</i>	AFLP, SSR, microsatellite markers (Liu <i>et al.</i> 2010; Liu <i>et al.</i> 2009; Shi <i>et al.</i> 2007)
<i>Laminaria digitata</i>	Microsatellite markers, RAPD (Billot <i>et al.</i> 1999; Billot <i>et al.</i> 1998)
Green Algae	
<i>Ulva pertusa</i>	ISSR (Zhao <i>et al.</i> 2010)
<i>Ulva intestinalis</i>	Microsatellite markers (Kostamo <i>et al.</i> 2008)
<i>Enteromorpha prolifera</i>	RAPD (Huh <i>et al.</i> 2004)
<i>Enteromorpha intestinalis</i>	Microsatellite markers (Alstrom-Rapaport and Leskinen 2002)
<i>Macrocystis pyrifera</i>	Microsatellite markers (Alberto <i>et al.</i> 2009)

Qin *et al.*, 1998), Rhodophyta (*Kappaphycus alvarezii*, *Porphyra miniata*, *Gracilaria changii*) (Gan *et al.*, 2003; Kuang *et al.*, 1998; Kurtzman and Cheney, 1991; Wang *et al.*, 2010), Chlorophyta (*Ulva lactuca*) (Huang *et al.*, 1996). To establish an effective expression system for seaweeds, some factors need to be taken into account such as (1) economic target, which includes the cost and profit calculating; (2) technological methods, including the vector's construction and selection, selection of transformants, etc; (3) engineering design, which means the bioreactor's design, running and maintenance; (4) safety issues on transgenic seaweeds. A genetic transformation model system of brown seaweed *Laminaria japonica* (kelp) was established successfully by applying land plant transgenic technology and also by modulating seaweed life cycle. This model demonstrated an integrative indoor cultivation system to produce valuable products by transgenic kelp's sporophyte, taking into account necessary factors for biosafety (Qin *et al.*, 2005). Recently, a gametophytic expression photobioreactor system for *Laminaria japonica* and *Undaria pinnatifida* was established and two functional genes (Human acidic fibroblast growth factor and tachyplesin genes) were successfully expressed in this system, in order to overcome the relative long time procedure of sporophytic transformation (Deng *et al.*, 2008, 2009; Zhang *et al.*, 2008).

Marker assisted selection, based on the DNA polymorphism, is very useful in seaweed breeding as well. Molecular

markers have several advantages over the traditional phenotypic markers that were previously available to breeders. Molecular markers offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait (Mohan *et al.*, 1997). The markers closely link with the genes of purpose, which could enhance the selection accuracy and shorten the breeding period thereby reducing the workload and raise the efficiency. At present, many effective molecular markers were developed for molecular breeding, including RFLP, AFLP, SSR, RAPD, etc. (Table 26.1).

## 26.3 Biotechnology of seaweeds in the chemical industry and pharmacy

Beginning with the late phycologist Prof. C.K. Tseng's research on phycocolloid as a process industry, the last 50 years of the 20th century were a period of great development in the production of new strains for the phycocolloid and food industries. China has established the world largest seaweed chemical industry (Tseng, 2001) but since the 1950s, and the United States, the United Kingdom, Norway, the Philippines and other countries have a developed a

phycocolloid industry as well. Three kinds of phycocolloids are involved in seaweed chemical industry: algin, carrageenan, and agar. Formerly, the source of algin in China was wild *Sargassum confusum* but the wild *Sargassum confusum* was practically depleted in a few years due to excess exploitation. Fortunately, the cultivation of *Laminaria japonica* was successfully established and *Laminaria* became the main raw material of this industry. Then the integrative seaweed chemical industry was established to produce algin, as well as iodine and mannitol as by-products (Ji, 1997; Ji *et al.*, 1963). Uses of algin have extended to various food industries as stabilizers, thickening agents in medical industries, impression material in dentistry and anticoagulant material in toothpaste (Ji, 1997), and also as materials in paper making, electric welding rods, etc. (Andres, 1987). At present, sources of algin come from many kinds of seaweeds, including *Macrocystis pyrifera*, *Laminaria japonica*, *Laminaria digitata*, *Laminaria hyperborean*, *Ecklonia maxima*, etc. The second phycocolloid industry is the production of carrageenan. For a long time, *Betaphycus* was treated as an agarophyte and employed as an amino material together with *Gelidium* in the production of agar. In recent years carrageenan has been produced independently from the raw materials, *Kappaphycus*, *Eucheuma*, and *Betaphycus* (Bixler, 1996; Tseng, 2001; Villanueva and Montano, 2003). The third phycocolloid industry is the production of agar (Tseng, 1944, 1946). Agar is the oldest phycocolloid produced in China, but now the smallest in production (Tseng, 2001). In recent years, *Gracilaria* is becoming increasingly important with the fast development of a cultivation industry. Two species of *Gracilaria* are involved, *G. lemaneiformis* and *G. tenuistipitata* var. *liui*. Old thalli of *Porphyra haite-nensis* are also employed as raw material (Ji, 1997).

Seaweeds also contain substances found to be useful as drugs. A successful example for a marine drug is propylene glycol alginate sodium sulfate (PSS) from algin; this is the first marine antiangiocardopathy drug in China, effective in heart and brain disease, which has made nearly over US \$140 million production value. There are also other drugs from seaweeds such as Carraguard (Dezzutti *et al.*, 2004), Halomon from *Portieria homerhannii* (Fuller *et al.*, 1994), Lobophorolide from *Lobophora variegata* (Kubanek *et al.*, 2003). Polyunsaturated fatty acids (PUFA) are promising bioactive compounds and exist universally and variously in seaweeds. The red algae (such as Cermiales, Cryptonemiales, Gigantinales, etc.) usually have a high content of 16:0, 20:5n-3 and 20:4n-6 PUFA. The green algae (such as *Ulva*, Bryopsidaceae, Codiaceae) usually have 16:0, 18:3n-3 and 18:4n-3 PUFA. The brown algae (Phaeophyta) usually have 16:0, 18:1n-9, 20:4n-6 and 20:5n-3 PUFA. The plentiful types of PUFA in seaweeds and the fast growth rate of seaweeds make them a good source

**Table 26.2** Some typical bioactive compounds in seaweeds

Type	Example compounds or drugs
Amino acids and their ramifications	Betaine Mycosporine-like amino acids
Polysaccharides	PSS Carraguard
Carotenoids	$\beta$ -carotene
PUFA	EPA (20:5n-3 PUFA)
Phytohormones	Auxin Cytokinins
EPA, eicosapentaenoic acid.	

for marine drugs, health food and mariculture feed (Brandsen *et al.*, 2005; Xu *et al.*, 2004). Betaine is another kind of bioactive compounds existing in seaweeds. Its role in modulating plant growth and high value in drugs R&D attract increasing attention (Blunden, 2001; Whapham *et al.*, 1993; Zeisel, 2006). Mycosporine-like amino acids (MAA) are UV-absorbing compounds distributed in marine heterotrophic bacteria, cyanobacteria, nearly all seaweeds and marine invertebrates (Carreto *et al.*, 1989, 2005). Table 26.2 summarizes the bioactive compounds found in seaweed.

## 26.4 Biotechnology of seaweeds in a changing world: their role in bioremediation and bioenergy

For centuries, aquaculture has been supporting human demands for fish products and is an important industry worldwide (Chopin and Yarishi, 1998; Naylor *et al.*, 2000). With the massive increase in world aquaculture production in 1990s, the current aquaculture industry is one of the fastest growing sectors in world food production. In recent years, fast growth in population and human activities, such as various agricultural practices, discharge of industrial wastewater, urban runoff, and burning of fossil fuels have resulted in the increase of nutrient inputs to aquatic environments that are many times more than that generated by natural processes (Neori *et al.*, 2004; Troell *et al.*, 2003).

In the sea, microalgae (such as diatoms) and seaweeds (Radmer, 1996) can uptake nutrients from water as they grow. Though microalgae grow well in nutrient-rich seawater, it seems that they do absorb a lot of nutrients from the surrounding seawater and act as a remover of

eutrophication, however, they would not last very long because of their short lifespan of only a few days. The nutrients from these dead algae will be released back into the water again by microbial degradation (Fei, 2004). Seaweeds, on the other hand, could accumulate considerable biomass within months and years over their relative long lifespan. This is especially so in the case for cultivated species during cultivation season. Cultivated seaweeds have high yields and are easy to harvest. The nutrients fixed by seaweeds are moved from seawater to land, which could dramatically decrease the nutrient content in aquacultural water area. Meanwhile, seaweeds have a relative long lifetime. This characteristic could let people cultivate different seaweeds such as *Gracilaria* and *Porphyra* in succession over one year in the same area. Many of the bioremediation studies have focused on the green seaweeds *Ulva* (Naldi and Viaroli, 2002; Suzuki *et al.*, 2005), the red seaweed *Gracilaria* (Anderson *et al.*, 1999; Yang *et al.*, 2006; Zhou *et al.*, 2006), the kelp, *Laminaria* (Chopin *et al.*, 2003), and recently the more high-valued seaweeds *Porphyra* (*P. katadai*, *P. yezoensis*, and *P. dentata* in Asia; and *P. dioca* in Europe) (Carmona *et al.*, 2006; Chung *et al.*, 2002; He *et al.*, 2008; Kraemer *et al.*, 2004; Pereira *et al.*, 2006).

Seaweed is one such source of aquatic biomass and potentially represents a significant source of renewable energy. The average photosynthetic efficiency of aquatic biomass is 6–8% (Ross *et al.*, 2008), which is much higher than that of terrestrial biomass (1.8–2.2%). Growth rates of seaweeds far exceed those of terrestrial biomass, mainly due to no water limitation (Gellenbeck and Chapman, 1983). Annual primary production rates (grams C per m<sup>2</sup> per year) are higher for the major seaweeds than for most terrestrial biomass.

Seaweeds are usually utilized to produce methane, biodiesel, and ethanol. A number of studies have investigated biodegradation of seaweeds for biogas (methane) production (Chynoweth *et al.*, 2001; Wise, 1981). Anaerobic digestion of seaweeds to produce biogas (methane) has been demonstrated with both high yields and conversion rates, although suitable conditions vary amongst different species and within the same species at different points in the growth cycle.

The extraction of biodiesel from a macroalga, *Chaetomorpha linum*, has been carried out comparing two technologies, supercritical CO<sub>2</sub> extraction and thermochemical liquefaction. Between the technologies used, thermochemical liquefaction seems to be more efficient than the extraction with sc-CO<sub>2</sub> from the quantitative point of view, but decomposition of the fatty acid may occur under the operative conditions. Nevertheless, the extract oil composition depends on the working temperature of thermochemical liquefaction, and the content of long chain fatty acids is higher at lower temperatures as decomposition may occur

at higher temperatures (Aresta *et al.*, 2005). Recently, *Enteromorpha prolifera*, one of the main algal genera for green tide in the Yellow Sea (Jiang *et al.*, 2008), was converted to bio-oil by hydrothermal liquefaction in a batch reactor at temperatures of 220–320°C (Zhou *et al.*, 2010).

A systematic approach is now being taken to secure a stable feedstock supply that meets the implementation target of cellulosic ethanol, which involves new biomass resources such as macroalgae and plantation residues. It is expected that cellulosic ethanol will be in supply from 2020, and by 2030 its use will effectively reduce total gasoline consumption in Korea by 10% (Kim *et al.*, 2010).

The coming decade is an exciting period that will promote seaweed biotechnology to an integrative stage involving all kinds of 'omics' technologies and will be more related to energy, global changes, and human health. We are all looking forward to seeing increasing progress on seaweed biotechnology.

## Acknowledgment

The authors thank the CAS/SAFEA International Partnership Program for Creative Research Teams 'Typical Process in Coastal Zone Area and Its Effects on Bio-resources', National Natural Science Foundation of China (Grant No. 40876082), Knowledge Innovation Program of the Chinese Academy of Sciences (Grant No. KZCX2-YW-209), and National High Technology Research and Development Program of China (Grant No. 2009AA10Z106) for financial support.

## References

- Alberto, F., Whitmer, A., Coelho, N.C., Zippay, M., Varela-Alvarez, E., Raimondi, P.T., Reed, D.C. *et al.* (2009) Microsatellite markers for the giant kelp *Macrocystis pyrifera*. *Conserv. Genet.*, **10**, 1915–1917.
- Alstrom-Rapaport, C. and Leskinen E. (2002) Development of microsatellite markers in the green algae *Enteromorpha intestinalis* (Chlorophyta). *Mol. Ecol. Notes*, **2**, 581–583.
- Anderson, R.J., Smit, A.J. and Levitt, G.J. (1999) Upwelling and fish-factory waste as nitrogen sources for suspended cultivation of *Gracilaria gracilis* in Saldanha Bay, South Africa. *Hydrobiologia*, **399**, 455–462.
- Andres, C. (1987) Expanding applications for alginate technologies. *Food Process*, **48**, 30–32.
- Araki, T., Hayakawa, M., Tamaru, Y., Yoshimatsu, K. and Morishita, T. (1994) Isolation and regeneration of haploid protoplasts from *Bangia atropurpurea* (Rhodophyta) with marine bacterial enzymes. *J. Phycol.*, **30**, 1040–1046.

- Aresta, M., Dibenedetto, A., Carone, M., Colonna, T. and Fragale, C. (2005) Production of biodiesel from macroalgae by supercritical CO<sub>2</sub> extraction and thermochemical liquefaction. *Environ. Chem. Lett.*, **3**, 136–139.
- Baweja, P., Sahoo, D., Garcia-Jimenez, P. and Robaina, R.R. (2009) Review: Seaweed tissue culture as applied to biotechnology: Problems, achievements and prospects. *Phycol. Res.*, **57**, 45–58.
- Billot, C., Boury, S., Benet, H. and Kloareg, B. (1999) Development of RAPD markers for parentage analysis in *Laminaria digitata*. *Bot. Mar.*, **42**, 307–314.
- Billot, C., Rousvoal, S., Estoup, A., Epplen, J.T., Saumitou-Laprade, P., Valero, M., Kloareg, B. *et al.* (1998) Isolation and characterization of microsatellite markers in the nuclear genome of the brown alga *Laminaria digitata* (Phaeophyceae). *Mol. Ecol.*, **7**, 1778–1780.
- Bixler, H.J. (1996) Recent developments in manufacturing and marketing carrageenan. *Hydrobiologia*, **326–327**, 35–57.
- Blunden, G. (2001) Biologically active compounds from marine organisms. *Phytother. Res.*, **15**, 89–94.
- Brandsen, M.P., Battaglene, S.C., Morehead, D.T., Dunstan, G.A. and Nichols, P.D. (2005) Effect of dietary 22:6n-3 on growth, survival and tissue fatty acid profile of striped trumpeter (*Latris lineata*) larvae fed enriched *Artemia*. *Aquaculture*, **243**, 331–344.
- Carmona, R., Kraemer, G.P. and Yarish, C. (2006) Exploring Northeast American and Asian species of *Porphyra* for use in an integrated finfish-algal aquaculture system. *Aquaculture*, **252**, 54–65.
- Carreto, J.I., Carignan, M.O. and Montoya, N.G. (2005) A high-resolution reverse-phase liquid chromatography method for the analysis of mycosporine-like amino acids (MAAs) in marine organisms. *Mar. Biol.*, **146**, 237–252.
- Carreto, J.I., De Marco, S.G. and Lutz, V.A. (1989) UV-absorbing pigments in the dinoflagellates *Alexandrium excavatum* and *Prorocentrum micans*. Effects of light intensity. In: *Red Tides: Biology, Environmental Science and Toxicology* (eds T. Okaichi, D.M. Anderson and T. Nemoto). Elsevier, New York, pp. 333–336.
- Chen, Y.C. and Chiang, Y.M. (1994) Development of protoplasts from *Grateloupia sparsa* and *G. filicina* (Halymeniaceae, Rhodophyta). *Bot. Mar.*, **37**, 361–366.
- Chopin, T., Bastarache, S., Belyea, E., Haya, K., Sephton, D., Martin, J.L. *et al.* (2003) Development of the cultivation of *Laminaria saccharina* as the extractive inorganic component of an integrated aquaculture system and monitoring of therapeutants and phycotoxins. *J. Phycol.*, **39**, 10.
- Chopin, T. and Yarishi, C. (1998) Nutrients or not nutrients? That is the question in seaweed aquaculture . . . and the answer depends on the type and purpose of the aquaculture system. *World Aquaculture*, **29**, 31–33, 60–61.
- Chung, I.K., Kang, Y.H., Yarish, C., Kraemer, G.P. and Lee, J.A. (2002) Application of seaweed cultivation to the bioremediation of nutrient-rich effluent. *Algae*, **17**, 187–194.
- Chynoweth, D.P., Owens, J.M. and Legrand, R. (2001) Renewable methane from anaerobic digestion of biomass. *Renew. Energ.*, **22**, 1–8.
- Coyer, J.A., Hoarau, G., Beszteri, B., Pearson, G. and Olsen, J.L. (2009) Expressed sequence tag-derived polymorphic SSR markers for *Fucus serratus* and amplification in other species of *Fucus*. *Mol. Ecol. Resour.*, **9**, 168–170.
- Deng, X., Zhang, Q., Jiang, P., Cui, Y. and Qin, S. (2008) The optimization for cell cultivation of transgenic *Laminaria japonica* gametophytes in a bubble-column bioreactor. *J. Biotechnol.*, **136**, 559–560.
- Deng, X.Y., Qin, S., Zhang, Q., Jiang, P., Cui, Y.L. and Li, X.K. (2009) Microprojectile bombardment of *Laminaria japonica* gametophytes and rapid propagation of transgenic lines within a bubble-column bioreactor. *Plant Cell Tiss. Org.*, **97**, 253–261.
- Dezzutti, C.S., James, V.N., Ramos, A., Sullivan, S.T., Siddig, A., Bush, T.J. *et al.* (2004) In vitro comparison of topical microbicides for prevention of human immunodeficiency virus type 1 transmission. *Antimicrob. Agents Ch.*, **48**, 3834–3844.
- Fei, X.G. (2004) Solving the coastal eutrophication problem by large scale seaweed cultivation. *Hydrobiologia*, **512**, 145–151.
- Fuller, R.W., Cardellina, J.H., Jurek, J., Scheuer, P.J., Alvaradolindner, B., McGuire, M. *et al.* (1994) Isolation and structure-activity features of halomon-related anti-tumor monoterpenes from the red alga *Portieria hornemannii*. *J. Med. Chem.*, **37**, 4407–4411.
- Gan, S.Y., Qin, S., Othman, R.Y., Yu, D.Z. and Phang, S.M. (2003) Transient expression of lacZ in particle bombarded *Gracilaria changii* (Gracilariales, Rhodophyta). *J. Appl. Phycol.*, **15**, 351–353.
- Gellenbeck, K.W. and Chapman, D.J. (1983) Seaweed uses – the outlook for mariculture. *Endeavour*, **7**, 31–37.
- Guillemin, M.L., Destombe, C., Faugeron, S., Correa, J.A. and Valero, M. (2005) Development of microsatellites DNA markers in the cultivated seaweed, *Gracilaria chilensis* (Gracilariales, Rhodophyta). *Mol. Ecol. Notes*, **5**, 155–157.
- He, P.M., Xu, S.N., Zhang, H.Y., Wen, S.S., Dai, Y.J., Lin, S.J. *et al.* (2008) Bioremediation efficiency in the removal of dissolved inorganic nutrients by the red seaweed, *Porphyra yezoensis*, cultivated in the open sea. *Water Res.*, **42**, 1281–1289.
- Heesch, S., Cho, G.Y., Peters, A.F., Le Corguille, G., Falentin, C., Boutet, G. *et al.* (2010) A sequence-tagged genetic map for the brown alga *Ectocarpus siliculosus* provides

- large-scale assembly of the genome sequence. *New Phytol.*, **188**, 42–51.
- Huang, X., Weber, J.C., Hinson, T.K., Mathieson, A.C. and Minocha, S.C. (1996) Transient expression of the GUS reporter gene in the protoplasts and partially digested cells of *Ulva lactuca* L. (Chlorophyta). *Bot. Mar.*, **39**, 467–474.
- Huh, M.K., Lee, H.Y., Lee, B.K. and Choi, J.S. (2004) Genetic diversity and relationships between wild and cultivated populations of the sea lettuce, *Enteromorpha prolifera*, in Korea revealed by RAPD markers. *Protistology*, **3**, 243–250.
- Ji, M.H. (ed.). (1997) *Seaweed Chemistry*. Science Press, Beijing.
- Ji, M.H., Shi, S.Y., Pu, S.Z. and Zhang, Y.X. (1963) Further studies on the comprehensive utilization of *Laminaria japonica* Aresch., (in Chinese with English abstract). *Stud. Mar. Sin.*, 77–101.
- Jia, J.H., Wang, P., Jin, D.M., Qu, X.P., Wang, Q., Li, C.Y. et al. (2000) The application of RAPD markers in diversity detection and variety identification of *Porphyra*. *Acta Bot. Sin.*, **42**, 403–407.
- Jiang, P., Qin, S. and Tseng, C.K. (2003) Expression of the lacZ reporter gene in sporophytes of the seaweed *Laminaria japonica* (Phaeophyceae) by gametophyte-targeted transformation. *Plant Cell Rep.*, **21**, 1211–1216.
- Jiang, P., Wang, J.F., Cui, Y.L., Li, Y.X., Lin, H.Z. and Qin, S. (2008) Molecular phylogenetic analysis of attached Ulvaceae species and free-floating *Enteromorpha* from Qingdao coasts in 2007. *Chin. J. Oceanol. Limnol.*, **26**, 276–279.
- Kim, J.S., Park, S.C., Kim, J.W., Park, J.C., Park, S.M. and Lee, J.S. (2010) Production of bioethanol from lignocellulose: Status and perspectives in Korea. *Biores. Technol.*, **101**, 4801–4805.
- Kong, F.N., Mao, Y.X., Yang, H., Qu, H.J., Yan, X.H. and Wang, L. (2009) Genetic analysis of *Porphyra yezoensis* using microsatellite markers. *Plant Mol. Biol. Rep.*, **27**, 496–502.
- Kostamo, K., Blomster, J., Korpelainen, H., Kelly, J., Maggs, C.A. and Mineur, F. (2008) New microsatellite markers for *Ulva intestinalis* (Chlorophyta) and the transferability of markers across species of Ulvaceae. *Phycologia*, **47**, 580–587.
- Kraemer, G.P., Carmona, R., Chopin, T., Neefus, C., Tang, X.R. and Yarish, C. (2004) Evaluation of the bioremediatory potential of several species of the red alga *Porphyra* using short-term measurements of nitrogen uptake as a rapid bioassay. *J. Appl. Phycol.*, **16**, 489–497.
- Kuang, M., Wang, S.-J., Li, Y., Shen, D.-L. and Zeng, C.-K. (1998) Transient expression of exogenous GUS gene in *Porphyra yezoensis* (Rhodophyta). *Chin. J. Oceanol. Limnol.*, **16**, 56–61.
- Kubaneck, J., Jensen, P.R., Keifer, P.A., Sullards, M.C., Collins, D.O. and Fenical, W. (2003) Seaweed resistance to microbial attack: A targeted chemical defense against marine fungi. *Proc. Natl Acad. Sci. USA*, **100**, 6916–6921.
- Kurtzman, A.L. and Cheney, D.P. (1991) Direct gene transfer and transient gene expression in a marine red alga using the biolistic method. *J. Phycol.*, **27**, 42.
- Li, X.-F., Sui, Z.-H. and Zhang, X.-C. (1998) Application of RAPD in genetic diversity study on *Gracilaria lemaneiformis* III. Phase and sex related markers. *Chin. J. Oceanol. and Limnol.*, **16**, 147–151.
- Liu, F.L., Shao, Z.R., Zhang, H.N., Liu, J.D., Wang, X.L. and Duan, D.L. (2010) QTL mapping for frond length and width in *Laminaria japonica* Aresch (Laminariales, Phaeophyta) using AFLP and SSR markers. *Mar. Biotechnol.*, **12**, 386–394.
- Liu, F.L., Wang, X.L., Liu, J.D., Fu, W.D., Duan, D.L. and Yang, Y.X. (2009) Genetic Mapping of the *Laminaria japonica* (Laminariales, Phaeophyta) using amplified fragment length polymorphism markers. *J. Phycol.*, **45**, 1228–1233.
- Luo, H., Morchen, M., Engel, C.R., Destombe, C., Epplen, J.T., Epplen, C. et al. (1999) Characterization of microsatellite markers in the red alga *Gracilaria gracilis*. *Mol. Ecol.*, **8**, 700–702.
- Mohan, M. and others. (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. *Mol. Breeding*, **3**, 87–103.
- Naldi, M. and Viaroli, P. (2002) Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po River Delta, Italy). *J. Exp. Mar. Biol. Ecol.*, **269**, 65–83.
- Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J. et al. (2000) Effect of aquaculture on world fish supplies. *Nature*, **405**, 1017–1024.
- Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C. et al. (2004) Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture*, **231**, 361–391.
- Pereira, R., Yarish, C. and Sousa-Pinto, I. (2006) The influence of stocking density, light and temperature on the growth, production and nutrient removal capacity of *Porphyra dioica* (Bangiales, Rhodophyta). *Aquaculture*, **252**, 66–78.
- Qin, S., Jiang, P., Li, X.-P., Wang, X.-H. and Zeng, C.-K. (1998) A transformation model for *Laminaria japonica* (Phaeophyta, Laminariales). *Chin. J. Oceanol. Limnol.*, **16**, 50–55.
- Qin, S., Jiang, P. and Tseng, C. (2005) Transforming kelp into a marine bioreactor. *Trends Biotechnol.*, **23**, 264–268.

- Radmer, R.J. (1996) Algal diversity and commercial algal products. *Bioscience*, **46**, 263–270.
- Ross, A.B., Jones, J.M., Kubacki, M.L. and Bridgeman, T. (2008) Classification of macroalgae as fuel and its thermochemical behaviour. *Biores. Technol.*, **99**, 6494–6504.
- Saito, M. and Fujita, Y. (1991) Optimization of fusion conditions in the polyethylene-glycol and the electric-stimulation methods for the protoplasts of *Porphyra*. *Nippon Suisan Gakk.*, **57**, 919–925.
- Shi, Y.Y., Yang, G.P., Liu, Y.J., Liao, M.J., Li, X.J. and Cong, Y.Z. (2007) Development of 18 polymorphic microsatellite DNA markers of *Laminaria japonica* (Phaeophyceae). *Mol. Ecol. Notes*, **7**, 620–622.
- Sim, M.C., Lim, P.E., Gan, S.Y. and Phang, S.M. (2007) Identification of random amplified polymorphic DNA (RAPD) marker for differentiating male from female and sporophytic thalli of *Gracilaria changii* (Rhodophyta). *J. Appl. Phycol.*, **19**, 763–769.
- Sun, J.W., Jin, D.M., Zhou, C.J., Yang, Q.K., Weng, M.L., Duan, D.L. *et al.* (2005) Identification of *Porphyra* lines (Rhodophyta) by AFLP DNA fingerprinting and molecular markers. *Plant Mol. Biol. Rep.*, **23**, 251–262.
- Sun, X., Zhang, X., Mao, Y., Sui, Z. and Qin, S. (2006) Identification of phase and sex-related ISSR markers of red alga *Gracilaria lemaneiformis*. *J. Ocean University of China*, **5**, 82–84.
- Suzuki, Y., Kametani, T. and Maruyama, T. (2005) Removal of heavy metals from aqueous solution by nonliving *Ulva* seaweed as biosorbent. *Water Res.*, **39**, 1803–1808.
- Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A.H., Kautsky, N. *et al.* (2003) Integrated mariculture: asking the right questions. *Aquaculture*, **226**, 69–90.
- Tseng, C.K. (1944) A valuable seaweed product. *Sci. Monthly*, **58**, 24–32.
- Tseng, C.K. (1946) Phycocolloids: useful seaweed polysaccharides. In: *Colloid Chemistry. Theoretical and Applied* (ed. J. Alexander). Reinhold Publishing Corporation, New York, pp. 629–734.
- Tseng, C.K. (2001) Algal biotechnology industries and research activities in China. *J. Appl. Phycol.*, **13**, 375–380.
- Villanueva, R.D. and Montano, M.N.E. (2003) Fine chemical structure of carrageenan from the commercially cultivated *Kappaphycus striatum* (sacol variety) (Solieriaceae, Gigartinales, Rhodophyta). *J. Phycol.*, **39**, 513–518.
- Wang, J., Jiang, P., Cui, Y., Deng, X., Li, F., Liu, J. *et al.* (2010) Genetic transformation in *Kappaphycus alvarezii* using micro-particle bombardment: a potential strategy for germplasm improvement. *Aquaculture Int.*, **18**.
- Wang, X.L., Yang, Y.X., Cong, Y.Z. and Duan, D.L. (2004) DNA fingerprinting of selected *Laminaria* (Phaeophyta) gametophytes by RAPD markers. *Aquaculture*, **238**, 143–153.
- Weng, M.L., Liu, B., Jin, D.M., Yang, Q.K., Zhao, G., Ma, J.H. *et al.* (2005) Identification of 27 *Porphyra* lines (Rhodophyta) by DNA fingerprinting and molecular markers. *J. Appl. Phycol.*, **17**, 91–97.
- Whapham, C.A., Blunden, G., Jenkins, T. and Hankins, S.D. (1993) Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *J. Appl. Phycol.*, **5**, 231–234.
- Wise, D.L. (1981) Probing the feasibility of large-scale aquatic biomass energy farms. *Solar Energy*, **26**, 455–457.
- Xu, W., Mai, K.S., Zhang, W.B., Liufu, Z.G., Tan, B.P., Ma, H.M. *et al.* (2004) Influence of dietary lipid sources on growth and fatty acid composition of juvenile abalone, *Haliotis discus hannai* Ino. *J. Shellfish Res.*, **23**, 1041–1044.
- Yang, Y.F., Fei, X.G., Song, J.M., Hu, H.Y., Wang, G.C. and Chung, I.K. (2006) Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. *Aquaculture*, **254**, 248–255.
- Zeisel, S.H. (2006) Betaine supplementation and blood lipids: Fact or artifact? *Nutr. Rev.*, **64**, 77–79.
- Zhang, Y.C., Jiang, P., Gao, J.T., Liao, J.M., Sun, S.L., Shen, Z.L. *et al.* (2008) Recombinant expression of rt-PA gene (encoding Reteplase) in gametophytes of the seaweed *Laminaria japonica* (Laminariales, Phaeophyta). *Sci. China Ser C*, **51**, 1116–1120.
- Zhao, J., Jiang, P., Li, N., Wang, J.F., Liu, Z.Y. and Qin, S. (2010) Analysis of genetic variation within and among *Ulva pertusa* (Ulvaceae, Chlorophyta) populations using ISSR markers. *Chinese Sci. Bull.*, **55**, 705–711.
- Zhou, D., Zhang, L.A., Zhang, S.C., Fu, H.B. and Chen, J.M. (2010) Hydrothermal Liquefaction of Macroalgae Enteromorpha prolifera to Bio-oil. *Energ. Fuel*, **24**, 4054–4061.
- Zhou, Y., Yang, H.S., Hu, H.Y., Liu, Y., Mao, Y.Z., Zhou, H. *et al.* (2006) Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. *Aquaculture*, **252**, 264–276.
- Zuo, Z., Chen, Y. and Li, B. (2006) Molecule tag in use for identifying a strain of *Porphyra haitanensis*, comprises using an amplified fragment length polymorphism marker technique. Univ. Xiamen.

# 27

## Current Trends and Future Prospects of Biotechnological Interventions Through Plant Tissue Culture in Seaweeds

**Abdul Bakrudeen Ali Ahmed and Rosna Mat Taha**

*Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia*

### 27.1 Introduction

Seaweed uses around the world include human foods, fertilizers, and the extraction of valuable products such as industrial gums and chemicals. Moreover, recent research has pointed to new opportunities, particularly in the field of medicine, associated with bioactive properties of molecules extracted from seaweeds. Seaweed may belong to one of several groups of multicellular algae: the red algae, green algae, and brown algae. As these three groups are not thought to have a common multicellular ancestor, the seaweeds are a polyphyletic group. In addition, some tuft-forming blue-green algae (cyanobacteria) are sometimes considered as seaweeds – “Seaweed” is a colloquial term and lacks a formal definition.

There are several ways to cultivate seaweed: fragments of plants, sporelings or spore can be seeded onto robes or other substrates and grown to maturity in the wild. An alternative to ocean-growing is the cultivation of seaweed in artificial enclosures, such as tanks or ponds, where seaweeds can be grown in high densities on otherwise low-value land. Both wild aquaculture and alternative grow-

ing methods are likely to be important for the growth of the industry. The seaweed research program is focusing initially on the potentially valuable native red seaweeds; however the skills and knowledge developed in the program will be readily transferable to a diversity of seaweeds.

The fundamental knowledge of the seaweed life cycle and the processes that affect tissue culture has borne fruit in improved seaweed tissue culture methods. Development of seed source from valuable red native seaweeds has taken place. The seaweeds have been induced to release spores in the laboratory with subsequent growth of sporelings. This is an important step in seaweed mariculture for both on growing in the wild or laboratory-based seaweed cultivation. The development of techniques for the culture of isolated plant organs, tissues and cells have led to several exciting opportunities in the area of plant biotechnology, and allowed widespread use of cell culture for *in vitro* genetic manipulation, plant propagation and production of commercially useful products (Cocking, 1990). Following the success achieved by application of these techniques in higher plants, tissue culture of seaweeds was initiated as early as

1978 (Chen and Taylor, 1978) with considerable interest and options to further enhance the economic prospects of seaweed resources as a whole. In addition, among the marine-living resources the macroalgae (seaweeds) are increasingly viewed as a major potential chemical resource, particularly for chemicals of industrial, pharmaceutical, and nutraceutical importance (Schnitzler *et al.*, 2001). The development of traditional macroalgal cultivation technology is aimed at the industrial scale production of biomass. This is labor intensive and requires a huge cultivable sea area for farming of seaweeds. The crop yields also are inconsistent and are subjected to several critical factors, such as quality of germplasm used for seeding, grazing, pathogens, mariculture practice, seasonal variation, and natural calamities like tsunamis and cyclones.

Many research articles have dealt at great length about the status, applications, potentials and needs in tissue culture of seaweeds (Aguirre-Lipperheide *et al.*, 1995). Since the major efforts have been made to develop basic background technologies for consistent production and regeneration of calluses from diverse groups of seaweeds (Rajakrishna Kumar, 2002). The techniques which have been so far described for propagation of seaweeds through tissue culture have been tested on the laboratory scale and have not been validated for their suitability in commercial scale production. However, the following aspects have to be critically studied if the economic prospects associated with *in vitro* cell culture technology are to be realized:

- Methods for producing viable axenic material with greater consistency are required.
- New tissue and callus culture methods need to be developed for obtaining callus from both pigmented and non-pigmented parts of thallus.
- Physiology of plant growth regulators in plant cell division and development needs to be studied if high callus induction rate and growth are required.
- Genotyping and selection of tissue culture progeny with desired stable traits must be studied.

In addition, reports describing true cell suspension cultures from seaweeds are rare and limited to the genus *Porphyra*. Techniques for developing cell suspension culture have to be carried out if high value secondary metabolites are to be produced *in vitro*. Therefore, the basic aspects of seaweed tissue culture are beyond the scope of this chapter.

## 27.2 Explants, sterilization and methods used in seaweed production

For the initiation of callus culture, the following factors are important: the origin of explants used for the establishment of callus cultures, the cellular/tissue differentiation status, external phytohormones, culture media and culture conditions (Yeoman and Yeoman, 1996). Macroscopic epiphytes are removed from the surface of the seaweeds under a dissecting microscope by gentle brushing with a camel-hair brush, sterile gauze or razor (Xue-Wu and Gordon, 1987). After the visible epiphytes have been removed, sonication and osmotic shock are optionally carried out, followed by treatment with a chemical agent as the primary sterilizer, the most common for seaweed development. This basic concept has not always been taken into account in Seaweed Tissue Culture (STC), as from the criteria used to select the explants, it is apparently assumed that any part of any alga is competent to plant growth regulators (PGRs), and this does not seem to be so. For example, explants from *Greteloupia doryphora* were not sensitive to any PGRs tested, as opposed to germinated carpospores and young sporelings that react to all PGRs (Garcia-Jimenez *et al.*, 1998). Cellular competence to plant hormones is understood as the status in which a cell must possess the ability to perceive a transducer and respond to a signal (Osborne and McManus, 2005). Seaweed plants produce equal or greater quantities of valuable agars and carrageens than do terrestrial plants. This step shows that marine farming of seaweed is feasible with no loss of commercial value of extracted products. Improved protocols for cleaning seaweed spores infected with bacteria and fungi have been developed. Successful culture of different types of seaweed on mussel farm long lines has been reported. Plants were sourced from the wild and successfully grown in marine farms (Baweja *et al.*, 2009).

Culture media concepts used for tissue culture were established for algal culture and can be found elsewhere (Stein, 1973), thus they will not be reviewed in this chapter. In contrast, a new approach and terminology distinguishes the work by Chen and Taylor in 1978 with *Chondrus crispus* from classical practices of algal cultivation. Two concepts were introduced: the explants, as the precise part of the thallus used for cultivation, and the requirement for aseptic or axenic conditions because a carbon source and PGRs were used. The techniques of cultivating cells and tissue have been referred to sometimes as 'aseptic culture of plants'. Therefore the absence of contaminants is assumed to be a fundamental requisite *in vitro*. Obtaining axenic explants from seaweeds is a more difficult process than in higher plants (Polne-Fuller, 1988) and in only a few cases has the

full axenicity of cultures been established (Fries, 1980). Seaweed explants that appear bacteria free under a dissecting microscope are regarded as sterile and are subsequently, so after epibiont removal, the tissue is further treated with antibiotics. After a minimum period of 2 weeks, the explants that appear bacteria free under a dissecting microscope are regarded as sterile and are subsequently transferred to a growth medium. In order to obtain a greater number of “new” plants, established calli are chopped into fine sections (Hurtado and Cheney, 2003); however, Wang (1993) claimed that he obtained a greater number of new plants from subcultures of chopped stock pieces than from shoots. Methods of tissue culture described in the present study are useful tools for the production of “new” plants that can serve as propagules in land and sea-based nurseries, and consequently for commercial farming. The concept of culturing the newly regenerated plantlets in bioreactors to mass produce individual plantlets in the future is worth exploring (Rorrer and Cheney, 2004; Baweja *et al.*, 2009).

Surface sterilization of seaweeds is difficult as they lack a thick protective surface, and therefore sodium hypochlorite and similar agents can easily damage the delicate tissue. The scope of these techniques has been extended for use in bioprocess technology for production of high value chemicals of immense commercial value in the pharmaceutical and nutraceutical sectors (Munoz *et al.*, 2006). Several protocols for routine callus induction and regeneration are now available in the literature for a wide range of seaweeds (Rajakrishna Kumar *et al.*, 2007; Baweja *et al.*, 2009). Therefore, *in vitro* cell culture technology facilitates development of new generation technologies in the area of seaweed cultivation and utilization that collectively help intensive cultivation of higher yielding strains, coupled with green processing technologies with controlled production of products of potential commercial applications at competitive rates.

### **27.2.1 Active chemicals and mechanism in seaweed production**

Plant cells are biosynthetically totipotent, which means that each cell in culture retains complete genetic information and hence is able to produce a range of chemicals found in the parent plant (Rao and Ravishankar, 2002). Nevertheless, the absolute control of growth and development as it is exerted by plant hormones and carbon sources in plant tissue culture is lacking. Carbon sources would be required whenever a rather high cell growth rate was needed. Therefore efforts should be focused on solving these two problematic topics, either by introducing new effective PGRs (like polyamines) on appropriate cells (competent cells), and adequately adjusting the culture medium towards the

addition of carbon sources, which furthermore should be metabolically compatible with algal cells. Seaweed protoplasts and derived free cell cultures could also be exploited in that way. To our point of view, effort must be driven in the direction for the purposeful use of STC including the application to seaweeds of molecular techniques, which are being considered with an increasing interest (Walker *et al.*, 2005). Doubtless, protoplast isolation and regeneration in marine seaweeds constitute the most promising and developed topic in STC. They may constitute the desired platform for STC. They may constitute the desired platform for STC, provided that spontaneous regeneration of cells into thalli could be controlled through PGR, and that mixo- or heterotrophic cell growth could also be achieved. As an example, incubation of microalgal cells with DNA is used for genetic transformation (Leon *et al.*, 2007; Baweja *et al.*, 2009).

### **27.2.2 Polyamines as growth promoters in seaweed production**

Chemical and substances are synthesized in particular cells and are transferred to other cells, which in extremely small quantities influence the development process. Like hormones, another group of PGRs, namely polyamines (PAs), are implicated in many biological processes in higher plants, including cell division, root and floral initiation, fruit development, senescence, and abiotic stress responses. PAs, spermidine, spermins, and their diamine obligate precursor putrescine, belong to a class of aliphatic amines that are commonly present in all living organisms and are labelled as a new class of growth substances or hormonal messengers. The occurrence of PAs has been reported in several intertidal marine macroalgae subjected to lethal hyposaline stress (Lee, 1998). Marian *et al.* (2000) have shown that the addition of  $\alpha$ -difluoromethylornithine, an irreversible inhibitor of polyamine synthesis, caused a decrease in growth rates and morphogenesis of sporelings of *Grateloupia* in glycerol-containing media. The endogenous levels of polyamines decreased in quantity from infertile status in *Grateloupia* sp. As well as correlating to variations at different stages of cystocarp maturation in *Gracilaria cornea*, spermine also promoted the maturation of cystocarps and the eventual liberation of spores from cultivated explants in both red species (Sacramento *et al.*, 2007). In tissue culture of carpospores of *Grateloupia*, spermine promoted callus formation when used alone, or regeneration when combined with the carbon source glycerol (Garcia-Jimenez *et al.*, 1998). Nevertheless PAs, or other unusual PGRs, that may include their own regulators are rarely used in seaweed tissue culture. Generally, putrescine and

spermidine are the most abundant while spermine only occurs in trace levels. Changes in environmental conditions influence the biosynthesis and accumulation of PAs in algae. This is an important consideration as many marine macroalgae grow in intertidal habitats where there are many environmental fluctuations that they need to tolerate in order to grow. For example, at a low salinity, six green macroalgae accumulated higher levels of putrescine (Lee, 1998). As in higher plants, PAs play an important role in cell growth and development of seaweeds, with an increase in endogenous concentrations just prior to cell division (Lee, 1998). PAs are present in plants in amount varying from micromolar to millimolar and thus their levels are significantly higher than those of plant hormones (Kakkar and Sawhney, 2002). These few examples suggest that PGRs (both hormones and polyamines) can potentially play a similar role in seaweed growth and development as in higher plants (Baweja *et al.*, 2009).

### 27.2.3 Plant growth regulators' role in seaweed production

Certain substances affect growth quite miraculously – the PGRs. PGRs, in the form of auxins and cytokinins, induce callus development in higher plants (Reinert and Bajaj, 1977), but this was refuted by Polne-Fuller and Gibor (1987), since their work did not affect growth morphology in any noticeable way. The right combination of auxin and cytokinins to be used is critical in callus induction and development (Dawes and Koch, 1991). PGRs influence callus induction not only by their concentration but also by providing changes in the sensitivity of cells for compound production (Trewavas and Cleland, 1983). Controversy arises since authors mostly center the discussion on the presence or absence of PGR, or go into trials as to whether it has an effect or not, thus missing other important concepts, such as: (i) Cellular competence of plant hormones in cultivated seaweeds; and (ii) the type of PGR to be used in STC. The role that PGRs have in multicellular algae is a controversial subject, and has been extensively reviewed (Tarakhovskaya *et al.*, 2007; Baweja *et al.*, 2009).

Auxins have many characteristic features such as polar translocation, root and shoot growth, root induction, delay in abscission, and differentiation of xylem elements. PGRs are involved in initial hormone binding and activation of the genes of meristematic cells by altering the secondary and tertiary messengers of a cellular cascade. In higher plant tissue culture, it is difficult to conceive genetic modification without the exquisite control of cell growth and development exerted by PGRs and a carbon source under aseptic conditions. The organic carbon source is essential to quickly

select the transformed cells from the population of cultivated cells, whereas PGRs organize growth to obtain the genetically modified plants (Pena, 2005). Multiple examples of the effects of PGRs on seaweeds offer a positive side to their controversial roles on marine seaweeds. Doubtless, Seaweed tissue culture must be ready for such biotechnological applications as PTC. Therefore, controlled cell growth and development, and consequently aseptic conditions, must not be disregarded (Baweja *et al.*, 2009).

Several PGRs can influence a single aspect of growth and development; a particular response probably results from a changing ratio of hormones rather than from the presence or absence of an individual hormone (Suri and Ramawat, 1995). Regarding the type of plant hormone to use, apart from the commonly used auxins (2,4 dichlorophenoxy acetic acid, 1-Naphthalene acetic acid, Indole butyric acid, indole-3-acetic acid, IAA); cytokinins (benzyladenine, isopentenyladenine, kinetin) and gibberellins (gibberellic acid) a recent line of evidence reflects that other plant hormones do exist and have important roles in marine microalgae. The first trials of cell and tissue culture with marine seaweeds have allowed us to define a plethora of applications that nowadays make it possible for us to select and define the source of tissue as explants, and to cultivate them axenically in enriched or artificial culture media. In such conditions, regeneration and even formation are achieved not with plant growth regulating substances, but as a result of the propagative capacity, the particular anatomy of seaweed thalli and/or the strong effect of the physical characteristic of the medium. In this state of the art method, STC may be already useful for certain biotechnological applications, such as clonal propagation of seed material for mariculture. Perhaps, PGRs may indirectly control gene expression through certain enzyme and messenger by acting during transcription, mRNA processing, mRNA stability, and at translational and post-translational modification (George *et al.*, 2008).

## 27.3 Micropropagation of seaweeds

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture method. Simple vegetative propagation of thallus segments (2–3 cm long) in laboratory culture is also utilized to ascertain various culture conditions and media required for successful tissue and protoplast culture in seaweeds. This practice is very useful, especially when one deals with species which are non responsive to tissue and protoplast culture. Seaweed tissue culture is, relatively, a developing area of research with just three decades of development and lags far behind that of

higher plants, which got started more than a century ago. The revolution made in plant cell and tissue culture studies has formed the foundation for the genetic engineering of crop plants (Cocking, 1990). Macropropagation using tissue culture methods are currently used for the large-scale propagation of clones with superior traits in a number of crop species. Studies have successfully employed these techniques for clonal propagation and maintenance of seed stock of economically important seaweeds for mariculture (Rajakrishna Kumar *et al.*, 2004). The methods described in the present study collectively assist in pursuing tissue culture research for other seaweeds while proving useful in clonal propagation of desired alga for field cultivation. Further callus obtained for these species provides an ideal option for maintenance and storage of germplasm as seed bank for cultivation (Reddy *et al.*, 2008).

Micropropagation is used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative reproduction. In higher plants, this disorganized growth was assigned to the effects of water potential of the medium on plant cell growth and development (Debergh, 1983). The regeneration of the explants (i.e., new thallus growth) is commonly achieved and it can even be exploited for marine agronomy (Dawes and Koch, 1991). The regeneration does not depend on whether the culture media are enriched or artificial seawater (Zuo-Mei, 1984). Sometimes, agar contributes to the matrix potential, whereas salinity affects the osmotic potential. Both are negative components of the water potential, thus retaining water, reducing cell growth rate and even inducing malformations. Such effects of the physical characteristics of the culture medium of seaweed growth *in vitro* were known to Robaina *et al.* (1990) working with three red seaweed species. Whether induced or not by the physical characteristic of the medium, certainly callus like structures are one of the outstanding features of seaweed tissue culture, being useful as a tool for further seaweed *in vitro* propagation. Asensi *et al.* (2001) induced morula like masses of pigmented cells from medullary explants from stipe of *Laminaria digitata* in Provasoli Enriched Seawater (PES) medium. When subcultured under continuous white light, they grew out as filaments, which tended to dissociate into spherical isolated cells, thus setting up a suspended cell filament culture. The ploidy and RAPD analysis of tissue culture progeny did not show any evidence for genetic variability. Dawes and Koch (1991) demonstrated the first successful branch of micropropagation and tissue culture of *Eucheuma denticulatum* and *Kappaphycus alvarezii* from the Philippines using modified protocols (Saga, 1986) and

combinations of auxins and cytokinins are plant growth regulators. The results of this study were further substantiated in Dawes *et al.*, 1993, 1994), wherein the same cultivars were tested for growth under laboratory and field conditions.

Regeneration or callusing may define a particular *in vitro* platform of seaweed cell and tissue culture techniques that could be exploited to propagate seaweed strains for mariculture or photo-bioreactor design (Rorrer and Cheney, 2004). Although these abilities of the seaweeds might be useful for propagation, STC must be ultimately adequate for plant genetic improvement. Reddy *et al.* (2003) and Rajakrishna Kumar *et al.* (2004) have used this phenomenon as a means of maintenance and clonal propagation of seed stock for mariculture of economically important seaweeds. Rorrer and Cheney (2004) employed micropropagules in bioreactor cultivation for production of low volume high value products from a variety of seaweeds. In addition, considering their utility in wide range of applications, some studies attempted to enhance the quantity of micropropagule production by developing innovative *in vitro* culture methods. Subculture of thin slices of pigments callus as embedded culture in 0.4% solidified PES medium enabled mass production of micropropagules from *Kappaphycus alvarezii* which is commercially cultivated worldwide for  $\kappa$ -carrageen.

Most recently, Titlyanov *et al.* (2006) while working on *Gelidium* described methods for mass generation of planting material for tank-bubbling and field cultivation using fragments and cell aggregates of apical meristem. Further, freeze-thawing of apical meristem tissues enabled the production of plantlets producing rhizoids that could be used for cultivation in the sea. Both these methods could effectively maximize the number of propagules per donor plant and further facilitate mass production of seed stock required for mariculture round the year. A similar approach has also been successfully demonstrated for producing mass plantlets and tetraspores from fragments and cell aggregates of meristematic and sub-meristematic tissue of *Palmaria palmata* (Titlyanov *et al.*, 2006; Reddy *et al.*, 2008).

## 27.4 Callus and cell suspension culture in seaweed production

Callus development in multicellular marine macroalgae has been related to the anatomical structure and cellular organization of the thallus (Aguirre-Lipperheide *et al.*, 1995). The earlier studies have often used the term “callus-like formations” to distinguish the callus of macroalgae (Yokoya *et al.*, 1993) from that of higher plants where the callus develops from differentiated tissues as a result of wounding (Yeoman, 1987). Despite the differences in cellular

organization among the red (pseudoparenchymatous type tissue) and brown (parenchymatous type tissue) seaweeds, the callus obtained from both groups showed more or less similar morphology, with uniseriate, pigmented, and branched filamentous outgrowths from both cortical and medullary tissues of explants. The callus in higher plants is friable and forms numerous cell aggregates when transferred to agitated liquid cultures. In contrast to this, the filamentous callus observed in seaweeds is rigid and regenerates rapidly into full plants when transferred to agitated liquid cultures and thereby limiting the scope of applications offered by cell suspension cultures.

The incidence of disorganized and callus-like growth has been reported frequently since the beginning of the STC initiative. Saga *et al.* (1978) described the formation of callus-like structures from *Laminaria augustata* for the first time. Chen and Taylor (1978) induced callus like structures from *Chondrus crispus* in solid media and regeneration in liquid media. Fries (1985) also reported that a colorless hair of *Fucus* can be developed into repeatedly branched filaments when incubated on agar medium. Polne-Fuller and Gibor (1987) reported callus formation from more than 20 species of seaweed, including green, brown, and red seaweeds, related to the tissue being cut and the stress on the interface between the solid surface and the seawater saturated air phase. Kimura *et al.* 1997 observed callusing in *Undaria undarioides* in ASP 12 nitriloacetic acid (NTA) medium. When these calli were further subcultured in liquid medium, sporophytes were regenerated. The initial objectives of developing tissue culture techniques for seaweeds has been either for understanding the fundamental aspects of callus formation, morphogenesis, role of plant growth regulators in morphogenesis, role of carbon sources on callus development and growth (Yokoya *et al.*, 2004). The plants with firm and thick thallus produced prominent callus tufts with abilities to proliferate in subcultures using both solid and liquid medium (Reddy *et al.*, 2008).

Seaweed calli, compared to those of higher plants, are generally slow growing and small in size. Furthermore, occurrence of calli in some seaweeds is sporadic, and the percentages obtained are often very low (Gusev *et al.*, 1987). In addition, the role of plant growth regulators and carbon sources on callus formation in seaweed explants is variable, and hence the occurrence of callus is suggested to be due to internal factors inherent to the explants rather than to the culture conditions employed (Aguirre-Lipperheide *et al.*, 1995). A number of studies have shown regeneration of micropropagules directly from callus and sometimes from explants of some red seaweed (Rajakrishna Kumar *et al.*, 2007; Baweja *et al.*, 2009).

The role of plant growth regulators on callus formation in seaweed explants is debatable and showed no definite

trend. Nevertheless, IAA, 2,4-dichlorophenoxy acetic acid (2,4-D) and kinetin had stimulatory role in callus formation, growth and regeneration both in intercalary and apical explants of *Gracilaria tenuis tipitata*, *G. perplexa* and *Grateloupia dichotoma* (Yokoya *et al.*, 2004). Kaczyna and Magnet (1993) also succeeded in induction of callus in *Gracilaria verrucosa* using a combination of auxins and cytokinins. The effect of PGRs on callus formation also varied with the seaweed and photon irradiance used during explant culture. The brown color morph of *Hypnea musciformis* had the highest rate of callus formation in high photon irradiance with low concentration of IAA, while the green color morph produced calli with 2,4-D irrespective of concentration studied and photon flux densities used. A water soluble extract from *Laurencia sp.* increased callus formation on explants of the same species (Robaina *et al.*, 1992). However, the type of substances involved in these responses and their mode of action are unknown. The subcultured callus tufts excised from *Turbinaria conoides* explants produced tiny somatic embryo-like colonies on pigmented filamentous cell with occasional friable callus clumps when cultured in soft agar. This finding perhaps highlights the need of innovative culture techniques for obtaining all categories of *in vitro* cell technology for seaweeds (Rajakrishna Kumar *et al.*, 2007).

*Kappaphycus alvarezii* plant was regenerated successfully from the callus. Most of the callus appeared to develop from the medullary tissue, as observed in previous studies like *Kappaphycus alvarezii* (Wang, 1993), *Eucheuma* (Hurtado and Cheney, 2003) and other carreegonophytes like *Chondrus crispus* (Chen and Taylor, 1978) and *Agardhiella subulata* (Huang *et al.*, 1998). The combination of PAA and zeatin at 1.0 mg/l proved to be sufficient in callus induction and development, and consequently for plantlet regeneration as demonstrated in *Eucheuma denticulatum* (Hurtado and Cheney, 2003). The work of Reddy *et al.* (2003) on *Kappaphycus alvarezii* demonstrated the regeneration of somatic embryos to whole plants from a pigmented uniseriate filamentous callus, similar to the results obtained by Cheney *et al.* (1987) in *Agardhiella subulata* and Polne-Fuller and Gibor (1987) in two species of *Eucheuma*. The tissue culture of *Gelidiella acerosa*, *Gracilaria corticata*, and *Tubinaria conoides* also produced calli that showed regeneration patterns quite similar to that of *Kappaphycus*, but the amount of propagules produced is comparatively less (Rajakrishna Kumar *et al.*, 2007; Reddy *et al.*, 2008).

## 27.5 Bioprocess technology and cell culture in seaweed production

Like other applications of biotechnology, modern bioprocess technology is an extension of ancient techniques for

developing useful product by taking advantage of natural biological activities. The development of techniques for the culture of isolated plant organs, tissues and cells have led to several exciting opportunities in the area of plant biotechnology, and allowed widespread use of cell cultures for *in vitro* genetic manipulation, plant propagation and production of commercially useful products (Cocking, 1990). The culture media and conditions generally employed for tissue and cell cultures are similar to those optimized for growing intact plants. Nevertheless, the selection of elite germplasm through clonal propagation is a continuous process and a substantial amount of harvest is utilized as seed material for subsequent cultivation. Further, isolation of useful mutants for cultivation through this process is very cumbersome and less likely to be successful as genetic variant cells in thallus are small in number and are veiled by more common non-variant cells (Garcia-Reina *et al.*, 1991). This has eventually led to the exploration of the possibilities of developing *in vitro* cell culture technology for this group of plants.

Bioprocess technology for the production of high value chemical from cell and tissue cultures of different macroalgae has been developed using specially designed photo-bioreactors (Rorrer and Cheney, 2004). The main advantage of photo-bioreactor cultivation of cell and tissue cultures of macroalgae is the enablement of continuous, steady and defined production of high yields of quality product, thereby circumventing the barriers of seasonality. Further, the downstream process used for recovery of products from cell culture is more environmental friendly as compared to conventional process that utilizes the whole plants as a source of raw material for seaweed extraction.

Bioprocess engineering of seaweeds is one of the most recent and exciting developments of seaweed biotechnology. This development has opened up new opportunities to produce and recover seaweed products directly from cells and cell aggregates in photo-bioreactors (Rorrer and Cheney, 2004; Munoz *et al.*, 2006), which dispense with the use of whole plants. The main advantage of photo-bioreactor cultivation of cell and tissue cultures of macroalgae is the enablement of continuous, steady and defined production of high yields of quality product, thereby circumventing the barriers of seasonality. Further, the downstream process used for recovery of products from cell culture is more environmentally friendly as compared to the conventional process that utilizes the whole plants as a source of raw material for extraction. The bioprocess technology consists of three main components: cell and tissue culture development, photo-bioreactor design, and identification of strategies for eliciting secondary metabolite biosynthesis. The former two components have been investigated using selective species known for biosynthesis of novel oxylipins and halogenated

terpenoids. Rorrer and Cheney (2004) also described a variety of methods of develop phototropic suspension culture suitable for *in vitro* cultivation in photo-bioreactor systems and assessed the factors limiting their process cultivation performance using different photo-bioreactor configurations. The photo-bioreactor cultivation of cell and tissue culture will no doubt form a potential technology platform for the controlled production of low volume high value products from macroalgal resources, including pharmaceuticals and nutraceuticals. Consequently, the macroalgal culture development providing a controlled growth environment suitable for secondary metabolite biosynthesis may outweigh the need for optimization of growth rate or minimize the inputs required for biomass production, thereby enhancing the efficiency of performance of photo-bioreactor cultivation (Reddy *et al.*, 2008).

The success of bioprocess technology for production of valuable compounds from macroalgae largely depends on the development of suitable *in vitro* tissue culture systems for bioreactor cultivation (Rorrer and Cheney, 2004). Although macroalgal tissue culture is underdeveloped relative to that of land plants, there are more than 40 species of seaweeds from which successful callus formation and subsequent plant regeneration have been accomplished (Rajakrishna Kumar, 2002). Furthermore, cell culture offers several advantages for the isolation of mutants over conventional selection procedures using whole plant material. Cell suspensions allow a very large number of cells to be screened simultaneously for a desired trait in a reasonable time frame and reproducible manner. In addition, regeneration of plants from callus can result in the recovery of new genetic variants as a consequence of the well known phenomenon of somaclonal variation (Evans and Sharp, 1983). Unlike higher plants, seaweeds show distinct alternation of generations (haploid and diploid) that can be effectively utilized for genetic improvement. Haploid tissues enable easy detection of mutants, while subsequent chromosome doubling produces fertile individuals homozygous at all loci and these provide pure breeding lines for selection and hybridization.

Successful regeneration of fresh plants from several seaweed species has been reported with a few dealing with inadvertent selection resulting from somaclonal variation. Yan (1984) obtained tissue culture progeny from calli of *Laminaria* and *Undaria* with rapid growth and high temperature tolerance for longer periods than the normal sporophytes. The *in vitro* culture of *Laminaria digitata* stipe medullary explants produced cell-filament suspension cultures which gave rise to normal sporophytic plants along with other forms. The genetic identity of these plants was confirmed with cell filament suspension using nine polymorphic micro-satellite markers, although flow cytometric

analysis of nuclei of cell filament and regenerated plants displayed 2C and 4C level (Asensi *et al.*, 2001). Garcia-Jimenez *et al.* (1998) described two types of callus of *Laurencia* obtained from the same plant with identical appearance in terms of pigmentation and texture. These differed markedly in regeneration potential and growth rate of the regenerated plants. The tissue culture progeny obtained from pigmented callus of *Kappaphycus* also showed enhanced growth rate as high as 1.5–1.8 times the rate of farmed plants propagated through vegetative means (Reddy *et al.*, 2003). The ploidy and RAPD analysis of tissue culture progeny did not show any evidence for genetic variability appearance in terms of pigmentation and texture. These differed markedly in regeneration and growth rate of the regenerated plants. The tissue culture progeny obtained from pigmented callus of *Kappaphycus* also showed enhanced growth rate as high as 1.5–1.8 times the rate of farmed plants propagated through vegetative means (Reddy *et al.*, 2003; Baweja *et al.*, 2009).

## 27.6 Remarks and conclusion

- The success achieved in *Chondrus crispus* (Chen and Taylor, 1978) and *Laminaria angustata* (Saga *et al.*, 1978) encouraged phycologists to develop new genetically improved strains through tissue culture and biotechnology of economically important species.
- Nowadays, the seaweed market has grown as predicted with prospects to go even further. Therefore potential improvements introduced through the application on *in vitro* techniques are expected to be even higher. The demand for the raw materials in food and phycocolloid industries is increasing day-by-day (Sahoo, 2000).
- Thus intensive work on new strain selection and improvement of an efficient mass culture system is clearly needed (Sahoo and Yarish, 2005). For the exploitation of seaweed at the cellular level, plant tissue culture constitutes a basic powerful tool (Rorrer and Cheney, 2004).
- Advances have been made in cell and tissue culture of seaweeds as a unique branch of *in vitro* techniques; however, they are lagging far behind those of land plants and are still in a state of development.
- Seaweed tissue culture techniques are expected to be developed enough in the near future that when combined with molecular genetics. This may give support to the same biotechnological applications as in higher plants in the genomic age, a field in which seaweed is also far behind higher plants (Grossman, 2005, Walker *et al.*, 2005).
- For that, we matched our description of the state of the art of Seaweed tissue culture to the milestones on the development of PTC as applied to biotechnology (Gamborg, 2002): (i) growing plant tissue or its cells on solidified gel or liquid nutrient media; (ii) plant regeneration from cultivated cells using PGRs, carbon sources and a precise formulation of the media; and (iii) genetic modification for plant improvement, growing and fashioning.
- Development of *in vitro* cell culture technology is of fundamental importance if seaweed biotechnology is to play a central role in the growth of global seaweed industry in future.
- Considering the present status of progress made in tissue culture of seaweeds, it would be realistic to believe that these techniques can help to the extent of generating genetic mutants of commercial importance. It is imperative to establish homozygous lines of economically important seaweeds for growth and phycocolloid yields. The benefits of *in vitro* cell manipulation techniques can be more effective and realized if such select genetic lines are used for improving the traits.
- The seaweeds growing demand for raw materials raises questions surrounding the sustainability of the new industry.
- The development of culture methods, particularly those for rare and slow-growing plants, is expected to have a significant environmental benefit by contributing to retention of biodiversity.

## References

- Aguirre-Lipperheide, M., Estrada-Rodriguez, F.J. and Evans, L.V. (1995) Facts, problems and needs in seaweed tissue culture: an appraisal. *J. Phycol.*, **31**, 677–688.
- Asensi, A., Gall, E.A., Marie, D., Billot, C., Dion, P. and Kloareg, B. (2001) Clonal propagation of *Laminaria digitata* (Phaeophyceae) sporophytes through a diploid cell-filament suspension. *J. Phycol.*, **37**, 411–417.
- Baweja, P., Sahoo, D., Garcia-Jimenez, P. and Robaina, R.R. (2009) Seaweed tissue culture as applied to biotechnology: Problems, achievements and prospects. *Phycol. Res.*, **57**, 45–58.
- Chen, L.C.M. and Taylor, A.R.A. (1978) Medullary tissue culture of the red alga *Chondrus crispus*. *Can. J. Bot.*, **56**, 883–886.
- Cheney, D.P., Luistro, A.H. and Bradley, P.M. (1987) Carrageenan analysis of tissue culture and whole plants of *Agardhiella subulata*. *Hydrobiologia*, **151/152**, 161–166.

- Cocking, E.C. (1990) All sorts of plant genetic manipulation. In: *Genetic Engineering of Crop Plants* (eds G.W. Lycett and D. Grierson). Butterworths, London, pp. 1–11.
- Dawes, C.J., Koch, E.W. (1991) Branch, micropropagule and tissue culture of the red algae *Eucheuma denticulatum* and *Kappaphycus alvarezii* farmed in the Philippines. *J. Appl. Phycol.* **3**, 247–257.
- Dawes, C.J., Trono, G.C. and Lluisma, A.O. (1993) Clonal propagation of *Eucheuma denticulatum* and *Kappaphycus alvarezii* for Philippine seaweed farms. *Hydrobiologia*, **260/261**, 379–383.
- Dawes, C.J., Lluisma, A.O. and Trono, G.C. (1994) Laboratory and field growth studies of commercial strains of *Eucheuma denticulatum* and *Kappaphycus alvarezii* in the Philippines. *J. Appl. Phycol.*, **6**, 21–24.
- Debergh, P. (1983) Effects of agar brand and concentration on the tissue culture medium. *Physiol. Plant.*, **59**, 270–276.
- Evans, D.A. and Sharp, W.R. (1983) Single gene mutations in tomato plants regenerated from tissue culture. *Science*, **221**, 949–951.
- Fries, L. (1980) Axenic tissue cultures from the sporophytes of *Laminaria digitata* and *Laminaria hyperborea*. *J. Phycol.*, **16**, 475–477.
- Fries, L. (1985) Propagation of *Fucus* (Fucales, Phaeophyta) by hairs with trichothallic growth. *Phycologia*, **24**, 481–484.
- Gamborg, O.L. (2002) Plant tissue culture and biotechnology milestones. *In Vitro Cell. Dev. Biol. - Plant.*, **38**, 84–92.
- Garcia-Jimenez, P., Rodrigo, M. and Robaina, R.R. (1998) Influence of plant growth regulators, polyamines and glycerol interaction on growth and morphogenesis of carposporelings of *Grateloupia* cultured in vitro. *J. Appl. Phycol.*, **10**, 95–100.
- Garcia-Reina, G.L., Gomez-Pinchetti, J.L., Robledo, D.R. and Sosa, P. (1991) Actual potential and speculative applications of seaweed cellular biotechnology: some specific comments of *Gelidium*. *Hydrobiologia*, **221**, 181–194.
- George, E.F., Hall, M.A. and De Klerk, G.J. (2008) Plant tissue culture procedure – background. In: *Plant Propagation by Tissue Culture*, Vol 1, 3rd edn (ed. E.F. George). pp. 508.
- Grossman, A.R. (2005) Paths towards algal genomics. *Plant Physiol.*, **137**, 410–427.
- Gusev, M.V., Tambiev, A.H., Kirikora, N.N., Shelyastina, N.N. and Aslanyan, R.R. (1987) Callus formation in seven species of agarophyte marine algae. *Bot. Mar.*, **95**, 593–597.
- Huang, Y.M., Maliakal, S., Cheney, D. and Rorrer, G. (1998) Comparison of development, photosynthesis and growth of filament clump and regenerated micro plantlet cultures of *Agardhiella subulata* (Rhodophyta Gigartinales). *J. Phycol.*, **34**, 893–901.
- Hurtado, A.Q. and Cheney, D.P. (2003) Propagule production of *Eucheuma denticulatum* (Burman) Collins et Harvey by tissue culture. *Bot. Mar.*, **46**, 338–341.
- Kaczyna, F. and Megnet, R. (1993) The effects of glyceron and plant growth regulators of *Garcilaria verrucosa* (Gigartinales, Rhodophyceae). *Hydrobiologia*, **268**, 57–64.
- Kakkar, R.K. and Sawhney, V.K. (2002) Polyamine research in plants – a changing perspective. *Physiol. Plant.*, **116**, 281–292.
- Kimura, H. and Notoya, M. (1997) Tissue culture of *Undaria undariodes* (Yendo) Okamura (Phaeophyta, Laminariales). *J. Mar. Biotechnol.*, **5**, 100–102.
- Lee, T.M. (1998) Investigations of some intertidal green macroalgae to hyposaline stress: Detrimental role of putrescine under extreme hyposaline conditions. *Plant Sci.*, **138**, 1–8.
- Leon, R., Galvan, A. and Fernandez, E. (2007) *Transgenic Microalgae as Green Factories. Advances in Experimental Medicine and Biology*. Springer, New York.
- Marian, F.E., Garcia-Jimenez, P., Robaina, R.R. (2000) Polyamines in marine macroalgae, levels of putrescine, spermidine and spermine in thalli and changes in their concentration during glycerol-induced cell growth in vitro. *Physiol. Plant.*, **110**, 530–534.
- Munoz, J., Armando, C., Cahue-Lopez Patino, R. and Robledo, D. (2006) Use of plant growth regulators in micropropagation of *Kappaphycus alvarezii* (Doty) in airlift bioreactors. *J. Appl. Phycol.*, **18**, 209–218.
- Osborne, D.J. and McManus, M.T. (2005) *Hormones, Signals and Target Cells in Plant Development*. Cambridge University Press, Cambridge.
- Pena, L. (2005) *Transgenic Plants. Methods and Protocols. Methods in Molecular Biology*, Vol. 286, Humana Press, New York.
- Polne-Fuller, M. (1988) The past, present and future of tissue culture and biotechnology of seaweeds. In: *Algal Biotechnology* (eds T. Stadler, J. Mollion, M.C. Verdu, Y. Karamanos, A. Morva and D. Christiaen). Elsevier, London, pp. 7–31.
- Polne-Fuller, M. and Gibor, A. (1987) Calluses and callus-like growth in seaweeds: induction and culture. *Hydrobiologia*, **151/152**, 131–138.
- Rajakrishna Kumar, G. (2002) Studies on the tissue culture of economic seaweeds. PhD thesis, Bhavnagar University, Bhavnagar, India.
- Rajakrishna Kumar, G., Reddy, C.R.K. and Bhavanath, J. (2007) Callus induction and thallus regeneration from the callus of phycocolloid yielding seaweeds from the Indian coast. *J. Appl. Phycol.*, **19**, 15–25.

- Rajakrishna Kumar, G., Reddy, C.R.K., Ganesan, M., *et al.* (2004) Tissue culture and regeneration of thallus from callus of *Gelidiella acerosa* (Gelidiales, Rhodophyta). *Phycologia*, **43**, 596–602.
- Rao, S.R. and Ravishankar, G.A. (2002) Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnol. Adv.*, **20**, 101–153.
- Ravishankar, G.A., Bhyalakshmi, N. and Rao, S.R. (1999) Production of food additives. In: *Biotechnology: Secondary Metabolites* (eds K.G. Ramawat and J.M. Merillon). Oxford IBH, New Delhi, p. 89.
- Reddy, C.R.K., Jha, B., Fujita, Y. And Ohno, M. (2008) Seaweed micropropagation techniques and their potentials: an overview. *J. Appl. Phycol.*, **20**, 609–617.
- Reddy, C.R.K., Raja Krishna Kumar, G., Eswaran, K., Siddhanta, A.K. and Tewari, A. (2003) *In vitro* somatic embryogenesis and regeneration of somatic embryos from pigmented callus of *Kappaphycus alvarezii* (Gigartinales, Rhodophyta). *J. Phycol.*, **39**, 610–616.
- Reinert, J. and Bajaj, Y.P.S. (1977) *Applied and Fundamental Aspects of Plant Cell, Tissues, and Organ Culture*. Springer-Verlag, Berlin, p. 803.
- Robaina, R.R., Garcia, J.P. and Luque, A. (1992) The growth pattern and structure of callus from the red alga *Laurencia* sp. (Rhodophyta, Ceramiales) compared to shoot regeneration. *Bot. Mar.*, **35**, 267–272.
- Robaina, R.R., Garcia, J.P., Garcia-Reina, G. and Luque, A. (1990) Morphogenetic effect of glycerol on tissue cultures of the red seaweed *Grateloupia doryphora*. *J. Appl. Phycol.*, **2**, 137–143.
- Rorrer, G.L. and Cheney, D.P. (2004) Bioprocess engineering of cell and tissue cultures for marine seaweeds. *Aquacultural Engineering*, **32**, 11–41.
- Sacramento, A.T., Garcia-Jimenez, P. and Robaina, R.T. (2007) The polyamine spermine induces cystocarp development in the seaweed *Grateloupia* (Rhodophyta). *Plant Growth Regul.*, **53**, 147–154.
- Saga, N. (1986) Pure culture of algae. In: *Plant Biotechnology* (eds Y. Yamada and Y. Okada). Tokyo Kaymkes Dojin, Tokyo, pp. 55–69.
- Saga, N., Uchida, T. and Sakai, Y. (1978) Clone *Laminaria* from single isolated cell. *Bull. Jap. Soc. Sci. Fish.*, **44**, 87.
- Sahoo, D.B. (2000) *Farming the Ocean: Seaweeds Cultivation and Utilization*. Aravali Book International, New Delhi.
- Sahoo, D.B. and Yarish, C. (2005) Mariculture of seaweeds. In: *Algal Culturing Techniques* (ed. R.A. Anderson). Academic Press, New York, pp. 1219–1237.
- Schnitzler, I., Pohnert, G., Hay, M. and Boland, W. (2001) Chemical defense of brown algae (*Dictyopteris* spp.) against the herbivorous amphipod *Longimana*. *Oecologia*, **126**, 515–521.
- Stein, J.R. (1973) *Handbook of Phycological Methods. Culture Methods and Growth Measurements*. Cambridge University Press, Cambridge.
- Suri, S.S. and Ramawat, K.G. (1995) *In vitro* hormonal regulation of laticifer differentiation in *Calotropis procera*. *Ann. Bot.*, **75**, 477–480.
- Tarakhovskaya, E.R., Maslov, Y.I. and Shishova, M.F. (2007) Phytohormones in algae. *Russ. J. Plant Physiol.*, **54**, 163–170.
- Titlyanov, E.V., Titlyanova, T.V., Kadel, P. and Luning, K. (2006) Obtaining of plantlets from apical meristem of the red alga *Gelidium* sp. *J. Appl. Phycol.*, **18**, 167–174.
- Trewavas, A.J. and Cleland, R.E. (1983) Is plant development regulated by changes in the concentration of growth substances or by changes in the sensitivity to growth substances? *Trends Biochem. Sci.*, **8**, 354–357.
- Walker, T.L., Collect, C. and Purton, S. (2005) Algal transgenic in the genomic era. *J. Phycol.*, **41**, 1077–1093.
- Wang, L.Z. (1993) Hybridization of macroscopic red algae by somatic cell fusion. Masters Thesis in Marine Biotechnology and Biology, Northeastern University, Boston, MA, pp. 72.
- Xue-Wu, L. and Gordon, E. (1987) Tissue and cell culture of New Zealand Pterocladia and Porphyra species. *Hydrobiologia*, **151/152**, 147–154.
- Yan, Z.M. (1984) Studies on tissue culture of *Laminaria japonica* and *Undaria pinnatifida*. *Hydrobiologia*, **116/117**, 314–316.
- Yeoman, M.M. (1987) Bypassing the plant. *Ann. Bot.*, **60**, 157–174.
- Yeoman, M.M. and Yeoman, C.L. (1996) Manipulating secondary metabolism in cultured plant cells. *New Phytol.*, **134**, 553–569.
- Yokoya, N.S., Guimaraes, M.P.B.S. and Handro, W. (1993) Development of callus like structures and plant regeneration in thallus segments of *Grateloupia filiformis* Kutzing (Rhodophyta). *Hydrobiologia*, **260/261**, 407–413.
- Yokoya, N.S., West, J.A. and Luchi, A.E. (2004) Effects of plant growth regulators on callus formation, growth and regeneration in axenic tissue culture of *Gracilaria tenuistipitata* and *Gracilaria perplexa* (Gracilariales, Rhodophyta). *Phycol. Res.*, **52**, 244–254.
- Zuo-Mei, Y. (1984) Studies on tissue culture of *Laminaria japonica* and *Undaria pinnatifida*. *Hydrobiologia*, **116/117**, 314–316.

# 28

## Detoxification Mechanisms of Heavy Metals by Algal-Bacteria Consortia

Enrique J. Peña-Salamanca\*, Ana Lucia Rengifo-Gallego and Neyla Benitez-Campo

*Applied Plant Biology Research Group Department of Biology, Universidad del Valle, Cali, Colombia*

### 28.1 Introduction

Heavy elements are defined as chemical elements whose density is at least five times heavier than that of water. Among 35 widely occurring metals, 23 are heavy elements or metals including Ag, As, Au, Bi, Cd, Ce, Cr, Co, Cu, Fe, Ga, Hg, Mn, Ni, Pb, Pt, Te, Th, Sb, Sn, U, V, and Zn (Kvesitadze *et al.*, 2006). In small amounts, most of these elements are indispensable for many organisms, but their enhanced doses induce acute or chronic poisoning. Ions most essential for life are the representative metal ions:  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ , and the transition metals: Mn, Fe, Co, Ni, Cu, Zn, Mo, and V. The essential metal ions have a variety of functions in biological systems. Their functions range from regulators of biological processes to important structural components in proteins (Nordberg *et al.*, 2005).

The toxicity of heavy metals is apparent in reducing growth and development in microorganisms and plants, which seriously harm the health of animals and humans. The deleterious effects of metal ions can be manifested in many ways, but their toxicity can be divided into five general groups (Kvesitadze *et al.*, 2006):

- 1 Displacing essential metal ions from biomolecules and other biologically functional units.
- 2 Blocking essential functional groups of biomolecules, including enzymes and polynucleotides.
- 3 Modifying the active conformation of biomolecules, especially enzymes and polynucleotides.
- 4 Disrupting the integrity of biomolecules.
- 5 Modifying some other biologically active agents.

The basis for the biological disruption by metal activities is basically based on their ability to bind strongly to oxygen, nitrogen and sulfur atoms, due to their abundance in biological systems, and their role to act as ligands to all essential metal ions (Nordberg *et al.* 2005). In addition, toxic metal ions can coordinate to essential functional groups of proteins which can render the protein inactive. This is especially true of Hg, which has a tremendously high affinity for sulfur, a common ion used to form amino-acid residues. In fact, the mechanisms of metal ion toxicity are directly related to the modes of metal ion binding in biological systems. A biomolecule that can bind a metal ion must possess a number of chemical characteristics, such as, a region that has a high concentration of oxygen, nitrogen or sulfur atoms, the number of donor atoms to stabilize the metal ion, and sufficient space in the metal ion space that allows an appropriate three-dimensional geometry about the metal ion (Gaur and Rai, 2001).

There are three major sources of heavy metals in most terrestrial ecosystems: the underlying parent material, the atmosphere, and the biosphere. Biotic sources of metals

are originally obtained from one of the other two sources. Particularly, inputs to a system from existing vegetation occur in different ways: inputs from above-ground biomass, from roots, and from below-ground biomass (Wang and Lewis, 1997; Perales-Vela *et al.* 2006). These inputs are also considered fluxes within the food chain of an ecosystem, since plants are the base of the uptake, transport, and accumulation of metal in biological systems (Kvesitadze *et al.*, 2006).

The need for a cost-effective process and safe methods for removing heavy metals from discharging effluents has resulted in search for other unconventional materials such as organic or inorganic sorbents (Loy *et al.*, 2004). The use of microbial biomass such as, fungi (Bang *et al.*, 2000), algae (Perales-Vela *et al.*, 2006), and bacteria (Loy *et al.*, 2004) for removal of heavy metals from aqueous solutions is gaining increasing attention. Recently, microbial systems have been successfully used as adsorbing agents for the removal of heavy metals. Microbial populations in metal polluted environments adapt to toxic concentrations and become metal resistant. Recently, there has been interest in the study of the metabolic capacity of plant-associated bacteria used for phytoremediation strategies. In the rhizosphere, many pesticides as well as trichloroethylene, polycyclic aromatic compounds, and petroleum hydrocarbons are degraded at accelerated rates. Although plant-associated bacteria have dynamic and possess varied metabolic capacities, current strategies on algal-bacteria consortia are little known and during the last years there have given a special attention to those interactions.

In this chapter, the strategies of algal-bacteria consortia to detoxify heavy metals and their potential of biotechnological applications on heavy metal treatments are discussed. Special attention to transformation processes of metal ions by algal associated bacteria is given.

## 28.2 Mechanisms used by algae in heavy metals tolerance and removal

The ability of eukaryotic algae to survive and reproduce in metal-polluted habitats may depend on genetic adaptation over extended time periods by mutation, genetic exchange, selection, and changes in physiology, resulting from metal exposure (Shaw, 1989; Peña *et al.*, 2005). The effects of heavy metal toxicity in algae may include:

- 1 An irreversible increase in plasmalemma permeability, leading to the loss of cell solutes (e.g.,  $K^+$ ) and changes in cell volume.

- 2 A reduction in photosynthetic electron transport and photosynthetic carbon fixation.
- 3 The inhibition of respiratory oxygen consumption.
- 4 The disruption of nutrient uptake processes.
- 5 Enzyme inhibition, due to displacement of essential metal ions
- 6 The inhibition of protein synthesis
- 7 Abnormal morphological development and ultrastructural changes.
- 8 The impairment of motility and loss of flagella in certain microalgae

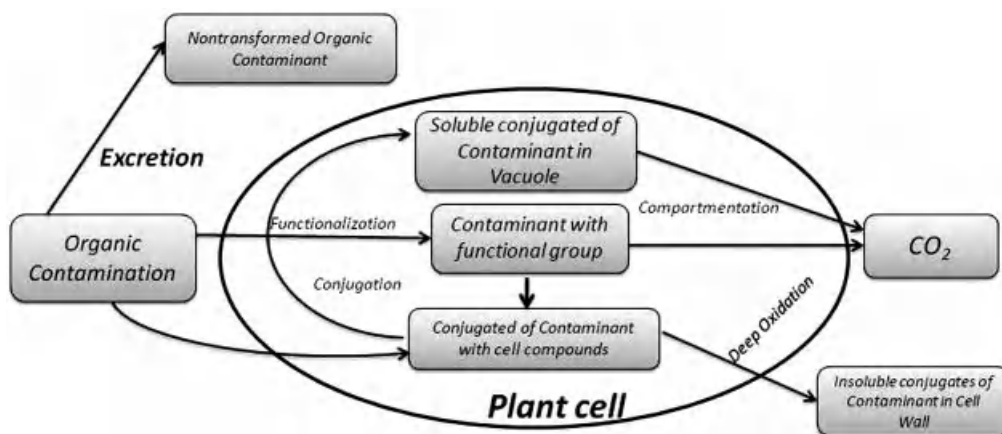
On the other hand, eukaryotic algae have developed some tolerance and detoxification mechanisms to allow them to resist metal ions inside their cells, which are shown in Figure 28.1.

### 28.2.1 Production of extracellular binding-polypeptides

One of the primary mechanisms observed in micro- and microalgae was related to the production of peptides capable to bind heavy metals (Nies, 1999). These molecules are further partitioned inside vacuoles to facilitate appropriate control of the cytoplasmic concentration of heavy metal ions (Cobbett and Goldsbrough, 2002). Those polypeptides are commonly named specific ion-chelators or siderophores. The complexing capacity of those ligands has been demonstrated primarily in cyanobacteria and freshwater microalgae (Butler *et al.*, 1980). They form large extracellular aggregates and possess anionic properties that are capable of binding metal cations. The peptides discussed can be grouped into two categories:

- 1 Short-chain polypeptides named phytochelatins (PCS) (class III metallothioneins, MT), found in higher plants, algae, and certain fungi (Nicholas *et al.*, 2003).
- 2 Specific proteins; class II MT (identified in cyanobacteria, algae and higher plants), and class I MT found in most vertebrates, observed in *Neurospora* and *Agaricus bisporus* (not reported in algae) (Robinson, 1989; Rauser, 1990).

The metal-binding polypeptides produced in algae are abundant in both sulfhydryl and carboxyl groups and could have affinity for a wide range of metal ions. In the case of MT they are low molecular weight, cysteine-rich metal



**Figure 28.1** Suggested mechanisms involved in plant detoxification for metal ions. Specific details are given in the case of algal–bacterial consortia, especially for the algal processes.

binding proteins. Class I and II MT are proteins which are encoded by structural genes (Rauser, 1990; Cobbett and Goldsbrough, 2002). Recently, the genes encoding for PCS activity were isolated (Perales-Vela *et al.*, 2006). Cysteine is part of the MT II chelating core and is an activator of the gene *PCS*. MT I and II biosynthesis can be induced by heavy metals such as  $\text{Cd}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Bi}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Au}^{2+}$ . MT III are synthesized in the cytosol and are subsequently transported into the chloroplast and mitochondria. This was first observed in *Euglena* organelles where almost 60% of the accumulated  $\text{Cd}^{2+}$  present inside the chloroplast was due to the Cd–MT III complexes (Schmitt *et al.*, 2001; Soldo *et al.*, 2005).

### 28.2.2 Exclusion mechanism

The slow phase of heavy metal accumulation by “binding sites” is often related with intraprotoplast uptake, or cell exclusion in contrast “rapid” physical binding or biosorption (Robinson, 1989; Mehta *et al.*, 2002). Indeed, changes in the affinity of binding sites within the cell wall matrix reflects, in part active (metabolism-dependent) uptake. Active transport systems have been described for several heavy metals in algae (Schiewer and Wong, 2000). Some metal-tolerant strains of microalgae may operate an exclusion mechanism, when reducing the internal accumulation capacity (Whitton, 1984). This process implies metal ions suffer antagonism, such as the case of iron and cadmium, in the marine diatom *Thalassiosira weissfloga* (Stauber and Florence, 1987). The exclusion mechanism may implicate a metal ion transport system in cadmium but an inhibition of iron. Consequently, a decreased internal accumulation

in iron appears as a tolerance of the metal by the alga (Peña *et al.*, 2004).

### 28.2.3 Internal detoxification

The study of internal detoxification of heavy metal in algae has received little attention than surface binding and transport. However, algae are able to activate a definite set of biochemical and physiological processes to resist the toxic action of environmental contaminants (Gaur and Rai, 2001). In microalgae such as the diatoms *Amphora* and *Navicula* copper is localized intracellularly in electron-dense spherical bodies corresponding to polyphosphate granules and in a dense irregular body containing sulfur, calcium, and copper (Ahner and Morel, 1990; Soldo *et al.* 2005). The main processes of detoxification are included: conjugation of the heavy metal with intracellular compounds, and further compartmentation of conjugates, degradation to common cell metabolites, and finally to carbon dioxide. Those processes can be regulated by environmental factors such as temperature, salinity, pH, and others, which implies the significance of those interactions with their self-resistance mechanisms (Ospina-Alvarez *et al.*, 2006).

### 28.2.4 Metal transformation

Algae can carry out chemical transformations of heavy metals such as oxidation, reduction, methylation, and demethylation. Those ones act as mechanisms of resistance, and most of them involved selective processes to eliminate non-essential metal ions for growth metabolism (Perales-Vela *et al.*, 2006; Lenis *et al.*, 2007). Methylation has been observed in brown algae, to detoxify Hg form aqueous

solution (Davis *et al.* 2003). The process implies degradation to less toxic compounds, as in the case of the transformation of tributyltin by *Chlorella*. The compound is debutylated to di- and monobutyltin molecules; although it should be stressed that light-stimulated photolysis also occurs (Toumi *et al.*, 2000). The transformation of those methyltin derivatives are also related with bacterial transformation. It has also been suggested that the decomposition products of arseno sugars from macroalgae lead to the formation of arsenobetaine. This compound is a ubiquitous component of marine animal tissues and the importance of algae as the initial source of this molecule in the marine environment is widely discussed (Davis *et al.*, 2003). Additionally, arsenic can be taken up by certain marine algae and converted to various organoarsenicals and such conversion may be a detoxification strategy (Schiewer and Wong, 2000).

## 28.3 Algal–bacterial mechanisms involved in heavy metal detoxification

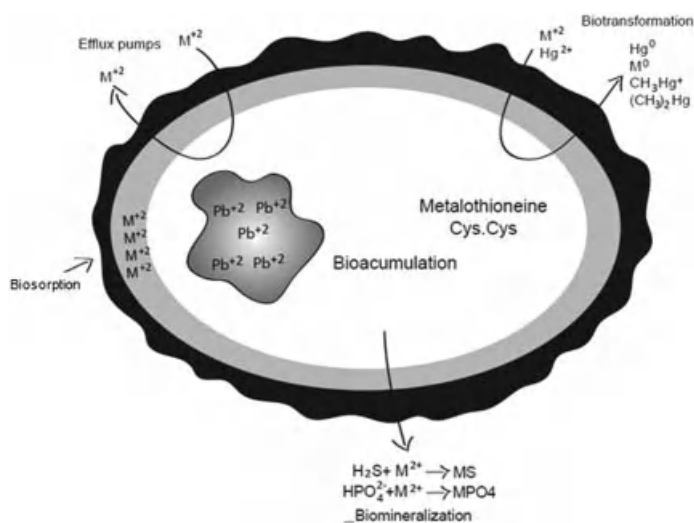
Recent studies have demonstrated that algal–bacterial consortia have an important role in the cycling of toxic metals and pollutants. Advances have been made in understanding metal–microbe interactions and new applications of these processes to the detoxification of metal and radionuclide contamination have been developed (Lloyd, 2003).

Overall, algae and bacteria share a variety of strategies for heavy metal tolerance. In some cases, metal tol-

erance is the outcome of their metabolism or is an intrinsic property related to their cell wall structure or the presence of extracellular polymeric substances. In other cases, the resistance mechanisms include active transport efflux pumps, or intra- and extracellular sequestration. In bacteria, they involve particularly enzymatic transformation, toxic chemical species by redox reactions, methylation, alkylation/dealkylation and reduction in the sensitivity of cellular targets to metal ions (Nies, 2003). In some plant bacterial association, these organisms underwent a variety of plasmid-mediated adaptation (Brinza *et al.*, 2007). The understanding of how those consortia resist metals can provide insight into strategies for detoxification or removal of pollutants from the environment. Both microorganisms and algae have adapted to the presence of different metal-toxic environments by developing a variety of mechanisms (Loutseti *et al.*, 2009). A number of mechanisms are proposed to explain how this consortium regulates the detoxification and transformation of essential and non-essential metal ions along with their biotechnological applications (Figure 28.2).

### 28.3.1 Biosorption

Recently microbial systems like fungi, bacteria, and algae have been successfully used as adsorbing agents for removal of heavy metals (Lee *et al.*, 2002; Wang and Cheng, 2009). Microbial populations in metal-polluted environments adapt to toxic concentrations of heavy metals and become metal resistant. Different species of *Aspergillus*, *Pseudomonas*, *Sporophyticus*, *Bacillus*, *Phanerochaete*, etc.,



**Figure 28.2** Main biological mechanisms involved in the detoxification of metals by plant-associated bacteria.

have been reported as efficient chromium and nickel reducers (Bapat *et al.*, 2003). Bacteria were used as biosorbents because of their small size, ubiquity, and ability to grow under controlled conditions; and their resilience to a wide range of environmental situations. Bacteria and algae have the ability to act as biological materials to accumulate heavy metals through metabolically mediated or physicochemical pathways of uptake or binding (Vieira and Volesky, 2000). These bioprocesses involve biosorptive (passive) uptake by dead biomass or bioaccumulation by living cells.

The main drawback in the use of algal–bacterial consortia as biosorptive materials is their ability to interact as ion-exchange synthetic resins and cell surface sequestration for metal ions. Nevertheless, biosorption methods seem to be more effective than their physicochemical counterparts in removing dissolved metals at low concentrations (below 2–10 mg/l) (Peña *et al.*, 2004) and demonstrate higher specificity, which avoids overloading of binding sites by alkaline-earth metals (Bunke and Buchholz, 1999).

### 28.3.2 Bioaccumulation

Nickel-resistant bacterial populations isolated from the green alga *Rhizoclonium riparium* (Cladophorales) exhibited reduced bioaccumulation when cells were in stationary phase (Peña *et al.*, 2004). In contrast, during the mid-log phase of cellular growth, metal uptake rate was higher, demonstrating the enhancing of Ni(II) removal by *Micrococcus* sp. The initial condition was a Ni(II) concentration of 50 mg/l, pH 7, temperature: 35 °C. Similar studies have been reported by *Enterobacter cloacae* (Leung *et al.*, 2000), *Bacillus circulans* (Tian-Wei *et al.*, 2004) and *Aspergillus* sp. (Nasseri *et al.*, 2002). All studies demonstrated that *Micrococcus* isolate were more effective for the removal of Cr(VI) and Ni(II) when compared with other microbial biomass reported. The initial metal ion concentration plays a role in determining the bioaccumulative capacity of bacterial isolate species. As the heavy metal concentrations increased, the cellular growth of all the isolates can be inhibited, depending on the metal ion kinetics (Prasenjiti and Sumathi, 2005).

### 28.3.3 Biotransformation and biomineralization

In microbial populations, the most widely studied biotransformation mechanism involves enzymatic reduction of metal ions to less toxic, volatile elemental (Nies, 1999). In addition to metal reduction, another strategy is the production of organic acids, and the generation of sulfuric acid through biooxidation of sulfur (e.g., by *Thiobacillus* spp.) (Gadd, 2000). A recent development has been the se-

quential extraction of copper by bacterial associated to root plants on macrophytes (e.g., by *Eichhornia crassipes*) (Peña *et al.*, 2005; Kumar-Rai, 2008). The mechanism involves preacidification by sulfur-oxidizing bacteria and the subsequent immobilization of the metals through organic acid production. In some bacteria, the transformation mechanism involves the presence of genes that form a specific ion-resistance operon (HgII), that not only detoxifies this ion but also transports and self-regulates resistance (Bruins *et al.*, 2000). This same set of genes also encodes the production of a periplasmic binding protein that regulates the biomineralization of mercury compounds to less toxic molecules which can be easily transport to cytoplasm for detoxification (Loy *et al.*, 2004).

## 28.4 Algal–bacteria consortia in the red algae *Bostrychia calliptera* (Rhodomelaceae)

During the last 20 years, the potential uses of macroalgae epiphytic on mangrove aerial roots have been studied as biomonitors of estuarine contamination (Peña, 1998; Peña, *et al.* 1999, 2005). Particularly, the metal concentrations of macroalgae and associated bacterial populations have extensively studied in the Buenaventura estuary, on the Pacific coast of Colombia (Peña *et al.*, 2004; Ospina-Alvarez *et al.* 2006; Peña, 2008; Rengifo, 2010). More recently, the percentage of chromium removal in the algae–bacterium association exposed to a set of different metal concentrations *in vitro* conditions were studied (Rengifo, 2010).

The monitoring of the estuarine pollution was motivated by the increase concerning of heavy metal pollution in the bay. The metals of concern, specifically chromium, copper, and lead, among others, enter waterways from a wide range of both natural and anthropogenic sources (Peña *et al.*, 2004). While external inputs of metals into estuaries are important, estuarine sediments themselves may become the main sources of contaminants to estuarine waters. Buenaventura bay is surrounded by extensive mangrove habitats that contain macroalgae attached to roots, tree trunks and mud surfaces (Peña, 1998). Despite the continual exposure of these algae to the high contaminant concentrations found within estuarine ecosystems, few studies have examined the effects of contaminants on the interactions in algal–bacterial communities in these tropical habitats.

Wild plants of *Bostrychia calliptera* associated with bacterial populations collected from Dagua River were monitored in the laboratory. The trial was conducted in synthetic seawater with two levels of chromium, 5 and 10 ppm, using bioreactors according to four treatments: unprocessed

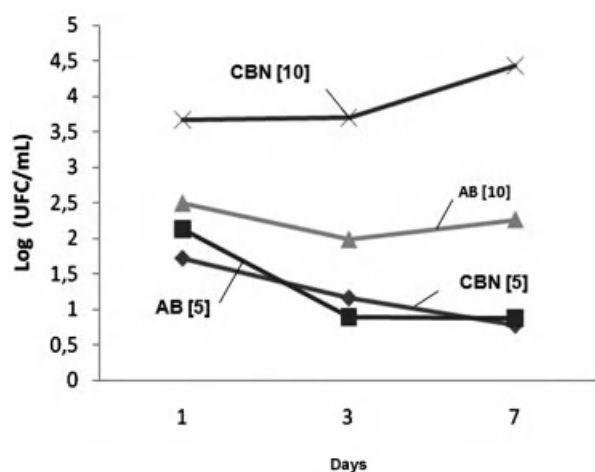
plant material (algae–bacteria), plant material with antibiotic (algae–antibiotic), sediment and/or suspended matter in surface algae (natural bacterial consortium or CBN), and the control without the presence of *B. calliptera* or bacteria. The experimental design followed a model of two factors (Concentration of chromium  $\times$  Types of combination) with repeated measures using one factor. The behavior of microbial populations and the chromium decrease concentration percentage was monitored by using atomic absorption spectroscopy (AAS).

Results showed greater bacterial growth at higher chromium concentrations (10 mg/l) compared to those with the treatment exposed at 5 mg/l. Additionally, significant differences were obtained for both, bacterial population to the total concentration of chromium in the algae–bacteria systems and CBN, algae–bacteria being the most efficient treatment to remove the metal at the highest metal concentration (Figure 28.3).

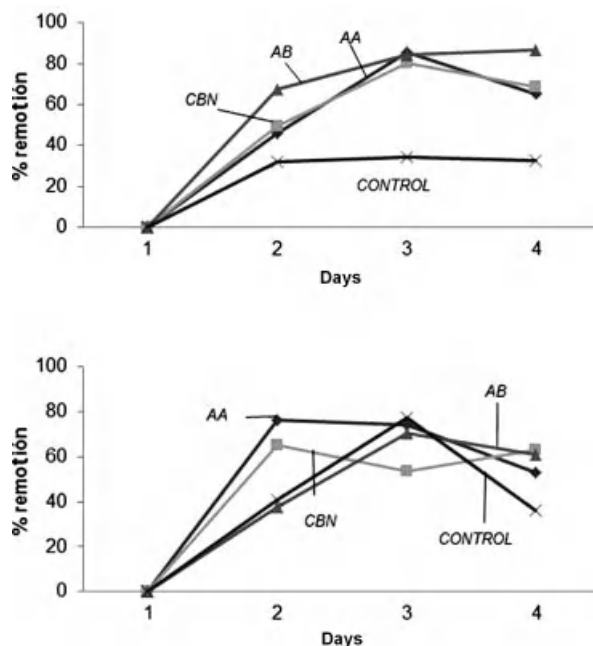
The natural consortia bacteria associated with the red algae (CBN–algae) showed higher chromium removal (Figure 28.4) suggesting their active role in the transformation processes of this metal in aqueous marine solutions at environmental levels.

## 28.5 Biological treatment of heavy metals

Conventional methods of heavy metal treatment are often expensive, hence alternative cost-effective technologies generally based on biological processes are being developed to



**Figure 28.3** Bacterial growth at different metal concentrations (5 and 10 mg/l). CBN (natural bacterial consortia); AB (isolated algal–bacteria strains).



**Figure 28.4** Percent of heavy metal removal by algal–bacteria consortia exposed to different chromium concentrations. (A) Percent removal at 5 mg/l. (B) Percent removal at 10 mg/l.

remediate heavy metal pollution (Vieria and Volesky, 2001). Bioremediation exploits microorganisms to deal with heavy metal pollution in a variety of methods such as bioleaching, biosorption, oxidation/reduction reactions, bioprecipitation and biomethylation. These techniques aim to change the speciation of the heavy metals, making them either more mobile in order to improve their removal, or decreasing their toxicity and mobility. Phytoremediation is a special situation in which plants and their associated microorganisms are used to assimilate and remove contaminants from the environment. Phytoremediation of heavy metals comprises several processes (Salt *et al.*, 1995). Although phytoremediation is a promising method, it is restricted to contamination at shallower depths and requires longer times compared to other methods (Peña *et al.*, 2005). Microorganisms can help plants to overcome heavy metal toxicity stress, either by decreasing metal toxicity or by counteracting the plant's stress response. In addition, they can assist the plants by rendering heavy metals more bioavailable, so improving their uptake. Recent advances demonstrated that all bioremediation/phytoremediation technologies rely on the genetic and biochemical capacities of the interactions of plant and microorganism to protect themselves against the toxic effects of heavy metals (Wang and Cheng, 2009). An understanding of the ways how algal–bacteria consortia cope with toxic concentrations of heavy metals is therefore essential in

order to exploit them for detoxification and removal of heavy metals.

## 28.6 Biotechnological applications

The efficiency of any biotechnological applications on heavy metal bioremediation depends on the activity of the microorganisms involved which is, in turn, affected by environmental conditions, operational parameters and the local composition of the overall algal–microbial community (Ospina-Alvarez *et al.*, 2006; Perales-Vela, 2006). When opting for a biological remediation strategy, important questions to be answered include:

- Are the organisms with the desired characteristics and activities present at the contaminated site?
- What is their activity?
- How is the algal–microbial community composition and function influenced by environmental parameters and process conditions?

Algae–bacterial associations have been traditionally used for pollution control, especially for the removal of inorganic nutrients (Toumi *et al.*, 2000). The most common arrangements used are high rate algal ponds (HRAP) and the patented Algal Turf Scrubber (ATS), which employs suspended biomass of common green algae (*Chlorella*, *Scenedesmus*, *Cladophora*), and bacteria (Cyanobacteria such as *Spirulina*, *Oscillatoria*, *Anabaena*) or consortia of both. The above mentioned algal systems have been tested for heavy metal removal (Peña *et al.*, 2005). Toumi *et al.* (2000) compared the heavy metal removal rates of traditional waste stabilization ponds (WSP) and a HRAP where both were receiving urban polluted water with trace concentrations of  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$ . It was found that HRAP had a higher removal rate per unit volume per day, with values up to 10 times more efficient in the case of  $\text{Cu}^{2+}$ . The values obtained could have resulted from the high pH achieved as a result of algal photosynthesis that enhances metal precipitation.

Adey *et al.* (1996) developed a system using consortia of filamentous cyanobacteria and suspended green algae for treating polluted underground waters. This research proved their advantage for the efficient removal of heavy metals, and also the removal of chlorinated and aromatic organic compounds was observed. The authors hypothesized that bacteria could have aided the biodegradation of aromatic compounds. Algae degradation of these chemicals has been recently reported and this is a growing field of research in environmental microbiology (Davis *et al.*, 2003).

The use of both living and death algal–microbial biomass for removal of heavy metals from aqueous solutions using biosorptive mechanisms is gaining increasing attention. Biosorption is regarded as a potential cost-effective biotechnology for the treatment of high volume low-concentration complex wastewaters containing heavy metals (Wang and Chen, 2009). It has been found that development of bioreactors with living cells for improving biosorption activity depends on properties of adsorbent and molecules in the transfer from the solution to the solid phase. It has also been reported that biosorption capacities for heavy metals are strongly pH sensitive and that adsorption increases as solution pH increases (Ospina-Alvarez *et al.*, 2006). It has been found that the plant-associated bacteria possessed maximum sorption capacity for the cationic metal ions at pH values between 4 and 6. At pH below 3, uptakes of copper, nickel and zinc were negligible, probably due to the cation competition effects with oxonium (hydronium) ion  $\text{H}_3\text{O}^+$  (Klimmek *et al.*, 2001).

In commercial applications, another factor affecting biosorption activity, beside pH are the multi-omponent metal solutions (Volesky and Naja, 2005). The sorption processes were found to be slower in a mixed-metal solution than in the single-component metal solutions, and equilibrium was reached after 5 h of the experiments. Reaching the equilibrium point, copper and zinc were bound 46 %, nickel 30 % and chromium 20 %. Moreover, during the next 5 hours there was no evidence in further uptake of metal ions (Volesky and Naja, 2005; Wang and Cheng, 2009). It can be concluded that the kinetics of biosorption appears to be faster in the single-component systems in the comparison with the multicomponent one. It is probably due to the absence of competitive processes between metals and biomass. Generally, for the future of biosorption technology, there are two trends of biosorption development for metal removal. One trend is to use hybrid technology (algae/bacteria biomass) for pollutants removal (Tsezos, 2001), especially using living cells. Another trend is to develop good commercial biosorbents, just like a kind of ion exchange resin, and to exploit the market with great endeavor (Volesky, 2007).

Recently, molecular and non-molecular methods for the identification and characterization of plant-associated bacteria and their specific properties have been used to assess the composition and activity of those consortia found at heavy metal-contaminated sites. These techniques promise to become complementary tools to classic chemical and physiological analysis (heavy metal concentrations and speciation, redox potential, etc.) for monitoring spatial and temporal changes in microbial community composition and function. Advances in understanding of the roles of these interactions in such processes, especially

for algal–bacterial consortia, together with the ability to fine-tune their activities using the tools of molecular biology, has led to the development of novel or improved metal bioremediation processes during the last years (Lloyd and Lovley, 2001).

## 28.7 Conclusions and future remarks

Significant advances have been made in understanding the roles of algae and bacteria in mineral cycling, and in the application of these processes to the bioremediation of metals. Additional advances are expected in the study of algal–bacterial interactions, focused on the use of new techniques, such as genomic approaches, which will undoubtedly make an impact in the area of environmental biotechnology.

Extensive surveys of heavy metal tolerant algal-associated bacteria are needed in order to obtain new data for specific strains that can be isolated for biotechnological applications such as biosorptive commercial designs. Studies that revise particular detoxification/resistance mechanisms should be verify to increase current knowledge of how they can involve in commercial applications for remediation of heavy metals in aqueous solutions. Especial attention is needed to identify candidate enzymes for genetic manipulation, responsible for the production and transportation of specific molecules involved in uptake and detoxification processes in algal–bacteria consortia.

## References

- Adey, W., Luckett, H. and Smith, C. (1996) Purification of industrially contaminated groundwaters using controlled ecosystems. *Ecol. Eng.*, **7**, 191–212.
- Ahner, B.A. and Morel, F.M. (1995) Phytochelatin production in marine algae. 2. induction by various metals. *Limnol. Oceanogr.*, **40**, 658–665.
- Bapat P. M., Kundu, S. and Wangikar, P. (2003) An optimized method for *Aspergillus niger* spore production on natural carrier substrates. *Biotechnol Prog.*, **19**, 1683–1688.
- Bang S.W., Clark, D.S. and Keasling, J.D. (2000) Engineering hydrogen sulfide production and cadmium removal by expression of the thiosulfate reductase gene (*phsABC*) from *Salmonella enterica* serovar typhimurium in *Escherichia coli*. *Appl. Environ. Microbiol.*, **66**, 3939–3944.
- Brinza, L., Dring, M.J., and Gavrilescu, M. (2007) Marine micro- and macro-algal species as biosorbents for heavy metals. *Environ. Eng. Manage. J.*, **6**, 237–251.
- Bunke, G., and Buchholz, R. (1999) *Metal Removal by Biomass: Physico-Chemical Elimination Methods*. Wiley-VCH Verlag, Weinheim. pp. 299–306.
- Butler, M., Haskew, A. E., and Young, M. (1980) Copper tolerance in the green algae *Chlorella vulgaris*. *Plant Cell. Environ.*, **3**, 119–128.
- Bruins, M.R., Kapil, S. and Oehme, F.W. (2000) Microbial resistance to metals in the environment. *Ecotox. Env. Safety*, **45**, 198–207.
- Cobbett, C. and Goldsbrough, P. (2002) Phytochelatin and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.*, **53**, 159–182.
- Davis, T., Volesky, B. and Mucci, A. (2003) A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res.*, **37**, 4311–4330.
- Gadd, G. (2000) Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Curr. Opin. Biotechnol.*, **11**, 271–279.
- Gaur, J.P. and Rai, L.C. (2001) Heavy metal tolerance in algae. In: *Algal Adaptation to Environmental Stresses. Physiological, Biochemical and Molecular Mechanisms* (eds L.C. Rai and J.P. Gaur). Springer-Verlag, Berlin, pp. 363–388.
- Klimmek, S., Stan, H.J., Wilke, A., Bunke, G. and Buchholz, R. (2001) Comparative analysis of the biosorption of cadmium, lead, nickel, and zinc by algae. *Env. Sci. Technol.*, **35**, 4283–4288.
- Kumar-Rai, P. (2008) Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: an eco-sustainable approach. *Int. J. Phytoremediation*, **10**, 133–160.
- Kvesitadze, G., Khatishashvili, G., Sadunishvili, T. and Ramsden, J.J. (2006) *Biochemical Mechanisms of Detoxification in Higher Plants*. Springer Verlag, Berlin. pp. 103–133.
- Lee, M.G., Lim, J.H. and Kam, S.K. (2002) Biosorption characteristics in the mixed heavy metal solution by biosorbents of marine brown algae. *Korean J Chem Eng.*, **19**, 277–284.
- Lenis, L.A., Benítez, R., Peña, E.J. and Chito, D.M. (2007) Extracción, separación y elucidación estructural de dos metabolitos secundarios del alga marina *Bostrychia caliptera*. *Scientia Et Technica*, **33**, 97–102.
- Leung, W.C., Wong, M.F., Chua, H., Lo, W., Yu, P.H.F. and Leung, C.K. (2000) Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater, *Wat. Sci. Technol.* **12**, 233–240.
- Loutseti, S., Danielidis, D., Economou-Amillia, A., Katsaros, Ch., and Santas, R. (2009) The application of a micro-algal/bacterial biofilter for the detoxification of copper and cadmium metal wastes. *Biores. Technol.*, **100**, 2099–2105.

- Lloyd, J.R. (2003) Microbial reduction of metals and radionuclides. *FEMS Microbiol. Rev.*, **27**, 411–425.
- Lloyd, J.R., and Lovley, D.R. (2001) Microbial detoxification of metals and radionuclides. *Curr. Opin. Biotechnol.*, **12**, 248–253.
- Loy, A., Lehner, K., Drake, H.L. and Wagner, M. (2004) Microarray and functional gene analyses of sulfate-reducing prokaryotes in low-sulfate, acidic fens reveal cooccurrence of recognized genera and novel lineages. *Appl. Environ. Microbiol.*, **70**, 6998–7009.
- Mehta, S.K., Tripathi, B.N. and Gaur, J.P. (2002) Enhanced sorption of  $\text{Cu}^{2+}$  and  $\text{Ni}^{2+}$  by acid-pretreated *Chorella vulgaris* from single and binary metal solutions. *J. Appl. Phycol.*, **14**, 267–273.
- Nasseri, S., Mazaheri, A.M., Noori, S.M., Rostami, K.H., Shariat, M. and Nadafi, K. (2002) Chromium removal from tanning effluent using biomass of *Aspergillus oryzae*. *Pak. J. Biol. Sci.*, **5**, 1056–1059.
- Nicholas, R.A., Stenberg, S.P.K., and Kathryn, C. (2003). Lead nickel removal using *Microspora* and *Lemna minor*. *Biores. Technol.*, **89**, 41–48.
- Nies, D.H. (1999) Microbial heavy-metal resistance. *Appl. Microbiol. Biotechnol.*, **51**, 730–750.
- Nies, D.H. (2003) Efflux mediated heavy metal resistance in prokaryotes. *FEMS Microbiol. Rev.*, **27**, 313–319.
- Nordberg, G.F., Fowler, B.A. and Nordberg, M. (2005) *Handbook on the Toxicology of Metals*, 3rd edn. AP Oxford, New York. pp. 487–510.
- Ospina-Alvarez, N, Peña, E.J. and Benítez, R. (2006) The effect of salinity on the bioaccumulation capacity of lead on green algae *Rhizoclonium riparium* (Roth) Harvey (Chlorophyceae, Cladophorales). *Actual Biol.* **28**, 17–25.
- Peña, E.J. (1998). Physiological ecology of mangrove associated macroalgae in a tropical estuary. PhD Thesis dissertation. University of South Carolina, EE.UU. 259 p.
- Peña, E. J. (2008) Dinámica espacial y temporal de la biomasa algal asociada a las raíces de mangle en la Bahía de Buenaventura, Costa Pacífica de Colombia. *Bol. Inv. Mar. Cost.*, **37**, 21–29.
- Peña, E.J., Zingmark, R. and Nietch, C. (1999) Comparative photosynthesis of two species of intertidal epiphytic macroalgae on mangrove roots during submersion and emersion. *J. Phycol.*, **35**, 1206–1214.
- Peña, E.J., Palacios M.L. and Ospina-Alvarez, N. (2005) *Algas como indicadores de contaminación*. Universidad del Valle, Cali. pp. 75–146.
- Peña, E.J., Ospina-Alvarez, N., and Benitez, R. (2004) Estudio de la contaminación por plomo, cobre y mercurio en la bahía de Buenaventura (Pacífico Colombiano) para la identificación de algas bénticas como organismos indicadores. *Pub. CYTED*, **10**, 167–176.
- Peña, J.M., Martínez-Jerónimo, F., Esparza-García, F. and Cañizares-Villanueva, R.O. (2004) Phenotypic plasticity in *Scenedesmus incrassatulus* (Chlorophyceae) in response to heavy metal stress. *Chemosphere*, **57**, 1629–1636.
- Perales-Vela, H.V., Peña-Castro J. and Cañizares-Villanueva, R.O. (2006) Heavy metal detoxification in eukaryotic microalgae. *Chemosphere*, **64**, 1–10.
- Prasenjit, B. and Sumathi, S. (2005) Uptake of chromium by *Aspergillus foetidus*, J. Mater. Cycles Waste Manag., **7**, 88–92.
- Rausser, W.E. (1990) Phytochelatin. *Annu. Rev. Biochem.*, **59**, 61–86.
- Rengifo, A. (2010) Caracterización bacteriana y evaluación del efecto de la asociación alga-bacteria (alga roja *Bostrychia calliptera* Rhodomelaceae) en el porcentaje de remoción de cromo. Tesis de pregrado, Universidad del Valle, Cali, Colombia. 58 p.
- Robinson, N.J. (1989) Metal-binding polypeptides in plants. In: *Heavy Metal Tolerance in Plants: Evolutionary Aspects* (ed Shaw, A.J.). CRC Press Inc., Boca Raton, FL, pp. 195–214.
- Salt, D.E., Blaylock, M., Kumar, M., Dushenkov, N.P., Ensley, V., Chet, B.D. and Raskin, I. (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology*, **13**, 468–474.
- Schiewer, S., and Wong, M. H. (2000) Ionic strength effects in biosorption of metals by marine algae. *Rev. Chemosphere.*, **41**, 271–282.
- Schmitt, D., Muller, A., Csogor, Z., Frimmel, F.H., and Posten, C. (2001) The absorption kinetics of metal ions onto different microalgae and siliceous earth. *Water Res.*, **35**, 779–785.
- Soldo, D., Hari, R., Sigg, L. and Behra, R. (2005) Tolerance of *Oocystis nephrocystioides* to copper: intracellular distribution and extracellular complexation of copper. *Aquat. Toxicol.*, **71**, 307–317.
- Stauber, J.L. and Florence, T.M. (1987) Mechanism of toxicity of ionic copper and copper complexes to algae. *Mar. Biol.*, **94**, 511–519.
- Tian-Wei, T., Hu, B. and Haijia, S. (2004) Adsorption of  $\text{Ni}^{2+}$  on amine-modified mycelium of *Penicillium chrysogenum*. *Enzyme Microb. Technol.*, **35**, 508–513.
- Toumi, A., Nejmeddine, A. and Hamouri, B. (2000) Heavy metal removal in waste stabilization ponds and high rate ponds. *Water Sci. Technol.*, **42**, 17–21.
- Tsezos M. (2001) Biosorption of metals. The experience accumulated and the outlook for technology development. *Hydrometallurgy*, **59**, 241–243.
- Vieira, R. and Volesky, B. (2000) Biosorption: a solution to pollution? *Int. Microbiol.*, **3**, 17–24.

- Volesky, B. (2007) Biosorption and me. *Water Res.*, **41**, 4017–4029.
- Volesky B., and Naja G. (2005). Biosorption: application strategies (eds. Harrison, S.T.L., Rawlings, D.E. & Petersen, J.). In: *16th International Biotechnology Symposium* Compress Co., Cape Town, South Africa. pp. 531–542.
- Wang, J. and C., Chen. (2009) Biosorbents for heavy metals removal and their future. *Biotech. Adv.*, **27**, 195–226.
- Wang, W. and Lewis, M. A. (1997) Metal accumulation by aquatic macrophytes. In: *Plants for Environmental Studies* (eds.: Wang, W., Gorsuch, J.W. & J.S. Hugkes). Lewis Publishers, New York, pp. 367–416.
- Whitton, B.A. (1984) Algae as monitors of heavy metals in freshwaters, In: *Algae as Ecological Indicators* (ed. Elliot, S. L.). Academic Press, New York, pp. 257–280.

# **PART V**

**Natural Resource Management and  
Industrial Applications of Seaweeds**

# 29

## Manufacturing Technology of Bioenergy Using Algae

**Gyung-Soo Kim**

*Biolsystems Corporation, 64-1 Umyeon-dong, Seocho-gu, Seoul, Republic of Korea*

### 29.1 Introduction

The word biomass comes from two root words, “bio” meaning life and “mass” meaning quantity. Biomass is the common name for organic materials used as renewable energy sources. Energy generated from biomass in the form of heat, electricity or as liquid/gas/solid fuel created via a biochemical/physical process, is called bioenergy. Although the term biomass generally refers to photosynthetic organisms, the energy industry also includes organic wastes such as manure and food waste into the definition of biomass. Biomass is classified according to the types of raw material as seen in Table 29.1.

It is estimated that 155 million tonnes of biomass is generated on Earth each year. This figure is estimated to rival the world’s crude oil reserves. Plants are the largest constituents of the above figure. As mentioned previously, biomass can be converted into bioenergy through biorefining which includes thermochemical and biochemical processes. Depending on the biomass used, the biorefinery can produce liquid fuels, such as bioethanol, biobutanol and biodiesel, or gaseous fuels such as hydrogen and methane.

### 29.2 Bioethanol types and characteristics

Currently, bioethanol feedstocks can be divided into three main categories: sugar-based (sugar cane, sugar beet, etc.),

starch based (corn, potato, yam, etc.) and woody (forest residues, waste wood, hay, etc.).

Sugar-based feedstocks can be refined into transportation ethanol at a relatively low cost through a simple pre-process followed by fermentation (Figure 29.1). However, use of sugar-based feedstock can only be utilized by countries such as Brazil, that possess vast amounts of arable land. Bioethanol production with sugar-based feedstock is not viable in nations such as Korea that do not meet this prerequisite. Starch-based feedstocks can easily be converted into glucose via saccharification. The glucose can then be used to produce bioethanol through the same process utilized by sugar-based feedstocks.

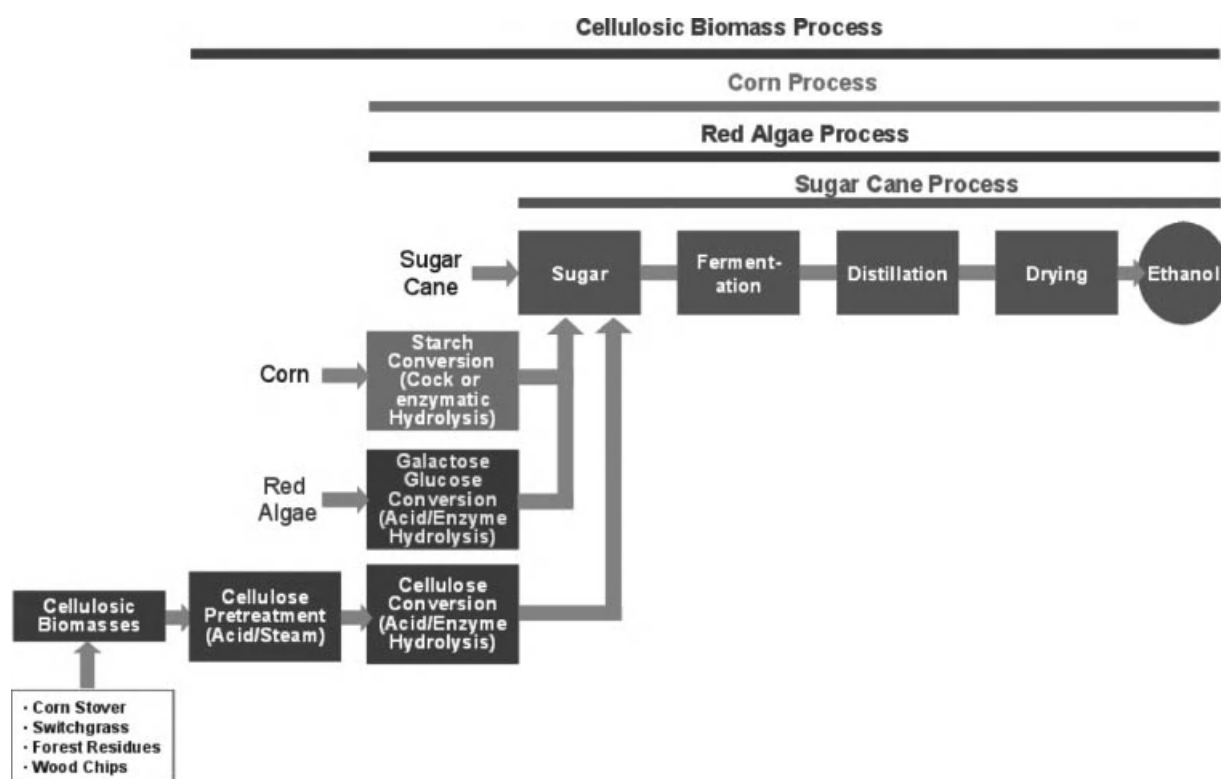
As stated above, current commercialized bioethanol production technologies use feedstocks that are used as food. In turn, the production methods above can have a negative impact on the world’s food supply and distribution in the long run. To overcome these issues, newer technologies are being researched to utilize woody feedstocks. This second-generation biomass has the advantage of being cheap and plentiful while avoiding the problem of competition with food supply. However, the lignin removal pretreatment, an essential step in the process, causes a considerable increase in the process cost. In addition, the strong hydrogen bonds present in cellulose molecules cause a lower saccharification yield. These obstacles need to be surpassed before woody feedstocks can be viable economically.

**Table 29.1** Main biomass types and uses

Biomass	Bioenergy	Application
Starch (corn, potato etc.)	Liquid fuel (ethanol, butanol)	Transportation fuel
Sugar (sugar cane, sugar beet etc.)	Solid fuel (pellet)	Heating fuel
Woody (forest residues, waste wood, hay etc.)	Syngas	Methanol
Animal waste, food waste, organic waste water	Methane	Local heating and cooling Electricity generation
Rape seed, soy, sunflower, Microalgae	Diesel	Transportation fuel
Organic waste water, sludge	Hydrogen	Fuel

## 29.3 Foreign and domestic bioethanol industries and technologies

The United States, along with Brazil, currently produces an estimated 95% of bioethanol. The feedstock of US bioethanol is mostly corn. Annual corn ethanol production worldwide is an estimated 19 850 000 liters (2007 statistics). The major producers are the United States, Europe and China. The United States produce 18 550 000 liters annually. In 1978, after the oil crisis, the United States passed the Energy Tax Act which grants a 4 cent per gallon federal tax break to fuel that contains up to 10% ethanol (E10, gasohol). This act has amplified the spread of mixed gasoline. The United States is looking to wean itself from foreign oil dependence by utilizing its vast arable land to grow feedstock and developing new renewable energy technologies. Brazil utilizes sugarcane as its main feedstock to produce ethanol. Of 17 820 000 liters of sugar-based ethanol produced worldwide, Brazil has accounted for 17 400 000 liters. Using the plentiful sugarcane feedstock, Brazil produces and distributes several varieties of gasohol. In 2003, FFVs (Flexible Fuel Vehicle) that can operate on any mixture of gasoline

**Figure 29.1** Bioethanol production process per feedstock type.

**Table 29.2** Status of bioethanol worldwide

Country	Policy	Production status
United States	E85, E10, E7	#1 in Worldwide production (19.85 million liters) Corn Feedstock Active research and production lead by DOE Tax break legislation in effect for renewable energy By 2017 20% of all transportation energy to be replaced by ethanol
Brazil	E100, E20	#2 in Worldwide production (17.82 million liters) Sugar cane Feedstock Government favored Proalcohol program in place. Flexible Fuel Vehicle 50% of new car market in 2005
China	E5	#3 in Worldwide production (3.8 million liters) Corn Feedstock Government subsidy and tax break benefits
Japan	E3	By 2030 10% of all transportation energy to be replaced by ethanol
Europe	E5	By 2030 20% of all transportation energy to be replaced by ethanol

and ethanol were released onto the market. By 2005, half of all consumer vehicles sold in Brazil were FFVs (Table 29.2).

Woody feedstock bioethanol production technologies have yet to reach the commercialization stage. Pilot process research is currently being conducted in the United States and Sweden. It is estimated that a practical process is around 5~10 years in the future. Domestic production of bioethanol is limited to the production of spirits from starch feedstocks for human consumption. As for research concerning the production of ethanol for fuel use, although various studies are underway currently, problems stemming from preprocessing and saccharification yield have prevented commercialization.

## 29.4 Algal biomass characteristics

Current commercialized bioethanol feedstocks, with the exception of woody feedstock, are biomass that has a direct effect on the world's food supply. As such, there are moral

issues at present as well as the question of if there will be enough supply to meet the rising energy demands. In addition, mass farming of corn, the current leading feedstock in world ethanol production, uses significant amounts of pesticides and nitrogen fertilizers, which in turn cause corrosion of arable land. Also, farming of any land-based plants cannot avoid the use of fresh water. The Stockholm Environment Institute warns of serious water scarcity in the near future. Therefore nations like Korea, that do not possess enough arable land and biomass, need to seek out alternate sources of biomass in order to produce bioenergy in sufficient quantities. The marine environment surrounding the Korean peninsula can be effectively used for both bioenergy production and CO<sub>2</sub> reduction.

Algae, compared to other biomasses, have a very fast cultivation cycle (In case of tropical climates, they can be harvested 4~6 times annually).

They are grown in the vast open sea eliminating the need for dry arable land. In addition they require very little or none of the costly resources that are required to grow other feedstock (e.g., fresh water, fertilizer etc.). Also, the preparation and saccharification processes are much simpler than that of woody feedstock as algae do not contain lignin. As the total conversion yield is high, if it were to be mass cultivated in Southeast Asia, bioethanol can be produced from algae at about the same level of process cost as sugar or starch based feedstock (Table 29.3).

## 29.5 Red algae bioethanol production technology

### 29.5.1 Overview

Algae are broadly classified into two categories: macroalgae and microalgae. Macroalgae are further divided into three categories: red algae, brown algae, and green algae.

**Table 29.3** Bioethanol production process cost per biomass type

Biomass	Feedstock cost (\$/1 kl)	Process cost (\$/1 kl)	Feedstock cost : process cost
Corn	390 <sup>a,b</sup> (69.8)	169 <sup>b</sup> (30.2)	7:3
Sugar cane	114 <sup>c</sup> (55.3)	92 <sup>c</sup> (44.7)	5.5:4.5
Switchgrass	140 <sup>d,e</sup> (32.8)	287 <sup>b</sup> (67.2)	3.2:6.7
Red algae	154 <sup>f</sup> (47.5)	148 (52.5)	4.7:5.2

<sup>a</sup>Park *et al.*, (2007), <sup>b</sup>Pimentel *et al.* (2005), <sup>c</sup>Novozymes (2007),

<sup>d</sup>Bangsund *et al.* (2008), <sup>e</sup>Joel (2007), <sup>f</sup>Kim (2009)

**Table 29.4** Chemical constituents of *Morracan Gelidium amansii*

Cellulose (fibers) (%)	Agar (galactan)		Others (protein, lipid, ash) (%)
	Galactose (%)	3,6-AHG (%)	
16.6	25.6	33.0	24.8

Bioethanol production technology with red algae feedstock will be the focus of this article. Bioethanol is gaining attention as one of the foremost bioenergies that can lower crude oil dependence. Some advantages of bioethanol include ease of transport/storage/use (compared with gaseous bioenergy) and ease of production (compared with butanol). The main reason why red algae are considered a suitable feedstock for ethanol production is due to its high carbohydrate content. For example, in the case of *Gelidium amansii*, 75.2% of its dry weight is composed of carbohydrates. The chemical constituents of *Gelidium amansii* are listed in Table 29.4 (Kim, 2007).

The red algae bioethanol production process is composed of [saccharification]–[fermentation]–[separation and refining]. The saccharification or hydrolysis process converts the polysaccharides present in red algae into monosaccharides that can in turn be used by various fermentation agents. The saccharification takes place through the use of enzymatic or acidic means. The saccharification product is then fermented into ethanol using the use of fer-

mentation agents. The fermented ethanol is then separated and refined to yield 99.5% purity fuel grade ethanol.

### 29.5.2 Saccharification process

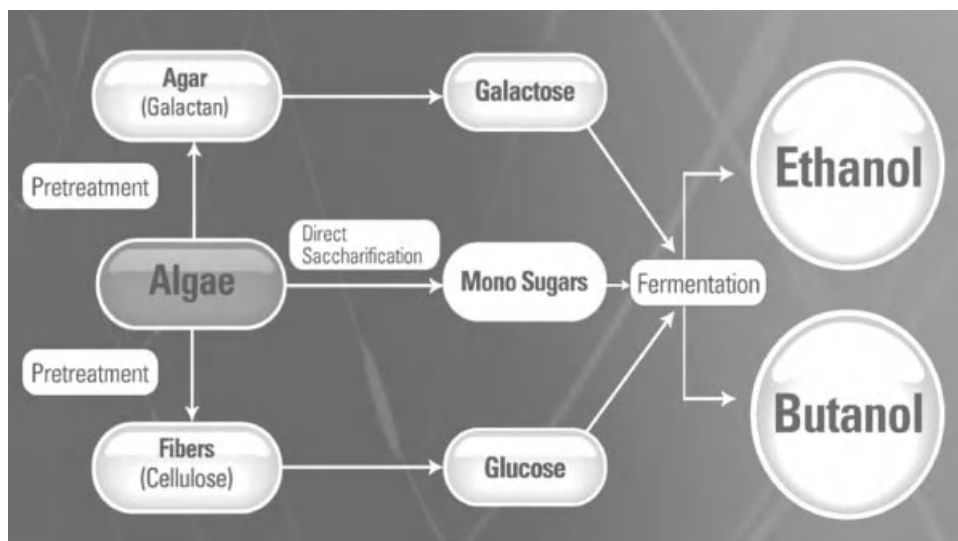
Compared to woody biomass that is mainly composed of lignin, C5 hemicellulose and cellulose, red algal biomass is mainly composed of galactan and cellulose. As such, the costly and troublesome lignin removal process, essential for woody bioethanol production, is not needed in red algae bioethanol production (Mosier *et al.*, 2005).

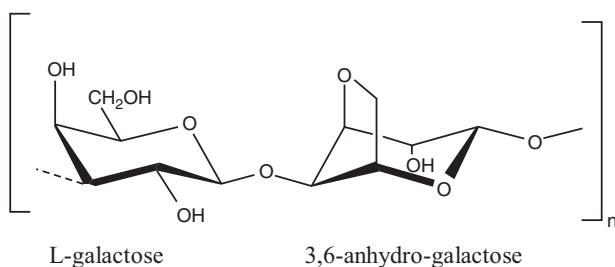
Red algal saccharification can be achieved through two different methods. The galactan–cellulose mixture can be saccharified together without separation (direct saccharification) as illustrated in Figure 29.2. Alternatively, galactan and cellulose can be separated then be saccharified independently using optimal conditions (indirect saccharification).

Galactan, present in red algae, is a galactose-based polysaccharide whose basic structure is composed of alternating chains of 3-linked  $\beta$ -D-galactopyranosyl units and 4-linked  $\alpha$ -3,6-anhydrogalactopyranosyl units. Depending on type, the L and D forms of 3,6-AHG exist; the former unit is called agarobiose while the latter unit is named carrabiose (Knutsen *et al.*, 1994).

Galactan, the agar present in many red algae, is roughly composed of 70% agarose and 30% agaropectin. Agarose is a linear polymer made up of repeating units of agarobiose (Figure 29.3).

The molecular weight of agarose is around 120 000. Agaropectin is irregularly modified with acidic side groups such as sulfates and pyruvates but share the same basic

**Figure 29.2** Algae bioethanol production process illustration.



**Figure 29.3** Agarose structure.

unit (agarobiose) with agarose. Cellulose, however, is a linear chain consisting of 4-linked  $\beta$ -D-glucopyranosyl units. Therefore, as pertains to red algae bioethanol production, saccharification involves producing D-galactose, D-glucose and 3,6-L-AHG by hydrolyzing the following bonds:  $\beta$ -1-4 glucosidic bond between D-galactose and 3,6-L-AHG,  $\alpha$ -1-3 glucosidic bond between 3,6-LAH and D-galactose,  $\beta$ -1-4 glucosidic bond between D-glucose and D-glucose (Table 29.5).

As red algae carbohydrates do not contain chemicals such as lignin that interfere with reactions with catalysts/enzymes, saccharification process methods involving acids or enzymes used in woody bioethanol production can be applied without the costly preprocessing.

Acid saccharification uses acids such as sulfuric acid to hydrolyze glucosidic bonds. While this method is simple to control and requires a relatively short reaction time, it also has some disadvantages. Some disadvantages include the possible yield of byproducts that prevent fermentation such as 5-(hydroxymethyl) furfural (5-HMF), cost to create the high temperature/pressure environment that is needed for the reaction to occur and the need for downstream processes that neutralize the pH and remove the gypsum. It follows that for effective acid saccharification, S/L (solid/liquid), acid type, acid concentration and temperature need to be all optimized. The author of this article

have found that in the case of red algae, as compared to the hydrolysis of woody biomass, can be effectively saccharified at lower temperatures (140–150 °C) and higher S/L ratios (20%). Also in these conditions, the levels of 5-HMF in the saccharification product was found to be harmless to the fermentation agents. Additionally, if galactan was to be extracted and put through acid saccharification, the entire reaction can be completed at 121 °C. In this case, if an acidic ionic liquid catalyst is used instead of sulfuric acid, it causes a high saccharification yield as well as a reduction of 5-HMF yield.

Enzymatic saccharification means using an enzyme that breaks glucosidic bonds, and when fitted suitable enzyme is used at high temperatures and pressures, polymers can be easily transferred into monomers without inhibitors. Since the wood bioethanol production is estimated to spend its unit cost mostly on enzymes, enzyme development research is actively in process around the world. Cellulose, one of the components of red algae, could be effectively hydrolyzed if enzymes like endo- $\beta$ -1,4-glucanase (EG), cellobiohydrolase (CBH), or  $\beta$ -glucosidase (BGL) act complementarily (Wong *et al.*, 1988; Medve *et al.*, 1988). EG randomly hydrolyzes  $\beta$ -1,4-bonds among the chains inside the cellulose, and CBH gradually hydrolyzes the reducing and non-reducing end chains of cellulose polymer, which are hydrolyzed by EG, and produces the combined bimolecular glucose, cellobiose. Cellobiose works as a strong restrainer for EG and CBH for their vitality, and BGL prevents this by hydrolyzing cellobiose. We found out that red algal cellulose and residuals from the primal acid saccharification could be easily transferred into glucose with the help of the enzymes, and obtained the mixture of red algae cellulose breakdown enzyme, enzyme improvement, and recombination mass production.

According to the research findings so far, saccharification of red algae has two paths: direct saccharification is suitable for acid saccharification or sequential application with enzymatic saccharification, and for the indirect one, acid saccharification for galactan and enzymatic saccharification for cellulose is favorable. The residues from saccharification are put into the latter ethanol fermentation group after some expensive separation unit process and pH control, such as filtration and centrifuging. Also, these kinds of saccharification processes can be used in other types of bioenergy manufacturing like butanol.

**Table 29.5** Carbohydrate makeup and monosaccharide contents of bioethanol feedstock

Feedstock	Carbohydrate	Monosaccharide
Sugar type	Sugar	Glucose, fructose
Starch type	Starch	Glucose
Wood type	Cellulose, hemicellulose, lignin	Glucose, C6 and C5
Red algae	Galactan, cellulose	Galactose, 3,6-AHG and glucose

### 29.5.3 Fermentation process

The production of alcohol using polysaccharides such as starch is one of the oldest fermentation technologies that humans have discovered. Yeast such as *Saccharomyces*



Operating modes of the fermentation process can be classified as batch, fed batch, or continuous modes. Batch process has been long been used to produce alcoholic beverages and has the advantages of simple structure and low contamination risk. Due to these advantages, most commercial processes utilize this operation. However, it also has some weaknesses such as low production yield, high cost of resources used for pre- and postprocess treatments and substrate inhibition (Chandal *et al.*, 2007). Accordingly, research to develop more effective fed batch and continuous processes is taking place. Simultaneously, techniques such as immobilization and cell recycling that can maintain high concentrations of bacteria strains are continually being reported.

In summary, for effective fermentation of red algae saccharification product into ethanol, two conditions must be met. First, a bacterial strain must be found that can effectively convert the galactose/glucose mixture and also must be resistant to salts and 5-HMF. Second, a new process needs to be developed that uses the aforementioned bacterial strain. The author of this article have found a strain of bacteria that can effectively metabolize galactose and also is resistant to inhibitors. In addition the authors are drawing up process conditions that are optimal for concentrated ethanol production

#### 29.5.4 Separation and distillation process

The fermentation product is put through the separation and distillation process after the yeast cells are removed using the centrifuge. The most typical ethanol separation technique is distillation, taking advantage of ethanol's boiling point (78.4 °C), which is considerably lower than that of water. However, because ethanol and water form a binary azeotrope, the purest ethanol that can yield from distillation is 95.7% (w/w). As 99.5% purity is required to utilize ethanol in an internal combustion engine and 99.8% purity is required to utilize ethanol as a chemical fuel, a subsequent dehydration process is required (Cho, 2004, First Edition Ethanol Guide, 2009). The traditional refining method is azeotropic distillation that involves adding benzene, which dehydrates the product by evaporation of the gaseous benzene-H<sub>2</sub>O mixture. However, this method requires a large energy cost. As such, other methods such as passing the ethanol/water vapor through a molecular sieve are being used. The type of molecular sieve used in ethanol refining is one whose pores are 0.3 nm in diameter. Since the size of water vapor molecule is greater than 0.3 nm and the size of the ethanol vapor molecule is smaller than 0.3 nm, the ethanol vapor passes freely through the molecular sieve while the water vapor is trapped within the sieve.

If the molecular sieve is used, there is an energy savings of 840 kJ/l ethanol (Madson and Monceaux, 2003).

## 29.6 Future technology outlook

A three-step strategy over 13 years from 2007–2020 with the goal of establishing a stable source of bioenergy by 2020 is in process in Korea. First, from 2007–2010, the focus has been on securing a viable bioethanol production technology. The resulting technology will then commercialize to replace domestic gasoline consumption by 5% in 2015 and 20% in 2020. If 20% of transportation fuel is replaced by bioethanol, US\$700 Million in crude oil import costs and US\$300 Million in CO<sub>2</sub> reduction costs can be combined to cut back US\$1 billion annually in energy costs. After 2020, the plan is to increase the replacement of gasoline to 40% and devise a pathway to export into the world's energy market. Meanwhile, as research related to bioenergy production is a complex blend of technologies comprised of fermentation research, metabolic studies and bioplatfrom technologies, we can predict that it will assist in stimulating energy industries and various researches. First and foremost, the proprietorship of bioethanol technology that allows domestic production and use will allow development of other biomass technologies that utilize newer varieties of biomass. Additionally, since saccharification, fermentation process modeling, optimization technology and process planning can be used not only by red algae bioethanol production process but can be universally applied toward all types of biorefinery technologies, it will have a major effect on the biotechnology industry sector as a whole. Through these effects, red algae bioethanol production will allow Korea to maintain its status as a producer nation by meeting the CO<sub>2</sub> emission regulations set by the Kyoto Protocol and further play a major role in allowing Korea to develop into a foreign energy independent nation.

## Acknowledgments

This report is contributed by the Biotech Policy Research Center of BIOIN ([www.bioin.or.kr](http://www.bioin.or.kr)).

## References

- Bangsund, D.A. DeVuyst, E.A. and Leistritz, F.L. (2008) Evaluation of breakeven farm-gate switchgrass prices in south central North Dakota, *Agribusiness and applied economics report No. 632-S*. North Dakota State University, North Dakota.

- Chandal, A.K., Chan, E.S., Rudravaram, R., Narasu, M.L., Rao, L.V. and Ravindra, P. (2007) Economics and environmental impact of bioethanol production technologies: an appraisal. *Biotechnol. Mol. Biol. Rev.*, **2**, 14–32.
- Cho, M.G. (2004) *Biological Pharmaceutical Process*. Yu Han Publishing Co., Seoul.
- First edition *Ethanol guidelines*, World Fuel Charter Committee, March 2009.
- Joel, K.B. (2007) Green dreams. *National Geographic Magazine*, National Geography Society, Washington, DC.
- Keating, J.D., Robinson, J., Bothast, R.J., Saddler, J.N. and Mansfield, S.D. (2004) Characterization of a unique ethanologenic yeast capable of fermenting galactose. *Enzyme Microbiol. Technol.*, **35**, 242–253.
- Kim, G.S. (2007) *Feasibility Study on the Utilization of Algae for the Bio-Energy Production*, Annual Report, Ministry of Commerce, Industry and Energy, Seoul.
- Kim, G.S. (2009) Development technique for the carbon source recycling in Southeast Asian Countries, *Technical report*, Korea Institute of Industrial Technology, Seoul.
- Knuttsen, S.H., Myslabodski, D.E., Larsen, B. and Usov, A. I. (1994) A modified system of nomenclature for red algal galactans. *Bot. Mar.*, **37**, 163–169.
- Kumar, S., Singh, S.P., Mishra, I.M. and Adhikari, D.K. (2009) Recent advances in production of bioethanol from lignocellulosic biomass. *Chem. Eng. Technol.*, **32**, 517–526.
- Madson, P.W. and Monceaux, D.A. (2003) *Fuel ethanol production*. KATZEN International Inc., Cincinnati, Ohio, USA.
- Medve, J., Karlsson, J., Lee, D. and Tjerneld, F. (1998) Hydrolysis of microcrystalline cellulose by cellobiohydrolase I and endoglucanase II from *Trichoderma reesei*: Adsorption, sugar production pattern, and synergism of the enzymes. *Biotechnol. Bioeng.*, **59**, 621–634.
- Minkevich, I.G., Andreyev, S.V. and Eroshin, V.K. (2000) The effect of two inhibiting substrates on growth kinetics and cell maintenance of the yeast *Candida valida*. *Proc. Biochem.*, **36**, 209–217.
- Mosier, N., Wyman, C., Dale, B.E., et al. (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. *Biores. Technol.*, **96**, 673–686.
- Novozymes (2007) Biofuel thematic paper. Alternative uses of biomass (energy), Novozymes Denmark.
- Park, H.T., Kim, Y.J., Lee S.M. and Han H.S. (2007) Policy issues and strategies to boost biomass utilization in agricultural sector, *Korea Rural Economic Institute Annual Report*. Korea.
- Pimentel, D. and Patzek, T.W. (2005) Ethanol production using corn, switchgrass, and wood; Biodiesel production using soybean and sunflower, *Nat. Resources Res.*, **14**, 65–76.
- Trumby, R.J. (1992) Glucose repression in the yeast *Saccharomyces cerevisiae*. *Mol. Microbiol.*, **6**, 15–21.
- Wong, K. K. Y., Tan, L. U. L. and Saddler, J. N. (1988) Multiplicity of  $\beta$ -1,4-xylanases in microorganism: Functions and applications, *Microbiol. Rev.*, **52**, 305–317.

## Seaweed as an Adsorbent to Treat Cr(VI)-Contaminated Wastewater

**Saroj Sundar Baral**

*Department of Chemical Engineering, Birla Institute of Technology & Science, Pilani- K. K. Birla Goa Campus, Goa, India*

### 30.1 Importance of chromium

Due to the increased use of various metals as different industrial and household materials, the load of toxic metal pollution in the environment is increasing. The effluents from metallurgical industries and mining sectors contain heavy metal ions, which are toxic to the living organisms. Some of these heavy metals, in traces, play significant roles in human metabolism. The demand of chromium has been increasing globally due to its extensive use in various metallurgical, chemical, and leather tanning industries because of its various physicochemical properties. The corrosion resistance property of chromium expanded its application in hardened steel, stainless steel and alloys. It is also used in electroplating to produce a hard, shining surface and prevent corrosion. Chromium is used in glass production to impart a greenish tint to glass. It is widely used in catalyst preparation. Because of its oxidizing nature  $K_2Cr_2O_7$  is used as an oxidizing agent. Lead chromate is used as yellow pigments. The other important uses of chromium are in the textile as a mordant, in the aircraft and other industries for anodizing and corrosion resistance and use as chromate in refractory for aluminum-forming bricks to give a defined shape, as it has a high melting point, moderate thermal expansion, and stable crystalline structure. Further it is used in open-hearth steel melting furnaces as a major component due to its high melting point (1700–1900 °C) and neutrality, being

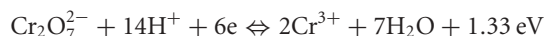
ideal for separating acidic and basic refractory compounds in wall linings.

Apart from industrial uses, Cr(III) plays an important role in our body (Wang and Li, 2004). Without Cr(III) in our diet, the body loses its ability to use sugar, protein, and fat properly, which may result in weight loss or impaired growth, poor function of the nervous system and induce diabetic conditions. The best-known nutritional effect of chromium is to assist insulin in regulating blood sugar (glucose) levels in human body as established through radiotracing. Insulin is a protein hormone that is released into the blood when blood glucose level is raised. Insulin then binds to the receptor cells outside, causing them to absorb more glucose from blood, returning blood glucose levels to normal.

### 30.2 Harmful effects of Cr(VI)

The waste and effluent stream of chromite mines and processing units contain chromium in two oxidation states: Cr(VI) and Cr(III) in aqueous solution. Of these, Cr(VI) is highly toxic in nature (Baral *et al.*, 2007a). The first report on harmful effects of Cr(VI) appeared in the 1930s because of the incidence of lung-cancer cases among workers in chromate handling industries. Machale and Gregorius (1948), and Mancuso and Hueper (1951) catalogued further

evidence in support of the harmful effects of chromate after detailed epidemiological studies. The relationship between trivalent and a hexavalent form of chromium is given by:



The difference in oxidizing potential of these two oxidation states show strong oxidizing power of the Cr(VI) species. On the other hand, high energy required to oxidize the trivalent form is not available with the biological systems and so almost all the Cr(VI) found in nature are derived from human activities. Cr(III), which generally occurs in the form of  $\text{Cr}^{3+}$ ,  $\text{Cr}(\text{OH})^{2+}$  or  $\text{Cr}(\text{OH})^{2+}$ , is adsorbed on the negatively charged soil particles and thus are less mobile (Deng and Bai, 2004). On the other hand, Cr(VI) is present in the aqueous solution in the form of dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ), hydrochromate ( $\text{HCrO}_4^-$ ), or chromate ( $\text{CrO}_4^{2-}$ ). These anionic species are generally poorly adsorbed by the negatively charged soil particles due to their repulsive electrostatic interaction. Therefore Cr(VI) is mobile and present in aqueous solution only. In experiments using cell culture, investigators found that Cr(VI) penetrates the cell membrane and gets into the cells much more easily than Cr(III). The latter do not normally enter into cells because of their slightly bigger size as compared to Cr(VI) ions. The Cr(VI) inside the cell is reduced rapidly to Cr(III). The process of Cr(VI) reduction can create reactive oxygen ( $\text{O}^-$ ) and other free radicals inside the cell. This combination of reactive intermediates has been postulated to be able to attack DNA leading to its damage. Since they are unstable, these intermediates are reduced to stable Cr(III), found on the DNA at the end of the process. DNA damage can lead to mutations, which in certain cancer-associated genes of the cell are believed to be the basis for initiating cancer.

Breathing in high levels of Cr(VI) -containing dust particles ( $>2 \mu\text{g}/\text{m}^3$ ) in the form of compounds like chromic acid or chromium trioxide, can cause irritation to the respiratory system. The symptoms include running nose, sneezing, itching, nose bleeds, ulcers, and damage to the nasal septum. These ailments are found in mining and industrial workers who handle Cr(VI) for a long period. A prolonged exposure of Cr(VI) is likely to cause allergic reaction consisting of severe redness and swelling of the skin leading to skin ulcer. In the animals that breathe high levels of Cr(VI), harmful effects on the respiratory system and a lower ability to fight diseases are noticed.

### 30.3 Different methods of treatment

The permissible limit of Cr(VI) is 0.05 and 0.1 mg/l (Baral *et al.*, 2006) for potable and industrial discharge water re-

spectively. Most of the chromite mine discharge water contains higher Cr(VI) concentration and the effluent from electroplating, ferrochromic, and leather tanning industries contain even higher Cr(VI) concentrations than the permissible limit. So Cr(VI) removal or reduction in mining and industrial effluents is important before discharge into the aquatic environment.

Various physical and chemical methods such as chemical methods, electrolytic methods, biological or microbiological methods, and adsorption methods are used to treat Cr(VI)-contaminated mining and industrial effluents. In chemical method a suitable chemical added to the Cr(VI) contaminated water to reduced Cr(VI) to Cr(III), which is not toxic. In most of the industrial effluent treatment ferrous sulfate is used to reduce Cr(VI) to Cr(III). In the electrolytic method, a carbon electrode is used to reduced the Cr(VI) to Cr(III). Similarly biological methods are used to remediate Cr(VI)-contaminated wastewater. However, these processes have a number of disadvantages including incomplete metal removal, high costs, and production of toxic chemical sludge or other waste products (Garg *et al.*, 2004; Preetha and Viruthagiri, 2007). Among other processes for removal of Cr(VI) from industrial/mining wastewater, an adsorption process is an economically feasible alternative.

#### 30.3.1 Adsorption method

Adsorption is a process in which a single or a group of ions/compounds accumulate on the surface of another solid or liquid. The substance on which the adsorption takes place is known as adsorbent and the substance, which gets adsorbed, is called the adsorbate. Based on the extent of attraction between the adsorbent and adsorbate, the adsorption process can be classified into two types:

- Physical adsorption or Van der Waal's adsorption
- Chemisorption

Adsorption, which can result from the Van der Waal's interaction force, is known as physical adsorption or Van der Waal's adsorption. In this type of adsorption, the process heat is of the order of 20–40 kJ/mol. The physical adsorption process is reversible and established rapidly. Physical adsorption can be of two types: monolayer adsorption and multilayer adsorption. In chemisorption, the chemical interaction or electrostatic force of attraction occur between the adsorbent surface and adsorbate molecules. In this process, the heat of adsorption usually varies from 40 to 400 kJ/mol. It is associated with appreciably high activation energy and therefore termed as activated adsorption. It is a relatively slow process.

Physical adsorption is a reversible process that occurs at a temperature lower or close to the critical temperature of an adsorbed substance. On the other hand chemisorption, in general, is an irreversible process because of strong electrostatic force of interaction between the adsorbent and adsorbate molecules. Physical adsorption is very effective, particularly at a temperature close to the critical temperature of a given fluid. Chemisorption occurs usually at temperatures much higher than the critical temperature and by contrast to physical adsorption, is a specific process, which can only take place on some solid surface for a given fluid. Contrary to physical adsorption, chemisorption leads to monolayer adsorption (Maron and Protton, 1971). Under favorable conditions, both the processes can occur simultaneously or alternately. Physical adsorption is accompanied by a decrease in free energy and entropy of the adsorption system and, this process is thus exothermic in nature.

Various forms of chemical adsorbents and materials of biological origin or biosorbents have been shown to be effective metal removers from the industrial and mining wastewater (Leyva-Ramos *et al.*, 1994; Panchanandikar and Das, 1994).

Due to ease of operation, adsorption techniques have been used widely to treat metal ion containing wastewater. Sawdust, being cheap and easily available, is used widely either as such or in treated form to remove metal ions from wastewater (Argun *et al.*, 2007; Chanah *et al.*, 2005). Sorption studies were carried out mostly in batch scale and various adsorption parameters affecting the overall process were studied. Use of activated carbon was also reported (Zhao *et al.*, 2005; Bailey *et al.*, 1999) to remove metal ions from wastewater. The activated carbon was made from various raw materials with high carbon content, including wood, sawdust, coconut shell, etc. It can be activated by thermal decomposition in a high-temperature oxidation or lower temperature chemical dehydration reaction. Activated carbon is widely used to treat wastewater to remove organic or inorganic pollutants because of its large specific surface area, high adsorption capacity, and special surface chemical properties (Park and Kim, 1999; Park and Jang, 2002). These physical and chemical properties of the activated carbon depend on pore size, pore distribution and number of surface oxygen groups. The pore size and pore volume can be controlled during the activation process such as activation time, activation agent and temperature. The surface oxygen also can be changed by using suitable oxidizing agents and thermal treatment in order to get surface functional groups such as carboxyl, phenolic, and lactonic groups attached to the carbon (Barton *et al.*, 1999). These groups can improve the adsorption capacity and selectivity on a certain adsorbate in either the gaseous or liquid phase (Pradhan and Sandle, 1999).

Adsorption studies were also carried out using various agricultural wastes like grape stalk waste (Fiol *et al.*, 2006), neem leaf powder (Sharma and Bhattacharya, 2004), waste acron (Malkoc *et al.*, 2006), rice husk (Guv *et al.*, 2002), wheat straw (Chun *et al.*, 2004), etc. In all these reports, adsorption studies were carried out either in stirred or up-flow reactors. Various adsorption parameters were studied to evaluate their effects on Cr(VI) removal efficiency. Among the adsorption parameters, pH was observed to be an important factor in determining the adsorption efficiency. Adsorption kinetics were observed to be reasonably faster and followed a dual rate, that is an initial faster rate followed by a slower one. The initial faster and latter slower rates might be due to surface and intraparticle diffusion processes, respectively.

Chitin, the waste polymer from the fishery industry, was reported to be a good adsorbent for Cr(VI) (Li *et al.*, 1997). It is a white, hard inelastic material containing nitrogenous polysaccharides derived from the outer skeleton of insects, crabs, shrimps, and other marine animals. Chitin is the second most abundant natural polymer (i.e., polysaccharide) and its estimated annual production is almost equal to cellulose (Li *et al.*, 1997). Chitin is converted to chitosan by alkaline hydrolysis using 50% (W/W) aqueous NaOH solution. Chitosan has many applications due to the presence of reactive  $-NH_2$  group at position 2 and two hydroxyl groups at positions 3 and 6. Due to the presence of these functional groups, chitosan is a good chelator (Li *et al.*, 1997) and forms complexes with almost all heavy metal ions. Further, due to its cationic nature, it adsorbs various anionic species. The solubility of chitosan in aqueous acids is a limiting factor for many such applications. It is therefore necessary to cross-link chitosan to render it insoluble in acid media. Chitosan is generally cross-linked using chemical reagents such as glutaraldehyde and epichlorohydrin (Rorrer *et al.*, 1993). Ramani and Sabharwal (2006) developed a gamma-radiation based method for cross-linking chitosan in the presence of carbon tetrachloride sensitizer. The cross-linking facilitated the desorption process. Hydrogel chitosan beads were reported to adsorb Cr(VI) (Modrzejewska *et al.*, 2006). The adsorbent was prepared by the phase inversion method. The process followed a pseudo second order rate. The uptake capacity of the adsorbent was observed to be high (1.1 g/g of chitosan). Cr(VI) adsorption studies were carried out (Hassan *et al.*, 2003) by using chitosan-coated perlite beads and under equilibrium as well as dynamic conditions. The uptake capacity was 452 mg/g of chitosan. Desorption studies were carried out by using alkali.

A number of chemical adsorbents were used to treat Cr(VI)-contaminated water. Stannous hydroxide was reported (Goswami and Ghosh, 2005) to have good Cr(VI) adsorptive capacity. The adsorbent was prepared by adding

hydrochloric acid to a solution of sodium stannate. Various adsorption parameters were studied to evaluate their effect. The adsorption process followed first order kinetics. Hydrotalcite  $\text{Mg}_6\text{Al}_2(\text{OH})_{16}\text{CO}_3 \cdot 4\text{H}_2\text{O}$  (HT) was also used to treat Cr(VI) contaminated water (Lazaridis and Asouhidou, 2003). HT is a double-layered mixed metal hydroxide. Due to the positive charge of HT, it is a potential adsorbent for various anions. Cr(VI) adsorption studies were carried out by using manganese nodule leach residue (Mallick *et al.*, 2006). Since the leached residue has a high surface area, it is a potential adsorbent. Its adsorption behavior was studied as a function of time, pH, temperature, concentration of adsorbate, and adsorbent dose. The adsorption process was endothermic in nature. Metallurgical wastes like red mud were also reported (Pradhan *et al.*, 1999) to be a good adsorbent for Cr(VI). Activated red mud was prepared by acid dissolution followed by ammonia precipitation and drying at 110 °C. The process was optimized by varying the adsorption parameters. Adsorption studies were also carried out using wollastonite (Sharma, 2001). Wollastonite is a clay mineral whose surface area was increased by heat treatment. The rate of adsorption was a function of temperature and pH. Ferrihydrite being a active adsorbent, was employed (Lehmann *et al.*, 2001) for Cr(VI) adsorption. Various other chemical adsorbents were used to treat Cr(VI) contaminated water such as clay (Lazaridis *et al.*, 2001), zeolite (Tahir *et al.*, 1998) and ion exchange resins (Rangaraj *et al.*, 2001).

A survey of the literature shows that, though tremendous efforts are continuing worldwide to improvise low-cost adsorbents having high loading capacities, it still remains an area of intensive R&D. Out of the various adsorbents used, many have very low adsorption capacity. Some adsorbents have very high adsorption capacity but at a relatively lower pH. Again the treatment of adsorbents in some cases may not be cost effective. Further, in all these cases the economic feasibility is dependent on the adsorption–desorption cycle. In most of the cases the cost of desorption is also very high.

Waste seaweed, usually in the form of dead cells, is also used as an alternative adsorbent for the treatment of heavy-metal containing wastewater. In this process biological materials accumulate heavy metals from wastewater by either metabolically mediated or purely physico-chemical pathways of uptake (Fourest and Roux, 1992). The major advantages of using seaweed as an adsorbent over other conventional treatment methods are:

- Low cost of adsorbent
- Easy availability of adsorbent
- Low operational cost

- Ease of operation compared to other processes
- Use of adsorbent after adsorption
- No regeneration of the adsorbent is required
- Capacity to remove heavy metal ions over a wide range of pH and to a much lower level
- Ability to remove complex form of metals that is generally not possible by other conventional method.

Microorganisms like algae can take up metal ions in numerous pathways. The mechanisms of biosorption have been discussed by Veglio and Beolchini (1997). Gadd (1988) and Brierley (1990) reviewed the unknown ways in which bacteria, fungi, and algae can take up toxic metals. The uptake of heavy metal ions can take place by entrapment in the cellular structure and subsequent sorption on to the binding sites present in the cellular structure.

Prakasham *et al.* (1999) used *Rhizophus arrhizus* biomass for the treatment of Cr(VI) contaminated wastewater. *R. arrhizus* was immobilized in 2% alginate solution. The prepared material was used further to establish the optimum conditions for removal of Cr(VI) from solution. Sag *et al.* (2000) reported equilibrium parameters for the single and multicomponent biosorption of Cr(VI) and Fe(III) ions on *R. arrhizus* in a packed bed column. In the above study, breakthrough curves for Cr(VI) adsorption in column were established. The Cr(VI) adsorption capacity decreased in presence of Fe(III), a common contaminant in Cr(VI) containing wastewater. Methylated yeast biomass was also reported (Seki *et al.*, 2005) to remove Cr(VI) from solution efficiently. The Cr(VI) adsorption was negligible at neutral pH but increased with the increase of degree of methylation. The optimum pH of adsorption varied in the range 4 to 6. A metal binding model was used to describe the adsorption. *Mucor hiemalis* was also reported (Tewari, 2005) to remove Cr(VI) from solutions. Detailed studies were made with regards to its kinetics and mechanism of adsorption. The competitive biosorption of Fe(III) and Cr(VI) on *C. vulgaris* from binary mixtures was investigated (Aksu and Acikel, 2000) in a single stage batch reactor by varying the solid/liquid ratio at an initial pH of 2.0. The batch adsorption was assumed to be a single stage equilibrium operation. The separation process was mathematically defined. For this purpose, the individual Langmuir constants evaluated from the non-competitive isotherms were used to find the competitive Langmuir model describing multicomponent adsorption equilibrium and thus predicting the equilibrium concentration at a given solid/liquid ratio. Biosorption of Cr(IV) from aqueous solutions on *Aeromonas caviae* particles was investigated (Louhido *et al.*, 2004) in a

well-stirred batch reactor. Equilibrium and kinetic studies were performed at various initial bulk concentrations, biomass loads, temperatures, and ionic background. The kinetics was observed to follow a pseudo-second order rate. A number of freshwater macrophytes like *Myriophyllum specatus*, *Potamogeton luteus*, *Salvinia herzogii*, *Ceratophyllum demersum*, *Eichhorria crassipes* (Schneider and Rubio, 1999; Mohanty, 2006) were reported to remove Cr(VI) from solution. In these cases the hydrophytes were dried, powdered, sieved and then used for biosorption. Biosorption studies were carried out in a batch reactor to evaluate the various adsorption parameters. Loukidon *et al.* (2004) used *Aeromonas caviae* to treat Cr(VI) contaminated wastewater. The adsorption studies were carried out in a stirred reactor. Equilibrium and kinetic experiments were carried out for various parameters like bulk concentration, biomass load, temperature, and ionic background. The isotherm followed a monolayer Langmuir model. The adsorption process followed controlled chemical sorption. The brown seaweed, *Ecklonia* sp., was reported (Park *et al.*, 2005) to reduce Cr(VI) to Cr(III). The Cr(VI)-reducing capacity was enhanced by various chemical treatment methods. The Cr(VI) reducing capacity was enhanced by modifying the amino and carboxyl group.

### 30.4 Case study on adsorptive removal of Cr(VI) from aqueous solution using seaweed *Hydrilla verticillata*

#### 30.4.1 Materials and method

##### Materials

The seaweed *Hydrilla verticillata* (local name: Chingudia Dala) used to removed Cr(VI) from aqueous solution. The seaweed was collected from Nairipentha side of Chilka, a semisalinity water body situated at a distance of ~90 km from Bhubaneswar, the capital city of Orissa. *Hydrilla verticillata* is a waste weed that grows profusely in the semisalinity water and removed periodically to improve the life of the water body. Chilka has a water span of 850–1000 km<sup>2</sup>. The weed is present all over the semisalinity portion (750 km<sup>2</sup>) barring the shore of the lake. It grows during the dry season (October to June) and gets totally submerged during monsoons when the water level rises, during which a part of the weed population perishes adding carbon to the silt. The collected seaweed was washed with water and dried in sunlight for five days followed by drying in an oven at 60 °C for 24 h. The resulting dried seaweed was crushed in a mill, sieved to different size fractions and stored in polyethylene bottles until use.

##### Method

The stock Cr(VI) solution of desired concentration was prepared by using calculated amount of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (Analytical grade). The pH of the solution was maintained at the desired value by adding dilute HCl or NaOH solution before adsorption. All experiments were carried out using AR/GR grade E Merck chemicals. Adsorption experiments were carried out in 500 ml volumetric flask using 250 ml Cr(VI) solution with the required amount of adsorbent. The mixtures were agitated by a Remi mechanical stirrer with speed regulator. Adsorption studies were carried out at different temperatures using an automatic temperature controlled water bath with an accuracy of  $\pm 1$  °C. For higher temperatures, the adsorption studies were carried out in a sealed unit to avoid loss due to evaporation. Five milliliters samples were drawn filtered through Whatman 42 filter paper at a regular interval. The residual Cr(VI) concentration in the filtrate was analyzed using an UV/visible spectrophotometer (Perkin Elmer Lambda-35) (Chun *et al.*, 2004).

#### 30.4.2 Results and discussion

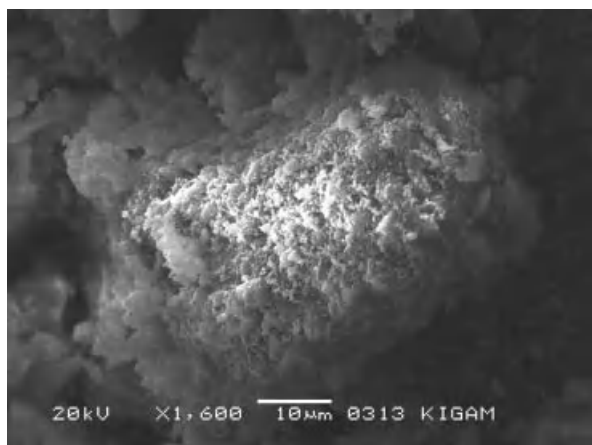
##### Characterization of adsorbent

The particle size and specific surface area of the adsorbent were analyzed using a Malvern particle size analyzer BET method in Quantasorb (Quanta Chrom-USA). The physicochemical characteristics of the adsorbent are given in Table 30.1.

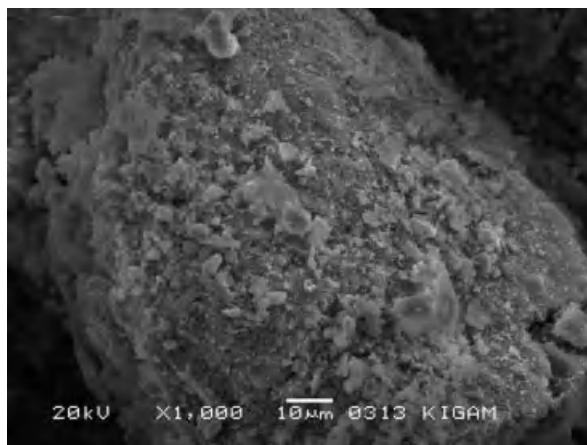
To find out the change in morphology after adsorption scanning electron microscope (SEM) analysis (SEM JXO-8100) at 300 $\times$  magnification was undertaken. The SEM images for the seaweed before and after adsorption are shown in Figures 30.1 and 30.2, respectively. It shows irregular

**Table 30.1** Physical properties of the seaweed *Hydrilla verticillata*

Parameters	Value
Specific gravity	0.65
Bulk density (g/cc)	0.55
Porosity (%)	74
Surface area (m <sup>2</sup> /g)	30.9
Average particle size	97.6 $\mu$ m
Moisture content (%)	63
Loss on ignition	95.4(w/w %)
Al <sub>2</sub> O <sub>3</sub>	1.5(w/w %)
SiO <sub>2</sub>	1.3(w/w %)
FeO <sub>2</sub>	0.22(w/w %)



**Figure 30.1** SEM image of seaweed before adsorption.



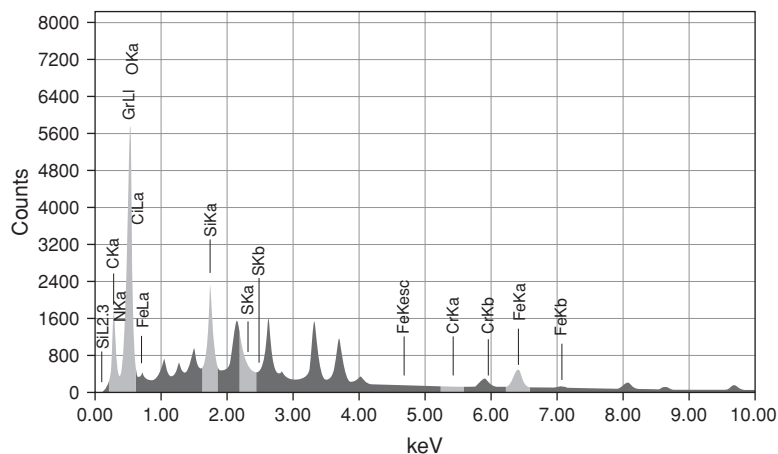
**Figure 30.2** SEM image of seaweed after adsorption.

surface initially, which turned smooth after adsorption. It is clearly observed that the pores and surfaces of the adsorbent were covered by Cr(VI) molecules and became smooth after adsorption. Further, adsorption of Cr(VI) on the surface of the adsorbent was confirmed from the elemental analysis by Energy Dispersive X-Ray Analysis (EDAX) method. From the EDAX analysis (Figures 30.3 and 30.4), the mass percentage of the chromium on the adsorbent surface before and after adsorption was found to be 0.54% and 40.97%, respectively. The higher mass percentage of chromium in the used adsorbent clearly indicates the adsorption of Cr(VI) on the surface of the adsorbent.

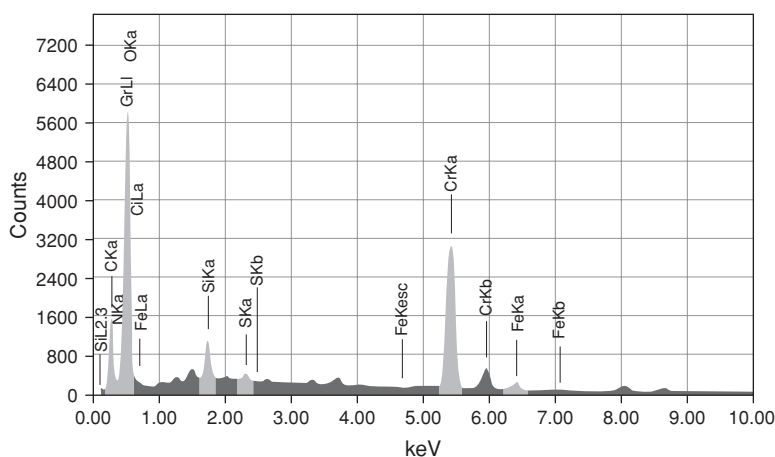
The adsorption on any adsorbent surface depends on the vibrational frequency changes of the functional groups presenting the adsorbent. The Fourier transform–infrared (FT-IR) spectra of the seaweed, before and after adsorption

of chromium were used to determine the vibrational frequency changes in the functional groups of the adsorbents. Infrared absorption spectra of the adsorbent before and after adsorption were obtained using a JASCO FT-IR- 3500 spectrometer. The conditions used were 16 scans at a resolution of  $4\text{ cm}^{-1}$  measured between  $600$  and  $4000\text{ cm}^{-1}$ . The FT-IR spectra of the adsorbents display a number of absorption peaks, indicating the complex nature of the studied adsorbents. Table 30.2 presents the fundamental peaks of the adsorbents before and after use.

In the seaweed before adsorption, the absorption peak around  $3430\text{ cm}^{-1}$  can be assigned to stretching vibration of OH and NH stretching. The peaks observed at  $3330\text{ cm}^{-1}$  indicate the H bond and OH group. The peaks around  $2920\text{ cm}^{-1}$  correspond to the  $\text{CH}_2$  asymmetric stretching vibration. The peak at  $1740\text{ cm}^{-1}$  can be assigned to  $\text{C}=\text{O}$



**Figure 30.3** EDAX image of seaweed before adsorption.



**Figure 30.4** EDAX image of seaweed after adsorption.

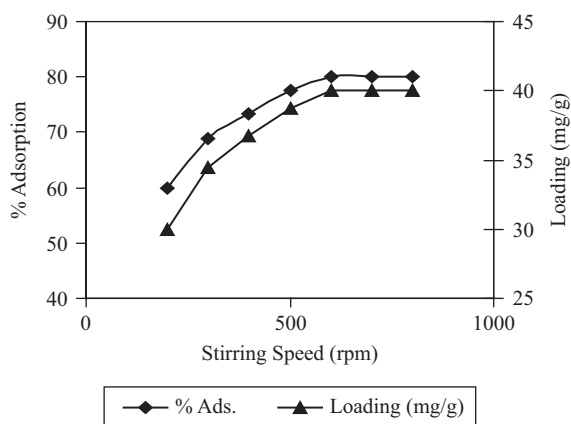
stretching. The broad band absorption peak observed at  $1570\text{ cm}^{-1}$  indicates the presence of a secondary amino group. The other absorbance bands for the seaweed as such showed three sharp peaks at  $1550\text{ cm}^{-1}$  (amide bonds),  $1415\text{ cm}^{-1}$  (C–O stretching) and  $1370\text{ cm}^{-1}$  (carboxyl group); one broad band at  $1035\text{ cm}^{-1}$  (C–O stretching and SiO stretching) and a small peak at  $875\text{ cm}^{-1}$  (aromatic CH). It is observed from Table 30.2 that the Cr(VI) adsorbed seaweed showed either a shift or reduction in absorption peak, suggesting the vital role played by the functional groups. These band shifts indicate that the bonded –OH groups and/or –NH stretching and carboxyl groups especially play a major role in Cr(VI) biosorption on seaweed. Similar observations related to Cr(VI) adsorption were observed by other researchers (Malkoc *et al.*, 2006; Baral *et al.*, 2007b).

### Effect of stirring speed

Adsorption of Cr(VI) on the surface of the adsorbent is governed by four consecutive steps (McKay, 1995): transport of adsorbate in the bulk solution, diffusion of adsorbate across the liquid film boundary surrounding the adsorbent particle, intraparticle diffusion of the adsorbate in the pores of the adsorbent, and adsorption–desorption within the particle and on the external surface. Among the above four steps, external transport, (i.e., transport in the bulk solution) and film diffusion are usually the rate limiting steps because of the poor mixing of the adsorbent particles in the solution. In order to find out the optimum stirring speed at which the external resistance to the mass transfer played insignificant role, adsorption experiments were carried out by varying the stirring speed. Cr(VI) adsorption studies

**Table 30.2** The FT-IR spectral characteristics of seaweed before and after adsorption

IR peak	Adsorption band ( $\text{cm}^{-1}$ )		Difference	Assignment
	Before adsorption	After adsorption		
1	3430	3444	14	OH and NH stretching
2	2920	—	—	$\text{CH}_2$ asymmetric stretching vibration
3	1740	1734	–6	C=O stretching
4	1672	1679	7	C=O stretching
5	1570	1580	10	Secondary amine group
6	1553	1559	6	Amide bond
7	1415	1420	5	C=O stretching
8	1367	1379	12	Carboxyl group
9	1034	1330	–4	C=O and SiO stretching
10	875	881	6	Aromatic CH



**Figure 30.5** Effect of stirring speed (conditions: pH 3.0; Adsorbent dose 2 g/l; Adsorbate concentration 100 mg/l; Temperature 27 °C).

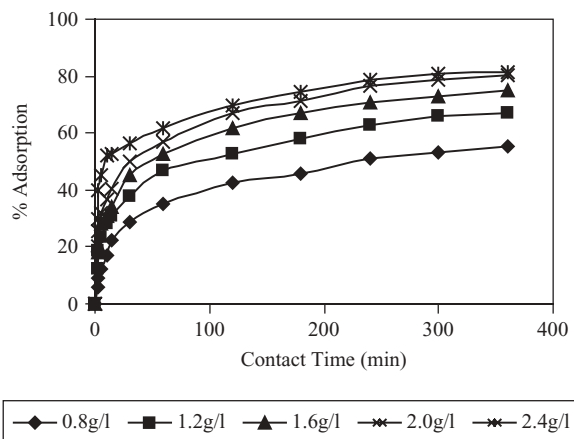
were carried out by varying the agitation speed from 200 to 800 rpm. and the results are shown in Figure 30.5. The percentage of adsorption and the loading capacity of the adsorbent were found to be increased from 60 to 80.1 mg/g and 30 to 40.05 mg/g, respectively when the stirring speed increased from 200 to 600 rpm. Thereafter, the adsorption process attained a steady state. As 600 rpm was found to be the optimum stirring speed, the rest of the experiments were carried out at this stirring speed.

#### Effect of contact time

Adsorption experiments were carried out over 900 min to find the equilibrium contact time. During the experiment, the other adsorption parameters such as stirring speed, adsorbent dose, adsorbate concentration, pH, and temperature of the solution were kept constant at 600 rpm, 2 g/l, 100 mg/l, 3.0 and 27 °C, respectively. The results are shown in Figure 30.6. It can be seen from this figure that the kinetics were very fast during the first 30 min followed by slower kinetics. The adsorption process attained equilibrium within 6 h and beyond that; there was hardly any change in concentration. Therefore, all further studies were carried out for 6 h. The intraparticle diffusion accounts for the slower kinetics at the later stage. Similar dual mechanisms are also reported by others.

#### Effect of pH

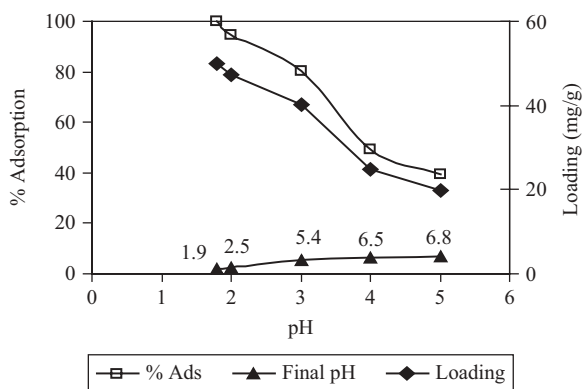
Earlier studies on biosorption of Cr(VI) showed that pH is an important parameter affecting the adsorption process (Park *et al.*, 2004; Holan and Volesky, 1994). Adsorption experiments were carried out by varying the pH between 1.8 and 5.0 to find out its effect on the percentage of adsorption and Cr(VI) uptake capacity of the adsorbent. The results are



**Figure 30.6** Effect of contact time (conditions: pH 3.0; stirring speed 600 rpm; adsorbate concentration 100 mg/l; temperature 27 °C).

shown in Figure 30.7. It was observed that the maximum adsorption occurred at pH 1.8. The sorption capacity of Cr(VI) at pH 1.8 by seaweed was 50 mg/g, which came down to 19.8 mg/g at pH 5. The optimum pH for the Cr(VI) adsorption process was found to be 1.8. But this lower pH (1.8) of the effluent water in the process needs another acid neutralization step which may not be economical. Therefore further adsorption studies were carried out at pH 3.

The mechanism by which metal ions are adsorbed onto the surface of the adsorbent has been a matter of considerable debate. Theories including ion exchange, surface adsorption, chemisorption, complexation, adsorption-complexation, and adsorption-reduction were reported in the literature. Opinions differ as to how complexation occurs between adsorbent and Cr(VI) ions. Evidence has also been found that chemisorption, a strong type of



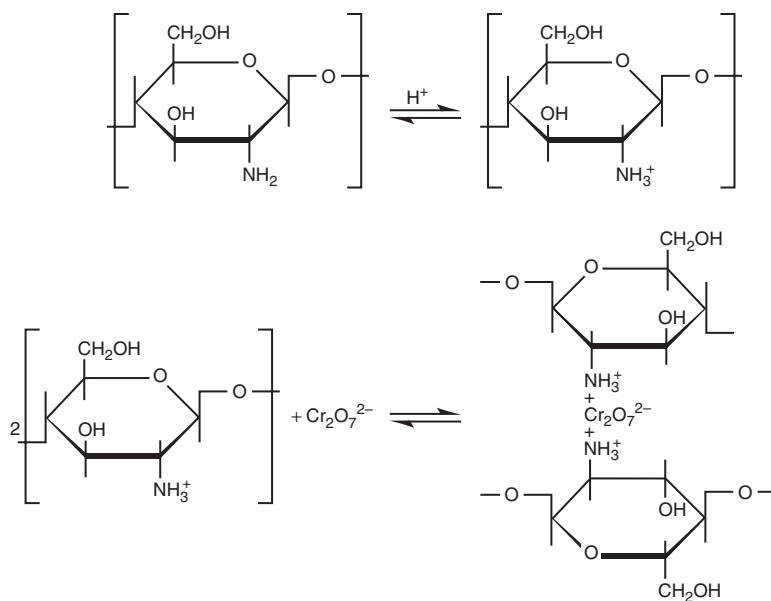
**Figure 30.7** Effect of pH (conditions: stirring speed 600 rpm; adsorbent dose 2 g/l; adsorbate concentration 100 mg/l; temperature 27 °C).

adsorption in which ions are not exchanged but electrons may be exchanged, can be involved in biomaterial–metal binding (Crist *et al.*, 1996). Different mechanisms, such as electrostatic forces, ion exchange, and chemical complexation, must be taken into account while examining the effect of pH on Cr(VI) sorption. One of the commonly proposed mechanisms is electrostatic attraction/repulsion between adsorbent and adsorbate. Many studies have claimed that Cr(VI) was removed from the aqueous phase through an adsorption mechanism, whereby anionic Cr(VI) ion species bind to the positively charged groups of nonliving biomass (Acar and Malkoc, 2004; Malkoc and Nuhoglu, 2003). Cr(VI) was completely reduced to Cr(III) in contact with biomass (Park *et al.*, 2005).

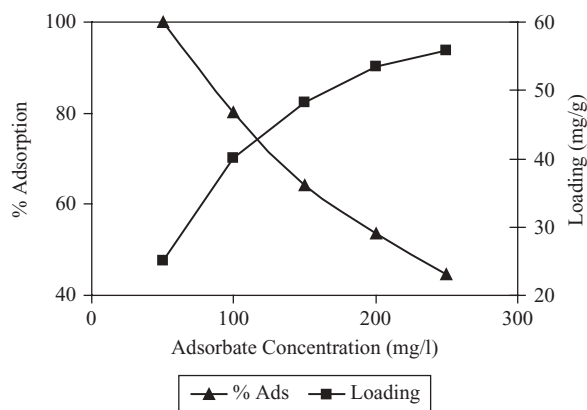
Cr(VI) can be removed from the aqueous phase by non-living biomass through two mechanisms: direct reduction and indirect reduction mechanisms. Cr(VI) is directly reduced to Cr(III) in the aqueous phase by contact with the electron-donor groups of the biomass – groups having lower reduction potential than that of Cr(VI). The indirect reduction consists of three steps: (1) binding of anionic Cr(VI) species to the positively charged groups present on the biomass surface; (2) reduction of Cr(VI) to Cr(III) by adjacent electron-donor groups; followed by (3) release of Cr(III) ions into the aqueous phase due to electronic repulsion from the positively charged groups, or complexation of Cr(III) with adjacent groups capable of binding (Park *et al.*, 2005). In the direct reduction mechanism, concentration of Cr(III) should increase with time. To find the possibility of direct reduction of Cr(VI) to Cr(III) in the solution during

the adsorption process, the initial and final concentrations of Cr(III) in the solution were estimated using an atomic absorption spectrometer (AAS). It is found that there is hardly any change in Cr(III) concentration in the solution. Therefore, it can be concluded that Cr(VI) was not removed from the aqueous phase through direct reduction. Amino and carboxyl groups being electron donors are capable of affecting indirect reduction. As the pH of the aqueous phase is lowered, a large number of hydrogen ions can easily coordinate with the amino and carboxyl groups present on the biomass surface.

The proposed adsorption mechanism of Cr(VI) on the protonated amine groups of the seaweed is shown in Figure 30.8. Thus, low pH makes the biomass surface more positively charged leading to faster removal rate of Cr(VI) in the aqueous phase since the binding of anionic Cr(VI) ion species to the positively charged groups is enhanced (Park *et al.*, 2005). The low pH also accelerates the reduction reaction, since the protons take part in this reaction. Thus, the solution pH is the most important controlling parameter in the practical use of nonliving biomass in the adsorption process (Nuhoglu and Oguz, 2003). Hence, lower pH of wastewater containing heavy metals is generally helpful in the process of adsorption over biomass. Meanwhile, if there are a small number of electron-donor groups in the biomass or protons in the aqueous phase, the chromium bound to the biomass can remain in the hexavalent state. Therefore, the adsorption mechanism depends on the biosorption parameters such as solution pH, temperature, biomass and Cr(VI) concentrations (Park *et al.*, 2005).



**Figure 30.8** Mechanism for Cr(VI) adsorption on seaweed (source: Boddu *et al.*, 2003).



**Figure 30.9** Effect of adsorbate concentration (conditions: pH 3.0; adsorbent dose 2 g/l; stirring speed 600 rpm; temperature 27 °C).

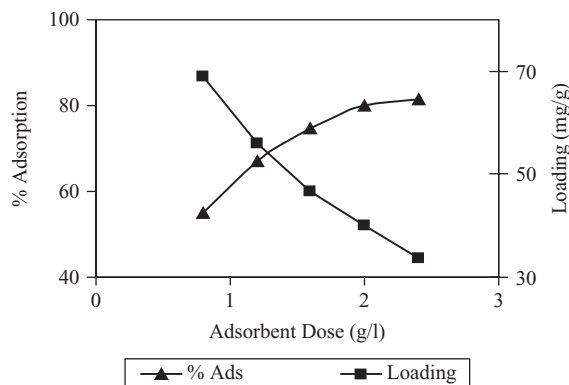
#### Effect of adsorbate concentration

Two different oxidation states ( $+3$  and  $+6$ ) dominate Cr chemistry. Cr(VI) can be present in two different anionic forms:  $\text{CrO}_4^{2-}$  and  $\text{Cr}_2\text{O}_7^{2-}$ , which are sensitive to the pH of the medium. While  $\text{CrO}_4^{2-}$  is the dominating form at  $\text{pH} > 8$ ,  $\text{Cr}_2\text{O}_7^{2-}$  is usually found in the pH range 2–6. In still higher acidic conditions ( $\text{pH} < 1$ ), it is converted to chromic acid ( $\text{H}_2\text{Cr}_2\text{O}_7$ ). Since the present studies were carried out in the pH range 1.8–5.0, all the Cr can safely be assumed to be in  $\text{Cr}_2\text{O}_7^{2-}$  form. (Bailar *et al.*, 1973).

It can be seen from Figure 30.9 that when the initial Cr(VI) concentration increased from 50 to 250 mg/l, Cr(VI) removal decreased from 100% to 44.6% and the Cr(VI) uptake capacity of the biomass increased from 25.0 to 55.8 mg/g. Probably higher concentration of Cr(VI) led to faster and more binding sites compared to lower initial Cr(VI) concentration at the same dose of adsorbent. Moreover, the higher initial Cr(VI) concentration increased the driving force to overcome the mass transfer resistance of metal ions between the aqueous and solid phases resulting in higher probability of collision between the adsorbates with adsorbents. This also results in higher metal uptake. The increases of Cr(VI) loading capacity of the biomass with increasing initial Cr(VI) concentration may also be due to higher interaction between the metal ions and adsorbent. However, the seaweed offered a finite number of surface binding sites and Cr(VI) adsorption showed a saturation trend at higher initial Cr(VI) concentration.

#### Effect of adsorbent dose

Adsorption experiments were carried out to evaluate the effect of adsorbent dose on Cr(VI) removal. The trend of adsorption at varying doses of adsorbent is shown in Figure 30.10. The percentage of Cr(VI) removal increased



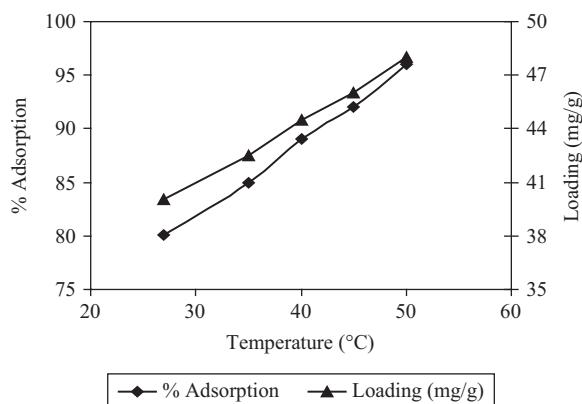
**Figure 30.10** Effect of adsorbent dose (conditions: pH 3.0; stirring speed 600 rpm; adsorbate concentration 100 mg/l; temperature 27 °C).

from 55.1 to 81.5 with increase in adsorbent dose from 0.8 to 2.4 g/l. The trend was as per expectations since more active sites of adsorbent were exposed when the adsorbent dose increased. However, the Cr(VI) uptake capacity of the adsorbent decreased from 68.9 to 33.7 mg/g when the adsorbent dose increased from 0.8 to 2.4 g/l. Further, it was observed that after rapid increase in percentage adsorption of Cr(VI) ions with increase in adsorbent dose up to 2.0 g/l, the rate of Cr(VI) removal attained an asymptotic value for larger doses of adsorbent. The sluggish rise in Cr(VI) removal beyond an optimum dose may be attributed to the attainment of equilibrium between adsorbate and adsorbent under the experimental conditions. This effect had been termed as “solid concentration effect”, or overcrowding of particles by Mehrotra *et al.* (1999).

#### Effect of temperature

The effect of temperature on the percentage of adsorption and Cr(VI) uptake capacity of the adsorbent is presented in Figure 30.11. It can be observed that the percentage of Cr(VI) adsorption increased from 80.1 to 96% when the temperature increased from 27 to 50 °C. Similarly, the uptake capacity of the adsorbent increased from 40.05 to 48 mg/g when the temperature increased from 27 to 50 °C. The increase in Cr(VI) uptake capacity may be attributed to the following:

- The increase in Cr(VI) adsorption capacity of the adsorbent with temperature indicates the endothermic nature of the adsorption process.
- The rise in sorption capacity with temperature is because of rise in the kinetic energy of adsorbent particles in which case, the collision frequency between adsorbent and adsorbate increases resulting in enhanced sorption on to the surface of the adsorbent.



**Figure 30.11** Effect of temperature (conditions: pH 3.0; adsorbent dose 2 g/l; adsorbate concentration 100 mg/l; stirring speed 600 rpm).

- Further, bond rupture of the functional groups on adsorbent surface at an elevated temperature may increase the number of active sorption sites, which may also lead to enhanced adsorption capacity of the adsorbent.
- The extent of protonation of the functional groups increases at higher temperatures resulting in an increase in the metal adsorption capacities at high temperature.

The effect of temperature on the equilibrium constant ( $K_C$ ) of the adsorption of metal ions onto the seaweed was also investigated. Equilibrium constants for Cr(VI) increased as temperature increases and hence adsorption increased with temperature. The thermodynamic parameters such as standard Gibbs free energy change ( $\Delta G^0$ ), enthalpy change ( $\Delta H^0$ ) and entropy change ( $\Delta S^0$ ) were estimated to evaluate the feasibility and nature of the adsorption process. The relationship between Gibb's free energy changes to the equilibrium constant is given by the equation:

$$\Delta G^0 = -RT \ln K_C \quad (30.1)$$

$$K_C = \frac{C_a}{C_e} \quad (30.2)$$

where:

$K_C$  = equilibrium constant

$C_a$  = mg of adsorbate adsorbed per liter of adsorbent

$C_e$  = equilibrium concentration of solution mg/l

$T$  = absolute temperature

$R$  = universal gas constant.

The values of Gibbs free energy decrease from  $-1.74$  to  $-6.67$  kJ/gmol when the temperature increases from  $27$  to  $50^\circ\text{C}$ . The adsorption process is endothermic; hence the amount adsorbed at equilibrium must increase with increasing temperature, which explains increasingly negative

$\Delta G^0$  values with the rise in temperature. The carboxylic and amine groups of the seaweed are partially protonated at all temperatures but their degree of protonation increases at higher temperatures resulting in an increase in the metal adsorption capacities at high temperatures. The "chelating effect" causes a large positive change in entropy, which means that the change of Gibbs free energy with temperature will be negative. Considering other thermodynamic factors, the entropy change in chelation reactions may have less bearing on temperature effects than the enthalpy of sorption. Thus, as the temperature increases, the resulting  $\Delta G^0$  will become more negative and so the equilibrium constant will increase slightly (Donais *et al.*, 1999; Cooney, 1999). Assuming that the activity coefficients are unity at low concentrations (Henry's law), thermodynamic parameters such as enthalpy change ( $\Delta H^0$ ) and entropy change ( $\Delta S^0$ ) were calculated using the following relations:

$$\ln K_C = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \quad (30.3)$$

The values of  $\Delta H^0$  and  $\Delta S^0$  were obtained from the linear Van't Hoff plot of  $\ln K_C$  versus  $1/T$  from Equation 30.3 and are found to be  $59.9$  J/gmol-K and  $0.204$  J/gmol-K respectively. The positive values of  $\Delta H^0$  indicate the endothermic nature of the process while the negative  $\Delta S^0$  corresponds to a decrease in the degree of freedom of the adsorbed species. The order of magnitude of the enthalpy change as well as the relatively slow rate of the adsorption suggests the adsorption to be of a chemical type. Similar results have been reported for the Cr(VI) adsorption onto activated carbon (Leyva-Ramos *et al.*, 1994). This may be attributed to a relative increase in adsorbing tendency of the solute from the adsorbent phase to the bulk phase with the rise in the temperature of the solution.

### Adsorption kinetics study

The study of adsorption kinetics in wastewater is significant as it provides valuable insight into the reaction pathways and into the mechanism of the reaction. Further, it is important to predict the time at which the adsorbate is removed from aqueous solution in order to design an appropriate sorption treatment plant. Any adsorption process is normally controlled by three diffusive transport processes for the adsorbate:

- From bulk solution to the film surrounding the adsorbent.
- From the film to the adsorbent surface
- From the surface to the internal sites followed by binding of the metal ions onto the active sites.

However, in kinetic modeling, all these three steps are grouped together and it is assumed that the difference between the average solid phase concentration and equilibrium concentration is the driving force for adsorption. Further, it is established from the experimental observations that at optimum agitation speed, the external boundaries have hardly any effect. So application of the kinetic model depends only on the initial and final concentrations of the solution at different time intervals. Several kinetic models have been proposed to clarify the mechanism of a solute sorption from aqueous solution onto an adsorbent:

- Pseudo first order/Lagergren kinetic model.
- First order reversible kinetic model.
- Ritchie's second order kinetic model.
- Pseudo second order kinetic model.

The adsorption kinetic parameters along with the regression coefficients are calculated for each kinetic model and are reported in Table 30.3. From Table 30.3 it can be concluded that the kinetics followed pseudo second order model.

**Table 30.3** Adsorption kinetics model parameters

Parameters	1st order reversible					Pseudo 1st order reversible		Ritchie 2nd order		Pseudo 2nd order reversible	
	$k_r$	$K_C$	$k_1$	$k_2$	$R^2$	$k$	$R^2$	$k_2$	$R^2$	$k_2$	$R^2$
Stirring speed (rpm)											
200	0.0093	0.75	0.0040	0.0053	0.948	0.0092	0.948	0.0704	0.791	0.0031	0.996
300	0.0108	1.11	0.0057	0.0051	0.972	0.0108	0.972	0.1008	0.819	0.0024	0.996
400	0.0115	1.38	0.0067	0.0048	0.971	0.0115	0.971	0.1278	0.788	0.0023	0.996
500	0.0128	1.72	0.0081	0.0047	0.976	0.0127	0.976	0.1967	0.696	0.0021	0.996
600	0.0117	2.01	0.0078	0.0039	0.982	0.0117	0.982	0.1313	0.789	0.0019	0.995
Initial pH											
2	0.0118	9.12	0.0106	0.0012	0.880	0.0117	0.878	0.2492	0.616	0.0044	0.999
3	0.0136	2.01	0.0091	0.0045	0.920	0.0136	0.919	0.3203	0.529	0.0069	0.995
4	0.0088	0.49	0.0029	0.0059	0.860	0.0088	0.862	0.0710	0.799	0.0020	0.998
5	0.0105	0.33	0.0026	0.0079	0.942	2.4456	0.942	0.0946	0.804	0.0056	0.998
Adsorbate concentration (mg/l)											
100	0.0115	2.013	0.0077	0.0038	0.97	0.0113	0.970	0.1232	0.789	0.0020	0.9953
150	0.0104	0.897	0.0049	0.0055	0.97	0.0104	0.970	0.0624	0.764	0.0015	0.9910
200	0.0098	0.575	0.0036	0.0062	0.95	0.0097	0.950	0.0854	0.773	0.0015	0.9901
250	0.0080	0.425	0.0024	0.0056	0.85	0.0069	0.904	0.1284	0.679	0.0014	0.9910
Adsorbent dose (g/l)											
0.8	0.0101	1.5340	0.0061	0.0040	0.98	0.0101	0.980	0.0688	0.830	0.0007	0.992
1.2	0.0114	1.7073	0.0072	0.0042	0.96	0.0113	0.960	0.1333	0.652	0.0011	0.992
1.6	0.0112	1.8552	0.0073	0.0039	0.98	0.0113	0.980	0.1047	0.815	0.0015	0.995
2	0.0116	2.0126	0.0077	0.0039	0.98	0.0115	0.980	0.1234	0.789	0.0019	0.995
2.4	0.0128	1.7064	0.0081	0.0047	0.95	0.0113	0.950	0.0250	0.988	0.0034	0.996
Temperature (°C)											
27	0.0117	2.0126	0.0078	0.0039	0.982	0.0117	0.98	0.5336	0.976	0.0019	0.995
35	0.0099	2.8333	0.0073	0.0026	0.970	0.0099	0.97	0.8177	0.999	0.0019	0.995
40	0.0107	4.0455	0.0086	0.0021	0.969	0.0108	0.97	1.6706	0.966	0.0019	0.995
45	0.0123	5.7500	0.0105	0.0018	0.959	0.0124	0.96	1.7448	0.967	0.0018	0.994
50	0.0124	12.0000	0.0114	0.0010	0.960	0.0124	0.97	0.2096	0.638	0.0017	0.994

**Rate controlling mechanism**

The adsorption of Cr(VI) on porous adsorbent can be governed by four consecutive steps: such as diffusion in the bulk solution, diffusion across the thin film surrounding the adsorbent particles, followed by intraparticle diffusion, and desorption within the particles. Any of the above steps or combinations of them may control the rate. To determine the exact mechanism, it is necessary to carry out experiments for pore/solid phase diffusion mechanisms. In many cases, intra-particle diffusion for which the mathematical

equation is given below was observed to be the rate-limiting step:

$$q_t = k_{id} t^{1/2} \quad (30.4)$$

where  $q_t$  is the uptake capacity (mg/g).

The plot of  $q_t$  vs  $t^{1/2}$  gives the coefficient of intraparticle diffusion ( $k_{id}$ ) for different adsorption parameters. The values of intra-particle diffusion coefficient ( $k_{id}$ ) along with the regression coefficient ( $R^2$ ) for the plot of  $q$  vs  $t^{1/2}$  under different adsorption parameters are given in Table 30.4.

**Table 30.4** Mass transfer model parameters

Parameters	$k_{id}$	$R^2$	$D_1 \times 10^{-12}$	$R^2$	$D_2 \times 10^{-12}$	$R^2$
Stirring (rpm)						
200	0.7617	0.999	0.4974	0.988	0.6252	0.956
300	0.8592	0.980	0.5098	0.973	0.7270	0.987
400	0.8693	0.970	0.5882	0.987	0.7851	0.986
500	0.9677	0.953	0.5489	0.986	0.9378	0.974
600	1.0257	0.956	0.4328	0.981	0.8215	0.989
pH						
1.8	0.5313	0.910	0.5762	0.897	0.7052	0.978
2	0.5868	0.955	0.6906	0.832	0.7342	0.828
3	0.9842	0.984	0.5246	0.988	1.1123	0.845
4	0.9716	0.972	0.6214	0.923	0.5089	0.894
5	0.9799	0.980	58.2183	0.965	0.6325	0.944
Adsorbate concentration (mg/l)						
50	0.3498	0.9405	0.4122	0.995	0.8360	0.904
100	1.0289	0.9626	0.5246	0.978	0.8215	0.988
150	1.4652	0.9827	0.2899	0.971	0.7851	0.983
200	1.5641	0.9836	0.3640	0.948	0.7415	0.956
250	1.261	0.9891	0.5690	0.984	0.5598	0.961
Adsorbent dose (g/l)						
0.8	2.3618	0.991	0.5762	0.990	0.6979	0.973
1.2	1.7201	0.9992	0.4338	0.965	0.8506	0.924
1.6	1.3588	0.9773	0.3931	0.979	0.7706	0.991
2	1.1326	0.976	0.4235	0.981	0.8215	0.978
2.4	0.8437	0.9888	0.2934	0.845	1.0541	0.939
Temperature ( $^{\circ}\text{C}$ )						
27	1.1364	0.972	0.4235	0.981	0.8215	0.989
35	1.1541	0.981	0.4061	0.985	0.6470	0.984
40	1.2101	0.995	0.3902	0.984	0.7561	0.964
45	1.3164	0.992	0.3991	0.985	0.9596	0.931
50	1.3343	0.995	0.3322	0.988	0.9669	0.931

**Table 30.5** Adsorption isotherm parameters

Langmuir adsorption isotherm				Freundlich adsorption isotherm				Temkin adsorption isotherm								
Parameter	$Q_0$	$b$	$R^2$	$q_e$ Exp.	$q_e$ Cal.	%Desv	$b_f$	$k_f$	$R^2$	$q_e$ Cal.	%Desv	$A$	$b$	$R^2$	$q_e$ Cal.	%Desv
Stirring speed (rpm)																
200	23.98	0.12	0.99	30.00	30.60	0.35	0.41	139.60	0.98	30.54	1.36	332.19	173.50	0.99	30.43	0.97
300				34.45	33.22					33.87					34.05	
400				36.70	35.54					36.13					36.30	
500				38.75	38.96					38.71					38.70	
600				40.05	42.43					40.72					40.47	
Initial pH																
1.8	20.41	0.26	0.96	50.00	50.00	6.80	0.34	90.76	0.87	50.00	8.92	471.22	226.03	0.92	67.92	8.21
2				47.40	81.00					51.91					49.73	
3				40.05	25.37					32.94					34.92	
4				24.75	22.11					24.02					24.65	
5				19.75	21.81					22.60					22.65	
Adsorbate concentration (mg/l)																
50	56.82	0.19	0.99	25.00	25.00	3.46	0.17	23.87	0.99	25.00	0.78	6.36	301.15	1.00	15.33	0.60
100				40.05	44.69					40.27					40.10	
150				48.15	51.63					47.90					48.32	
200				53.50	53.70					52.73					52.87	
250				55.75	54.69					56.54					56.17	
Adsorbent dose (g/l)																
0.8	196.08	0.01	0.99	68.88	69.10	0.21	0.75	4.09	0.98	70.18	3.29	66.78	7.17	0.99	68.49	2.26
1.2				56.00	55.77					55.50					56.76	
1.6				46.75	45.87					45.58					46.92	
2				40.05	38.10					38.20					38.10	
2.4				33.96	35.91					36.18					35.37	
Temperature (°C)																
27	38.46	0.85	1.00	40.05	40.87	1.67	0.11	56.78	0.93	40.97	1.59	97525.50	517.65	0.94	40.94	1.42
35				42.50	41.72					42.26					42.30	
40				44.50	43.05					43.71					43.80	
45				46.00	45.07					45.26					45.33	
50				48.00	54.42					48.81					48.67	

Again, from the plot between  $t$  (time) and percentage adsorption at different adsorption parameters, was found to be nonlinear over the entire time range. In that case, more than one step may affect the adsorption process. So the adsorption process can be divided into two distinct steps: the initial curved portion relates to film diffusion ( $D_1$ ) and the latter linear portion relates to the diffusion within the adsorbent. The equation for  $D_1$  and  $D_2$  are given by;

$$\frac{q_t}{q_e} = 6 \left( \frac{D_1}{\pi a^2} \right)^{\frac{1}{2}} t^{\frac{1}{2}} \quad (30.5)$$

$$\ln \left( 1 - \frac{q_t}{q_e} \right) = \ln \left( \frac{6}{\pi^2} \right) - \left( \frac{D_2 \pi^2 t}{a^2} \right) \quad (30.6)$$

where  $q_e$  is the equilibrium uptake capacity.

The values of  $D_1$  along with the  $R^2$  under different adsorption parameters were calculated from the slope of the plot between  $q_t/q_e$  vs  $t^{1/2}$  for the initial curved portion and are given in Table 30.4. Similarly,  $D_2$  values were calculated from the slope of the curve between  $\ln(1 - q_t/q_e)$  vs  $t$  under different adsorption parameters and are given in Table 30.4. From Table 30.4 it is observed that the  $D_1$  values are always less than those of  $D_2$ . So it can be concluded that the surface diffusion is the rate controlling mechanism, as it is the slowest rate.

### Adsorption isotherm study

An adsorption isotherm can be utilized to obtain information concerning the desorption mechanism strictly connected with interaction between the adsorbent and adsorbate molecules. Therefore, the efficiency of an industrial adsorbent can be assessed through an adsorption isotherm curve. The adsorption isotherm thus developed provides useful information for estimating performance in a full-scale process stream. First, they help to determine the possibility to reach a desired purity level for a given adsorbent. Secondly, the isotherm allows calculation of uptake ( $q_e$ ) at equilibrium, which has a major impact on the process economy. It can also be used to predict the relative performance of different types of adsorbents. A number of isotherm equations were proposed by different investigators. Some of those in frequent use are:

- Freundlich adsorption isotherm
- Langmuir adsorption isotherm
- Temkin adsorption isotherm.

The model parameters for each adsorption isotherm model were calculated and are reported in Table 30.4. The

**Table 30.6** Comparison of adsorption parameters and uptake capacity with other reported adsorbents

Adsorbent	Uptake capacity	pH	Initial concentration (mg/l)
<i>Chlorella vulgaris</i>	24	2	25–250
<i>Zooglera ramigera</i>	3	2	25–400
<i>Halimeda opuntia</i>	40	4.1	25–400
<i>Rhizopus arrhizus</i>	62	2	25–400
<i>Rhizopus arrhizus</i>	8.8	2	
<i>Sargassum</i>	40	2	
<i>Spirogyra</i>	14.7	2	25–400
Tyres activated carbon	58.50	2	60
Coconut shell carbon	20	2	—
Coconut shell carbon	10.88	4	25
Sawdust activated carbon	44.05	2	200
<i>Hydrilla verticillata</i>	247	3	100

other statistical parameters such as correlation coefficient ( $R^2$ ) and the average absolute percentage deviation between calculated equilibrium capacity ( $q_{e(cal)}$ ) and experiments ( $q_{e(exp)}$ ) for the models are also reported in Table 30.5. It can be concluded from Table 30.5 that the experimental data were well fit to the Langmuir adsorption isotherm model.

The seaweed used in this study for the removal of Cr(VI) from waste water is easily available and can be processed to get a low-cost adsorbent. There are a number of advantages of using this seaweed as an adsorbent for the treatment of Cr(VI) contaminated wastewater as compare to other conventional adsorbent. The adsorption capacity of the present adsorbent was compared with other similar adsorbents for Cr(VI) reported in literature. The comparison is shown in Table 30.6, from which it can be concluded that the present adsorbent is efficient in treating Cr(VI) contaminated water.

## References

- Acar, F.N. and Malkoc, E. (2004) The removal of chromium(VI) from aqueous solutions by *Fagus orientalis* L. *Biores. Technol.*, **94**, 13–15.
- Aksu, Z. and Acikel, U. (2000) Modelling of a single-stage bioseparation process for simultaneous removal of Fe (III) and Cr(VI) by using *C. vulgaris*. *Biochem. Eng. J.*, **4**, 229–238.
- Argun, M.E., Dursun, S., Ozdemir, C. and Karatan, M. (2007) Heavy metal adsorption by modified *Oak* saw dust: Thermodynamics and Kinetics. *J. Hazard. Mater.*, **141**, 77–85.

- Bailar, J.C. Jr., Emeleus, H.J., Nyholm, R. and Trotman-Dickenson A.F. (1973) *Comprehensive Inorganic Chemistry* Volume 3. Pergamon, Oxford, p. 693.
- Bailey, S.E., Olin, T.J., Bricker, R.M. and Adrin, D.D. (1999) A review of potentially low-cost sorbents for heavy metals. *Wat. Res.*, **33**, 2469–2479.
- Baral, S.S., Das, S.N. and Rath, P. (2006) Hexavalent chromium removal from aqueous solution by adsorption on treated sawdust, *Biochem. Eng. J.*, **31**, 216–222.
- Baral, S.S., Das, S.N., Rath, P. and Chaudhury, G.R. (2007a) Chromium (VI) removal by Calcined Bauxite. *Biochem. Eng. J.*, **34**, 69–75.
- Baral, S.S., Das, S.N., Rath, P., Chaudhury, G.R. and Swamy, Y.V. (2007b) Removal of Cr(VI) from aqueous solution using waste weed, *Salvinia cucullata*. *Chem. Ecol.*, **23**, 105–117.
- Barton, S.S., Evans, M.J.B., Halliop, E. and MacDonald, J.A.F. (1999) Acidic and basic sites on the surface of porous carbon. *Carbon*, **35**, 1361–1366.
- Boddu, V.M., Abburi, K., Talbott J.L. and Smith, E.D. (2003) Removal of hexavalent chromium from wastewater using a new composit chitosan biosorbent. *Env. Sci. Technol.*, **37**, 4449–4456.
- Brierley, C.L. (1990) Bioremediation of metal contaminated surface and groundwastes. *Geomicrobiol. J.*, **8**, 201–223.
- Chanah, T.G., Jumasiah, A., Azni, I., Katyon S. and Choong, S.Y.T. (2005) Rice husk as a potential low-cost biosorbents for heavy metal and dye removal: an overview. *Desalination*, **175**, 305–316.
- Chun, L., Hongzhang, C. and Zuohu, M.L. (2004) Adsorptive removal of Cr (VI) by Fe-modified steam exploded wheat straw. *Proc. Biochem.*, **39**, 541–545.
- Cooney, D.O. (1999) *Adsorption Design for Wastewater Treatment*. Lewis Publishers, London, pp. 1–87.
- Crist, R.H., Martin, J.R. Chonko, J. and Crist, D.R. (1996) Uptake of metals on peat moss: an ion exchange process. *Env. Sci. Technol.*, **30**, 2456–2461.
- Deng, S., and Bai, R. (2004) Removal of trivalent and hexavalent Chromium with aminated polyacrylonitrile fibers, performance and mechanism. *Wat. Res.*, **38**, 2424–2432.
- Donais, M.K., Henry, R. and Rettberg, T. (1999) Chromium speciation using an automated liquid handling system with inductively coupled plasma–mass spectrometric detection. *Talanta*, **49**, 1045–1050.
- Fiol, N., Escudero, E., Poch, J. and Villaescusa, I. (2006) Preliminary studies on Cr(VI) removal from aqueous solution using grape stalk encapsulated in calcium alginate beads in a packed bed up-flow column. *React. Funct. Polym.*, **66**, 795–807.
- Fourest, E. and Roux, J.C. (1992) Heavy metal biosorption by fungal mycelial byproducts: mechanisms and influence of pH. *Appl. Microbiol.-Biotechnol.*, **37**, 399–403.
- Gadd, G. M. (1988) *Accumulation of Metals by Microorganisms and Algae, Biotechnology: A Complete Treatise*. VCH Publishers, Weinheim.
- Garg, V.K., Gupta, R., Kumar, R. and Gupta, R.K. (2004) Adsorption of Cr from aqueous solution on treated saw dust. *Biores. Technol.*, **92**, 78–81.
- Goswami, S. and Ghosh, V. C. (2005) Studies on adsorption behavior of Cr(VI) onto synthetic hydrous stannic oxide. *Water WA*, **31**, 597–602.
- Guv Y., Qi, J., Yang, S., Yu, K., Wang, Z. and Xu, H. (2002) Adsorption of Cr(VI) on micro and mesoporous rice husk based active carbon, *Mater. Chem. Phys.*, **78**, 132–137.
- Hassan, S., Krishnaiah, A., Ghosh, T.K., Viswanath, D.S., Boddu, V. M. and Smith, E.D. (2003) Adsorption of Cr(VI) on chitosan coated perlite. *Separat. Sci. Technol.*, **38**, 3775–3793.
- Holan, Z.R. and Volesky, B. (1994) Biosorption of lead and nickel by biomass of marine algae. *Biotechnol. Bioeng.*, **43**, 1001–1009.
- Lazaridis, N.K. and Asouhidou, D.D. (2003) A. Kinetic of sorptive removal of Cr(VI) from aqueous solutions by calcined Mg-Al CO<sub>3</sub> hydrotalcite, *Wat. Res.*, **37**, 2875–2882.
- Lazaridis, N.K., Matis, K.A. and Webb, M. (2001) Floatation of metal loaded clay anion exchangers Part I: the case of chromates. *Chemosphere*, **42**, 373–378.
- Lehmann, M., Zouboulis, A.I. and Matis, K.A. (2001) Modeling the sorption of metals from aqueous solutions on goethite fixed bed. *Env. Poll.*, **113**, 121–128.
- Leyva-Ramos, R., Juarez-Martinez, A. and Guerrero-Coronado, R.M. (1994) Adsorption of chromium(VI) from aqueous solutions on activated carbon. *Wat. Sci. Technol.*, **30**, 191–197.
- Li, Q., Dunn, E.T., Grandmaison, E.W. and Goosen, M.F.A. (1997) Applications and properties of chitosan: *Application of Chitin and Chitosan* (ed. M.F.A. Goosen). Lancaster, USA, pp. 3–29.
- Louhido, M.X., Zouboulis, A.I., Karapantsios, T.D. and Matis, K.A. (2004) Equilibrium and kinetic modeling of Cr(VI) biosorption by *Aeromonas calire*. *Colloids Surfaces A: Physicochemical Engineering Aspects*, **242**, 93–104.
- Machale, W. and Gregorius, F. (1948) US Public Health Report, **63**, 1114–1127.
- Malkoc, E. and Nuhoglu, Y. (2003) The Removal of chromium(VI) from synthetic wastewater by *Ulothrix zonata* Fresen. *Env. Bull.*, **12**, 376–381.
- Malkoc, E., Nuhoglu, Y. and Abali, Y. (2006) Cr(VI) adsorption by waste acron of *Query ithaburensis* in fixed bed : predication of breakthrough curve. *Chem. Eng. J.*, **119**, 61–68.
- Malkoc, E., Nuhoglu, Y. and Dundar, M. (2006) Adsorption of chromium(VI) on pomace- An olive oil industry

- waste: Batch and column studies. *J. Hazard. Mater.*, **B138**, 142–151.
- Mallick, S., Dash, S.S. and Parida, K.M. (2006) Adsorption of Cr(VI) on manganese nodule leached residue obtained from  $\text{NH}_3$ - $\text{SO}_2$  leaching. *J. Colloid Interf. Sci.*, **297**, 419–425.
- Mancuso, T. and Hueper, W.C. (1951) Occupational cancer and other health hazards in a chromate plant: a medical appraisal. I. Lung cancers in chromate workers. *Indian Medical Engineering*, **20**, 358–363.
- Maron, S.H. and Protton, C.F. (1971) *Principles of Physical Chemistry*, 4th edition. Macmillan, New York.
- McKay, G. (1995) *Use of Adsorbents for the Removal of Pollutants from Wastewaters*. CRC Press, Boca Raton.
- Mehrotra, A., Gopal, K. and Seth, P.K. (1999). Annual Report VIO Hyderabad State, Indian Council of Agriculture Research, ICAR, New Delhi.
- Modrzejewska, Z., Sujka, W., Dorabalska, M. and Zarzycki, R. (2006) Adsorption of Cr(VI) on cross-linked Chitosan bead. *Separ. Sci. Technol.*, **41**, 111–122.
- Mohanty, K., Jha, M., Meikap, B.C. and Biswas, M.N. (2006) Biosorption of Cr(VI) from aqueous solution by *Eichhornia crassipes*. *Chem. Eng. J.*, **117**, 71–77.
- Nuhoglu, Y. and Oguz, E. (2003) Removal of copper(II) from aqueous solutions by biosorption on the cone biomass of *Thuja orientalis*. *Proc. Biochem.*, **38**, 1627–1631.
- Panchanadikar, V. V. and Das, R. P. (1994) Biosorption process for removing Pb (II) ions from aqueous effluent using *Pseudomonas* Sp. *Int. J. Env. Studies*, **46**, 243–250.
- Park, D., Yun, Y.S. and Park, J.M. (2004) Reduction of hexavalent chromium with the brown seaweed *Ecklonia* biomass. *Env. Sci. Technol.*, **38**, 4860–4864.
- Park, D., Yun, S.Y. and Park, J.M. (2005) Studies on hexavalent chromium biosorption by chemically treated biomass of *Ecklonia* sp. *Chemosphere*, **60**, 1356–1364.
- Park, S.J. and Jang, Y.S. (2002) Pore structure and surface properties of chemically modified activated carbons for adsorption mechanisms and rate of Cr(VI). *J. Colloid Interf. Sci.*, **249**, 458–463.
- Park, S.J. and Kim, K.D. (1999) Adsorption behaviours of  $\text{CO}_2$  and  $\text{NH}_3$  on chemically surface treated activated carbons. *J. Colloid Interf. Sci.*, **212**, 186–189.
- Pradhan J., Das, S.N. and Thakur, R.S. (1999) Adsorption of Cr(VI) from aqueous solution by using activated red mud, *J. Colloid Interf. Sci.*, **217**, 137–141.
- Pradhan, B.K. and Sandle, N.K. (1999) Effect of different oxidizing agent treatments on the surface properties of activated carbons. *Carbon*, **37**, 1323–1332.
- Prakasham, R.S., Merrie, J.S., Sheela, S.R.N. and Ramkrishna, S.V. (1999) Biosorption of Cr(VI) by free and immobilized *R. arrhizus*. *Env. Poll.*, **104**, 421–427.
- Preetha, B. and Viruthagiri, T. (2007) Batch and continuous biosorption of chromium(VI) by *Rhizopus arrhizus*. *Separ. Purific. Technol.*, **57**, 126–133.
- Ramani, S. P. and Sabharwal, S. (2006) Adsorption behavior of Cr(VI) onto radiation cross linked chitosan and its possible application for the treatment of wastewater containing Cr(VI). *Reactive Funct. Polym.*, **66**, 902–909.
- Rangaraj, S., Yeon, K. H. and Moon, S. H. (2001) Removal of Cr from water and wastewater by ion-exchange resin. *J. Hazard. Mater. B*, **87**, 273–287.
- Rorrer, G.L., Hsien, T. Y. and Way, J. D. (1993) Synthesis of porous magnetic chitosan beads for removal of cadmium ion from wastewater. *Ind. Eng. Chem. Res.*, **32**, 2170–2178.
- Sag, Y., Atacoglu, I. and Kutsal, T. (2000) Equilibrium parameters for the single and multicomponent biosorption of Cr(VI) and Fe (III) ions on *R. arrhizus* in a packed bed column. *Hydrometallurgy*, **55**, 165–179.
- Schneider, I. A. H. and Rubio, J. (1999) Sorption of heavy metal ions by the non living biomass of freshwater macrophytes. *Env. Sci. Technol.*, **33**, 2213–2217.
- Seki, H., Suzuki, A. and Marugama, H. (2005) Biosorption of Cr(VI) and As(V) onto methylated yeast biomass. *J. Colloid Interf. Sci.*, **281**, 261–266.
- Sharma, A. and Bhattacharya, K.G. (2004) Adsorption of Cr(VI) on *Azadirachta indica* (neem) leaf powder. *Adsorption*, **10**, 327–338.
- Sharma, Y.C. (2001) Effect of temperature on interfacial adsorption of Cr(VI) on Wollastonite. *J. Colloid Interf. Sci.*, **233**, 265–270.
- Tahir, H., Saleem, M., Afzaq, M., Ahmad, H., Hussain, S.T. and Afzal, J. (1998) Estimation and removal of Cr ion from tannery waste using Zeolite-3A. *Adsorp. Sci. Technol.*, **16**, 153–161.
- Tewari, N., Vasudevan, P. and Gupta, B.K. (2005) Study on biosorption of Cr (VI) by *M. hiemalis*. *Biochem. Eng. J.*, **23**, 185–192.
- Veglio, F. and Beolcini, F. (1997) Removal of metals by biosorption: a review. *Hydrometallurgy*, **44**, 301.
- Wang, T. and Li, Z. (2004) High temp reduction of Cr(VI) in solid alkali. *J. Hazard. Mater. B*, **112**, 63–69.
- Zhao, N., Wei, N., Li, J., Qiao, Z., Cui, J. and He, F. (2005) Surface properties of chemically modified activated carbons for adsorption rate of Cr(VI). *Chem. Eng. J.*, **115**, 133–138.

# 31

## Using the Biomass of Seaweeds in the Production of Components of Feed and Fertilizers

Katarzyna Chojnacka

*Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology, Poland*

### 31.1 Introduction

Seaweeds have recently been used as a renewable resource – food, feed and fertilizer (Hong *et al.*, 2007; Sivasankari *et al.*, 2006). Utilized species include, for example, *Ulva*, *Caulerpa*, *Gelidiella*, *Laurencia*, *Hypnea*, or *Porphyra* sp. (Hong *et al.*, 2007). Their value is related with high content of proteins, lipids (polyunsaturated fatty acids), vitamins, pigments, macro- and microelements (Hong *et al.*, 2007). Other bioactive compounds incorporate fucoidans, phlorotannins – algal polyphenols – antiviral, antibacterial, and antioxidative compounds (Takashi and Takahiko, 2009). Seaweeds are rich in polysaccharides and antioxidants and therefore are valuable dietary and pharmaceutical supplements (Takashi and Takahiko, 2009). For instance, extract from *Sargassum* sp. is rich in potassium, magnesium, and calcium, as well as in high molecular weight water-soluble polysaccharides (alginates), which give its extracts high viscosity (Takashi and Takahiko, 2009). Of importance is the content of phycocolloids – agar, alginic acid, and carrageenan (Sivasankari *et al.*, 2006). Fertilizer applications are similar to farmyard manure. Liquid extracts from seaweeds can be used as foliar fertilizers, which are either sprayed on seeds, leaves or added to soil (Sivasankari *et al.*, 2006). These formulations contain hormones that promote plant growth: IAA and IBA,

cytokinins, trace elements (Fe, Mn, Cu, Co, Zn, Mo and Ni), vitamins, and amino acids (Sivasankari *et al.*, 2006).

Seaweeds can be easily collected and handled (de Oliveira *et al.*, 2009). The biomass is deposited on beaches; however its utilization is difficult, because of possible contamination with wastes (Castlehouse *et al.*, 2003). Macroalgae can also be cultivated by floating raft, fixed off-bottom monolines, broadcasting seedlings, or fixed off-bottom nets (Hong *et al.*, 2007).

### 31.2 Seaweeds in fertilizers

#### 31.2.1 General aspects of using seaweeds and their extracts as fertilizers

As the world population permanently increases, it is estimated that the global demand for fertilizer will reach 220 Tg/year by the middle of the century (Kawashima *et al.*, 1997). Conventional nitrogen fertilizers still play a major role on the market, but on the other hand they disturb the natural nitrogen cycle resulting in increased nitrification and denitrification and consequent generation of nitrous oxide (Kawashima *et al.*, 1997). The excess use of fertilizers causes environmental problems, such as eutrophication

(Kawashima *et al.*, 1997). A sustainable solution of the problem could be fertilizers from seaweeds, the biomass of which is a side product of this process.

Seaweeds have been collected and utilized in coastal areas since ancient times, particularly in the Far East (Hong *et al.*, 2007; Takashi and Takahiko, 2009) and in ancient Rome (Thirumaran *et al.*, 2009). They were also popular in Britain, France, Spain, Japan, and China (Thirumaran *et al.*, 2009). Nowadays, inedible seaweeds can be resources of bio-active compounds and also used for silage and fertilizer manufacture (Takashi and Takahiko, 2009). Many seaweed fertilizers are manufactured from the biomass of *Ascophyllum nodosum* from North Atlantic and Arctic Ocean (Bateman and Simon, 2007).

Fertilizers are harvested fresh, chopped, dried, or milled into seaweed meal, to minimize the loss of volatile compounds (if dried naturally) (Bateman and Simon, 2007). Seaweeds can be applied directly to soils and allowed to compost or dried and ground (meal) and then applied (Lee, 2008). Fresh seaweeds can be used as soil amendments (Thirumaran *et al.*, 2009). Both (raw seaweeds and meal) act as slow-release fertilizer, condition and aerate the soil. Other methods of fertilization by seaweeds include the production of extracts through hydrolysis of macerated seaweed in a pressure chamber under cold or alkaline conditions (Bateman and Simon, 2007).

The advantages of fertilizers from seaweeds are cost-effectiveness as compared to synthetic fertilizers and the presence of biostimulants for plant growth (Wajahatullah *et al.*, 2009). Seaweeds have good fertilizer properties because contain all the nutrients and hormones required by plants: plant growth hormones, regulators and promoters (Thirumaran *et al.*, 2009). Good fertilizer properties of seaweeds are related to the presence of macronutrients (N, P, K), but also trace elements and metabolites (Sivasankari *et al.*, 2006; Lee, 2008). Seaweed fertilizers contain high levels of K and low levels of N and P as compared with manure (Thirumaran *et al.*, 2009). The presence of organic matter favors retention of water and minerals in topsoil, and thus availability to plant through the root system (Sivasankari *et al.*, 2006). Additionally, seaweeds do not contain toxic elements at the level found in conventional fertilizers (As, Cd) (Thirumaran *et al.*, 2009).

### 31.2.2 Seaweed extracts as fertilizers

Seaweed extracts recently became popular in sustainable agriculture (Chouliaras *et al.*, 2009) and are used as foliar fertilizers for cereals, vegetables, fruits, orchards, and horticultural plants (Thirumaran *et al.*, 2009). It was proved that seaweed fertilizers increase crop yield, quality and uptake of inorganic nutrients from soil, and are resistant

to stress conditions, fungi, and insects, all of which reduces the costs (Chouliaras *et al.*, 2009). Seaweed extracts contain growth-promoting hormones (IAA and IBA), cytokinins, gibberellins, trace elements vitamins, amino acids, antibiotics, and micronutrients (Thirumaran *et al.*, 2009). Important in good fertilizer properties are trace elements and metabolites, as well as organic matter (e.g., carbohydrates), which improves moisture retaining capacity of soils (Thirumaran *et al.*, 2009).

### 31.2.3 Plant biostimulants from seaweeds

Biostimulants are metabolic enhancers – materials other than fertilizers, promoting growth of plants if applied in small quantities – diluted 1:1000 (Wajahatullah *et al.*, 2009). Seaweed biostimulants contain: nutrients (macro- and microelements), amino acids, vitamins, cytokinins, auxins, abscisic acid-like growth substances (Wajahatullah *et al.*, 2009). Also, laminarin stimulates the natural defense of plants against pathogens (e.g., antimicrobial agents) (Wajahatullah *et al.*, 2009). Auxins promote root activity; betains alleviate osmotic stress and enhance chlorophyll production (Wajahatullah *et al.*, 2009).

### 31.2.4 Commercial seaweed fertilizers

On the market, several seaweed powder and liquid fertilizer concentrates (Table 31.1) are available (Lee, 2008). The following seaweeds are the components: in the northern (*Ascophyllum nodosum*, *Fucus* sp., *Sargassum* sp. and *Laminaria* sp.) and southern (*Ecklonia* and *Durvillea*) hemispheres (Lee, 2008). Seaweeds and seaweed products are permitted in organic cultivation in European Union, as specified by Council Regulation (EEC) No. 2092/91 (Bateman and Simon, 2007).

### 31.2.5 Studies on cultivation of plants on seaweed derived fertilizers

Mulbry *et al.* (2007) prepared slow release nitrogen biofertilizer from algal biomass from treatment of dairy and swine manure. It was found that the algal biomass contained 40% of plant-available N and P. The experiments were carried out on corn and cucumber (Mulbry *et al.*, 2007).

Chouliaras *et al.* (2009) applied seaweed fertilizer foliarly in the cultivation of olive trees, together with soil fertilization with N and B. Productivity and the content of oil increased, together with accelerated maturation of olive fruits (Chouliaras *et al.*, 2009). Tree productivity was higher, nutritional status was improved, and quality parameters of olive oil were more advantageous (Chouliaras *et al.*,

**Table 31.1** Commercially available seaweed preparations for plants

Seaweed based fertilizers and their manufacturers (Bateman and Simon, 2007):
Seaweed extract – Grower, Maxicrop
Seaweed based liquid feed – Greenfingers, Vitax,
Natural seaweed meal – Maxicrop
Seaweed based liquid feed – B&Q
Seaweed based fertilizer – Grower.
Seaweed extracts (Sivasankari <i>et al.</i> , 2006):
Maxicrop (Sea born),
Algifert (marinure)
Goemar GA14
Kelpak 66,
Seaspray
Seasol
SM3
Cytex
Seacrop 16
Species of seaweeds used in plant growth stimulants and plant biostimulants production and their manufacturers (Wajahatullah <i>et al.</i> , 2009):
<i>Ascophyllum nodosum</i> – brand name (company) Acadian (Acadian Agritech), Agri-Gro Ultra (Agri-Gro Marketing Inc.), Alg-A-Mic (BioBizz Worldwide N.V.), Bio-Genesis (Green Air Products, Inc.), Guarantee (Mainstream Organics), Kelp meal (Acadian Seaplants Ltd.)
<i>Ecklonia maxima</i> – Kelpak (BASF)
<i>Durvillea antarctica</i> – Profert (BASF)
<i>Durvillea potatorium</i> – Seasol (Seasol International Pty Ltd.)
<i>Macrocystis pyrifera</i> – AgroKelp (Algas y Bioderivados Marinos S.A.)
<i>Lithothamnium calcareum</i> – Acid Buf (Chance & Hunt Limited)
<i>Ascophyllum nodosum</i> – Tasco (Acadian Agritech)

2009). Seaweed fertilizer caused an increase of the content of K, Fe, Cu in olive leaves. The concentration in olive oil of linolenic and oleic acids increased and palmitoleic, stearic and linoleic acids, as well as total phenols decreased (Chouliaras *et al.*, 2009).

Other examples of the application of seaweed fertilizer embrace: *Ascophyllum nodosum* extract on spinach (Cassan *et al.*, 1992), in nitrogen fixing trees (Berlyn and Russo, 1990), greenhouse tomato plants (Crouch and van Staden, 1992), cucumber (Passam *et al.*, 1993), kiwi (Chouliaras *et al.*, 1997), mandarin and orange (Fornes *et al.*, 2002). Advantageous effects of seaweed extracts on fruit and veg-

etable growth, weight, and maturation were proved. In all cases crop yield and storage properties increased.

An advantageous effect of *Sargassum wightii* used foliarly on *Zizyphus mauritania* was related to increased crop yield and quality of fruits (Rama Rao, 1991). Sivasankari *et al.* (2006) investigated the effect of seaweed liquid fertilizer on *Vigna sinensis*. The fertilizer was manufactured from *Sargassum wightii* and *Caulerpa chemnitzia*. The seeds were soaked in the extract (Sivasankari *et al.*, 2006). Improved germination was noted as compared with the control (Sivasankari *et al.*, 2006).

Other results of studies on plants cultivation on fertilizers derived from seaweeds are as follows:

- Soil application of seaweed liquid fertilizer (SLF) from *Enteromorpha clathrata* and *Hypnea musciformis* increased growth and yield of green gram, black gram and rice (Kannan and Tamilselvan, 1990).
- Seaweed concentrate (Kelpak®) increased growth and yield of potassium-stressed wheat (Beckett and van Staden, 1989).
- Foliar spray of SLF from *Sargassum wightii* on *Zizyphus mauritania* increased yield and quality of fruits (Bhosle *et al.*, 1975).
- SLF from *Ascophyllum nodosum* increased chlorophyll in cucumber cotyledons and tomato plants (Whapham *et al.*, 1993).
- SLF from *Rosenvingea intricata* increased growth, yield, seed germination, and pigment concentration of *Abelmoschus esculents* (Thirumaran *et al.*, 2009).

### 31.2.6 Seaweed fertilizer as value-added product from manure

Algae can be grown on anaerobically digested dairy manure. In this case, N and P are transformed into algal biomass (Mulbry *et al.*, 2005). Such a concept can be considered as recycling of manure nutrients to produce slow release fertilizer, since algal biomass contains 33% of available to plant N. Seaweeds are not able to fix nitrogen and assimilate by absorption of nitrate and ammonium (Bateman and Simon, 2007). Corn seedlings cultivated on such fertilizer (dry algal biomass) contained 46–60% of available N and 38–60% of P. Seedlings dry weight and nutrient content was similar as in the control grown on conventional NP fertilizer (Mulbry *et al.*, 2005). Algae can thus be used as soil conditioning amendments and biofertilizer (Mulbry *et al.*, 2005).

Algal systems can also be used to generate energy from manure (Mulbry *et al.*, 2007). Algal byproducts can be used as soil conditioning amendment and biofertilizer in rice cultivation (Mulbry *et al.*, 2007).

### 31.3 Seaweeds in feeds for animals

#### 31.3.1 General aspects of using seaweeds and their extracts in animal diet

Commercial applications as the components of feeds are less widespread as fertilizers (Lee, 2008). Seaweeds can also be considered as a source of biologically active compounds for animals and represent a potential feed alternative. For this reason they are recommended as dietary supplements and have an economic potential in animal nutrition, since may compensate nutritional deficiencies of diets (Taboada *et al.*, 2010). Many types of seaweed are also authorized for human consumption (e.g., *Ulva* sp.) (Taboada *et al.*, 2010). Seaweeds are generally accepted by the consumers as safe (Bach *et al.*, 2008), and also by several institutions: US Food and Drug Administration (FDA), Canadian Food Inspection Agency (CFIA), to be used as the components of feed (Bach *et al.*, 2008).

Seaweeds contain various compounds that are beneficial for the health of animals: antibacterial, antioxidant, anti-inflammatory, antiviral agents, and dietary fiber (O'Sullivan *et al.*, 2010). Nutritional properties of seaweeds are not fully known as well as of terrestrial plants (de Oliveira *et al.*, 2009). Seaweeds contain low levels of lipids (<1%), high levels of protein (10–15%) and ash (13–25%), non-starch polysaccharides (dietary fiber) and vitamins (de Oliveira *et al.*, 2009). Literature reports that brown seaweeds contain more bioactive compounds than those of red and green algae (Gardiner *et al.*, 2008). The advantageous properties take account of improvements of the immune system, carcass characteristics, and the extended shelf life of meat (Bach *et al.*, 2008). Macroalgae are used in the fodder of domestic animals as a substitute for animal ingredients (pigments) to improve resistance to diseases and egg production (Cruz-Suarez *et al.*, 2009). Also, aquatic animals are fed with seaweeds: sea bass, snakehead, and shrimp (Cruz-Suarez *et al.*, 2009).

Seaweeds are rich in polysaccharides, which are not digested by animals themselves but are degraded by bacteria (Lynch *et al.*, 2010). Those polysaccharides are prebiotics – non-digestible compounds that stimulate gut microflora – fucoidan, laminarin, and alginate (O'Sullivan *et al.*, 2010). These compounds are the components of cell walls and also play a role as food reserves and provide strength and flexibility to cope with waves (O'Sullivan *et al.*, 2010). Also,

seaweed extracts find an application in feeding animals, since they contain bioactive compounds that improve activity of the immune system and have advantageous effects on the gastrointestinal flora (Leonard *et al.*, 2010).

The disadvantages include fiber-gelling properties, which increase food retention, slower digestion, and absorption (Lee, 2008). Of concern is the elevated level of arsenic in the biomass of seaweeds (Feldmann *et al.*, 2000).

#### 31.3.2 Seaweeds in feeds – historical aspects

Seaweeds have been used for centuries as feed supplements in livestock diet (Hansen *et al.*, 2003). Ancient Romans fed horses with seaweeds. Also, in Iceland, Norway, and France, domestic animals were fed with macroalgae (Craigie, 2011). Importance is that they possess high level of minerals, vitamins, non-digestible polysaccharides (dietary fiber), high level of protein, and low level of lipids, as compared with terrestrial plants (Hansen *et al.*, 2003; Takashi and Takahiko, 2009). Seaweeds were found useful in diet of ruminants, including sheep or goats (Hansen *et al.*, 2003).

*Palmaria palmata* and *Alaria esculenta* have been used as winter fodder since the fifth century in north-western Europe (coastal areas) in feeding domestic animals: sheep, cattle and horses (Balasse *et al.*, 2005). *A. nodosum* was extensively investigated as fodder for dairy cows, sheep, hogs and poultry and was showed to improve growth of animals if supplemented on the level lower than 10% (Craigie, 2011).

There are sheep which graze solely on seaweeds (e.g., brown kelp), since the biomass is available throughout the whole year: in winter is driven ashore by storms, in summer – by tides (Balasse *et al.*, 2005). These sheep developed physiological adaptation and have unique microflora differing from non-adapted sheep (Balasse *et al.*, 2005). Cellulolytic microorganisms are absent. Instead present are xylan and laminarin decomposers, because these compounds are found in *Palmaria palmata* (Balasse *et al.*, 2005).

Identification of which animals are fed on seaweeds is possible by the analysis of their tooth characteristics. An example could be the work of Balasse *et al.* (2005), who investigated tooth enamel as the method of identification if sheep grazed on seaweeds in prehistoric times by isotope analysis, using the difference in <sup>13</sup>C and <sup>18</sup>O between terrestrial plants and seaweeds (Balasse *et al.*, 2005). Identification of seaweed-rich fodder is also possible by dental microwear analysis (Ingrid and Mainland, 2000). Different microwear pattern is attributed to different forces and movements in mastication, due to different texture (Ingrid and Mainland, 2000).

### 31.3.3 Nutritional properties of seaweeds

It is reported that seaweed feeds are beneficial for beef, dairy cows, lambs, and pigs – improved performance of growth, quality of carcass, resistance to diseases, nutritive properties of meat, were confirmed by several studies (Braden *et al.*, 2007; Saker *et al.*, 2003; Turner *et al.* 2002; Franklin *et al.*, 1999; Lee, 2008). Various literature reports agree that the presence of seaweeds or their extracts in livestock diet improves health, reproduction, milk productivity, gastrointestinal flora, increases resistance to diseases (Craigie, 2011). Several studies showed that mass of eggs laid by hens fed with seaweed supplements increased (Carrillo *et al.*, 2008). As supplements, growth performance and the quality of carcass was improved, similarly as nutritional value of meat (Lee, 2008). In sheep, wool production was improved (Craigie, 2011).

### 31.3.4 Seaweed nutraceuticals

Several studies confirmed that seaweeds and their extracts can be used as alternatives to nutritional antibiotics (Dierick *et al.*, 2010). The compounds also have antioxidant properties (Dierick *et al.*, 2010). Non-digestible polysaccharides support the activity of fermentative bacteria, favoring the production of short chain fatty acids, which can be absorbed as the source of energy, reduce pH in the gut and affect the population of microflora (Gardiner *et al.*, 2008). Also, pure compounds isolated from algae (polyketides) are used as supplements of daily diet for poultry and cattle (Cardozo *et al.*, 2007).

Seaweeds contain polysaccharides which are resistant to hydrolysis and are dietary fiber (Deville *et al.*, 2004). *In vivo* and *in vitro* studies showed that seaweeds and their extracts improve performance and reduce pathogenic bacteria in livestock (Gardiner *et al.*, 2008). Brown seaweeds contain the following polysaccharides: laminarin, fucoidan, and alginic acid (Zvyagintseva *et al.*, 2003). Laminarins are low molecular weight glucans that are soluble in water and are isolated from Phaeophyta (Zvyagintseva *et al.*, 1999). Gahan *et al.* (2009) showed that these substances acted as a substrate for *Bifidobacteria* and *Lactobacilli* sp. (O'Connell *et al.*, 2005) and thus can be used as a substitute for lactose (a substrate for microflora of large intestine). This maintains high performance of post-weaned piglets fed with promoter-free diets (Gahan *et al.*, 2009).

The advantageous properties of laminarin consist of antimicrobial properties since it influences adherence and translocation of bacteria across epithelial cell walls, modulates metabolism in intestine (mucus composition, pH, short chain fatty acids, e.g. butyrate) (O'Doherty *et al.*, 2010). Fucoidans have antitumor, antiviral, and antibacte-

rial properties (O'Doherty *et al.*, 2010). Both, laminarin and fucoidan can be used in antibiotic growth promoter-free diets and reduce the counts of *Escherichia coli* (O'Doherty *et al.*, 2010). *Ascophyllum nodosum* contain lectins that inhibit attachment of microorganisms to the wall of gut, which reduces the prevalence of some pathogenic microorganisms, which reduces the risk of diarrhea in weanling pigs fed with antibiotics-free diets (Gardiner *et al.*, 2008).

### 31.3.5 Studies on animal breeding using seaweed meals

Various species of macroalgae are edible to animals: *Ascophyllum nodosum*, *Schizochytrium* sp., *Laminaria* sp., *Durvillea* sp. Bull kelp (*Durvillea* sp.) is used as feed for livestock, in particular dairy and beef cattle (Lee, 2008). *Ulva* was used as feed supplement for abalones (Daume, 2006; Boarder 2001; Robertson-Andersson *et al.*, 2006) or shrimps (Neori and Shpigel, 2007; Braud, 2006). *Gracilaria* is applied as feed in growing marine animals, such as direct feed for abalone (Tseng, 2001). Extracts from seaweeds are also useful as the components of growth media for microalgae, which are then used as feed for mollusks, larvae of crustaceans, and fish (Alvarado *et al.*, 2008).

### Poultry

Carrillo *et al.* (2008) investigated the effect of *Macrocystis pyrifera*, *Sargassum sinicola* and *Eneromorpha* sp. on the quality, lipid content, the level of *n*-3 fatty acids, and consumer acceptability of eggs fed with seaweeds. Algae contain fatty acids: linoleic,  $\alpha$ -linolenic, arachidonic, eicosapentaenoic, docosahexaenoic. The content of crude protein in seaweed meal is 6.6–14.1%, metabolizable energy 2.2–2.5 kcal/g. The level of minerals is as follows: Ca (1.2–3.2%), sodium (9.2–31.1%), potassium (1.8–5.8%), magnesium (0.5–0.9%) and phosphate (0.01–3.5%) (Carrillo *et al.*, 2008). In hens fed with the diet containing 10% seaweed *M. pyrifera*, similarly as *n*-3 fatty acids, the albumen height and yolk color was improved. The mass of egg and the flavor were not affected (Carrillo *et al.*, 2008).

Seaweeds were used as the source of iodine for laying hens (Kaufmann *et al.*, 1998). It is hypothesized that biofortification of eggs with iodine would improve the problem of iodine deficiency in humans (Kaufmann *et al.*, 1998).

In another study, laying hens were fed with seaweed meal containing a carotenoid fucoxanthin, the transfer of which to egg yolks was investigated (Strand *et al.*, 1998). The level of dietary supplementation was 15%. Although fucoxanthin itself was not transferred to egg yolks, its metabolites improved the level of pigmentation (Strand *et al.*, 1998).

### Rats

Taboada *et al.* (2010) fed experimental rats with diet supplemented with an edible alga *Ulva rigida* (90 g of rodent feed was supplemented with 10 g of dried alga). Also, chemical and biochemical analyses of the seaweed were performed. It was found that the alga was a rich source of protein, carbohydrates, fiber, vitamins and minerals, with low level of lipids. Studies on animals showed that *Ulva* was well accepted by the animals. No change in nutritional parameters and reduction of LDL cholesterol was observed (Taboada *et al.*, 2010). In another study it was reported that *Ulva reticulata* advantageously influenced metabolism of fatty acids in mice (Hong *et al.*, 2007).

### Sheep

Sheep fed with only seaweed (brown kelps *Laminaria digitata* and *Laminaria hyperborea*) *ad libitum*, with adapted and non-adapted gut microflora were examined (Hansen *et al.*, 2003). Sheep consumed 1.4 kg of fresh seaweed/day (Hansen *et al.*, 2003). Seaweed uptake did not differ statistically between the groups. Adapted, as well as non-adapted sheep, showed high values of digestibility and were able to obtain energy only from seaweed fodder (Hansen *et al.*, 2003).

### Cattle and lambs

Acadian Seaplants Ltd. (Canada) produces a preparation based on sun-dried and ground brown kelp *Ascophyllum nodosum* (Bach *et al.*, 2008). The supplement was included in the feed for cattle and lambs. The level of supplementation was 0–20 g/kg. In animals containing seaweeds in their diet, the presence of pathogens in feces and *E. coli* shedding was lower (Bach *et al.*, 2008). Average daily gain, feed intake, feed efficiency, and carcass traits, as well as animal growth did not differ between the experimental and the control groups (Bach *et al.*, 2008).

### Pigs

Dierick *et al.* (2010) investigated the effect of *Ascophyllum nodosum* on gut microflora of piglets under *in vitro* conditions. It was confirmed that in fact the algae improved gut microflora and also gastrointestinal health status and the differences were statistically significant (Dierick *et al.*, 2010).

Meal of *A. nodosum* was fed to healthy weanling piglets in the quantity 10–20 g/kg of basal feed (Dierick *et al.*, 2009). Statistically significantly lower levels of *E. coli* in small intestine were encountered together with increased ratio of

*Lactobacillus/E. coli* (supplementation 10 g/kg). No effect on the final weight was found (Dierick *et al.*, 2009).

### Aquatic animals

In South Africa, natural kelp *Ecklonia maxima* is harvested to feed abalone and to produce a liquid growth stimulant for crops – Kelpak® (Troell *et al.*, 2006). Since the biomass of the kelp has found so many applications, a concern arose about its presence in the ecosystem (Troell *et al.*, 2006).

Shrimp can be fed with *Macrocystis pyrifera*, *Ascophyllum nodosum* or *Sargassum* sp. The presence of seaweed in fodder gives it some advantageous properties: integrity, water-holding capacity, texture, water stability, and lower leaching of nutrients, which results in higher feed intake and better growth performance (Cruz-Suarez *et al.*, 2009; Immanuel *et al.*, 2010).

### Other animals

There are snails which feed solely on seaweeds; for example *Norrisia norrisi* consumes *Macrocystis pyrifera* (Wakefield and Murray, 1998). It was found that snails prefer seaweeds that contain high levels of nitrogen. Important is also higher edibility and palatability (lower structural toughness (measured by penetrometer) and lower levels of phlorotannins) (Wakefield and Murray, 1998).

## 31.3.6 Studies on animal breeding using seaweed extracts

### Rats

The diet of rats was supplemented with 2.5% alginate, which increased counts of *Bifidobacterium* and *Lactobacillus* (O'Sullivan *et al.*, 2010). Supplementation of rodents' diet with laminarin protected from lipopolysaccharide-induced liver toxicity (O'Sullivan *et al.*, 2010).

### Lambs

Extract from brown seaweed *Ascophyllum nodosum* was used in feeding lambs (Saker *et al.*, 2004). The extract improved functioning of immune system and protected from prolonged heat-induced oxidative stress (Saker *et al.*, 2004). Seaweed extracts can be thus considered to be immunomodulatory (Saker *et al.*, 2004).

### Pigs

Leonard *et al.* (2010) studied the effect of supplementation with seaweed extract of maternal diet of weaned pigs. The pigs' performance, intestinal morphology, microflora,

and immunological status were investigated. A higher daily gain in the experimental group was noted as was lower fecal *E. coli*. Generally, the presence of seaweed extracts in maternal diet influenced advantageously gastrointestinal environment and performance of weaned piglets by the reduction of the population of Enterobacteriaceae (Leonard *et al.*, 2010).

Lynch *et al.* (2010) investigated the effect of seaweed extract from *Laminaria* sp. containing the prebiotics laminarin and fucoidan on boars. Nutrient digestibility, intestinal fermentation and microbial populations were studied (Lynch *et al.*, 2010). The reduction of *Enterobacterium* sp. and increase of *Lactobacilli* sp. in intestine showed the potential of the extract to improve gut health of pigs (Lynch *et al.*, 2010). It is hypothesized that seaweed extract modulates the environment of gut and immunity, which reduces diarrhea and improves productivity (Lynch *et al.*, 2010).

O'Doherty *et al.* (2010) investigated the effect of seaweed extract from *Laminaria* sp. containing laminarin and fucoidan on growth performance, digestibility and microbial populations in feces of weanling pigs. Higher average daily gain, gain to feed ratio in pigs, the diet of which was supplemented with seaweed extract was found and the differences were statistically significant (O'Doherty *et al.*, 2010). The supplementation of feed with seaweed extract resulted in increased nitrogen apparent digestibility, gross energy and reduced the population of *E. coli* in feces (O'Doherty *et al.*, 2010).

### 31.3.7 Integrated processes – aquaculture

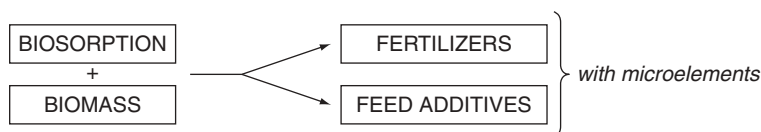
Macroalgal cultures can be integrated with mariculture of fish or invertebrates to remove nutrients from wastewaters which stays in accordance with sustainable development (Zhou *et al.*, 2006). *Gracilaria* (Rhodophyta) gives high yields and is an efficient nutrient pump (Zhou *et al.*, 2006). Such integrated aquaculture systems enable to simultaneously remove waste nutrients from fish culture – soluble compounds of carbon, nitrogen and phosphorus. The biomass can be used as fodder for shellfish such as abalone (Zhou *et al.*, 2006). A pilot system where annually 70 t of fresh seaweed biomass is harvested (9 t dry mass)

with the simultaneous removal of the following quantities of waste nutrients: 0.2 t N, 0.03 t P was constructed (Zhou *et al.*, 2006).

Seaweeds can be integrated in marine aquaculture systems (Chopin *et al.*, 2001). The wastes of one resource become a resource (fertilizer or food) for another stage (Chopin *et al.*, 2001). In such balanced polytrophic ecosystems, organisms are cocultured and many value-added crops are produced (Chopin *et al.*, 2001). Seaweeds play a role of renewable biological nutrient scrubber, because can assimilate 90% of ammonia in water (Chopin *et al.*, 2001). This shows the necessity of assurance of reasonable harvesting rate – periodic removal of tissues to allow growth of new biomass (Chopin *et al.*, 2001). Since seaweeds can reduce nutrient concentrations in seawater, controlled harvesting of their biomass would participate in reduced fertility of waters and would be a method to cope with the problem of eutrophication – green tides – extensive blooms of macroalgae: *Enteromorpha*, *Ulva* and *Cladophora* (Chopin *et al.*, 2001). The harvested biomass can be used as NK fertilizer.

## 31.4 Using the biomass of seaweeds enriched with microelements by biosorption in nutrition of plants and animals

Macroalgae are well known biosorbents of metal cations. This property is used in wastewater treatment processes, but it can also be employed in the elaboration of new products – fertilizer and feed components with microelements, whereby seaweed biomass serves as biological carrier of nutritionally important elements (Figure 31.1). Although biosorption is a well-known process, discussed in many papers and patent databases, its application so far has been confined only to toxic metals removal from wastewaters. Such an approach is an attempt to solve the problem of microelements scarcity in the trophic chain. The concept of the production of biological fertilizer concentrates with microelements by biosorption is new. In the production of seaweed fertilizers components with micronutrients,



**Figure 31.1** Biosorption as the method of manufacture of products with microelements.

the effect of combined biosorptive and biostimulating properties of seaweeds is attained.

### 31.4.1 Microelement hunger

Deficiency of microelements in the diet of humans, similarly as in plants and animals, according to Liebig's law of minimum causes the phenomenon of hidden hunger. This problem concerns globally over 2 billion people (World Health Organization, 1992; Graham and Welch, 1996). Therefore, in recent years attempts have been made to enrich food with microelements, to improve its nutritional value. The new approach was termed farming for health. Biofortification is a new concept describing the manufacture of food products with increased content of minerals and vitamins (Johns and Pablo, 2007). In the case of consumption of such food, supplementation of diet with microelement preparations would not be necessary.

At present, deficiency of microelements in human diet is treated with preparations in the form of inorganic salts, mainly oxides, sulfates, nitrates, chlorides (e.g., zinc oxide, cupric sulfate, manganese sulfate, chromium chloride). Microelements in such form possess low availability and are of transit character, frequently posing toxicity.

### 31.4.2 Biofortification of food

In the literature, only few years ago, the first information appeared on food biofortified with microelements. Results of clinical studies investigating bioavailability of microelements either from biofortified food or dietary supplements has not been found in the available literature. Such studies are essential and can be carried out, for example on swine, which can be used as a model organism (instead of humans). This would give preliminary information on bioavailability of microelements to human, because from swine, invasive matrices (e.g., internal tissues) could be sampled.

It is possible to produce a new generation of food biofortified with microelements by using unique biosorption and bioaccumulation properties of algae to bind metal cations in the production of biologically bound and thus highly bioavailable form of microelements of feeding and fertilizing significance (Chojnacka, 2008). It is possible to obtain by these methods biological components of feed and fertilizers (Figure 31.1) whereby microelement cations (Cu(II), Fe(II), Mn(II), Zn(II), Co(II)) are bound with the functional groups exposed on the surface of macroalgal cell walls, such as carboxyl. The chemistry of this process is similar to commercially available preparations (chelates), which are costly formulations used as both feed supplements and as micronutrient fertilizers.

### 31.4.3 Using biosorption to increase bioavailability of microelements

Microelements supplemented in inorganic form are absorbed on very low level – a few percent. Therefore are of transitory character. The majority is excreted from an organism with urine or feces and this poses environmental concern. Because of higher absorption of microelements bound with the biomass, the problem mentioned above can be eliminated. The biomass of algae possesses natural properties of binding microelement ions – natural cation-exchange properties.

Biological carriers of micronutrients can be seaweeds, for instance *Enteromorpha prolifera*, *Cladophora rupestris* and *Ulva lactuca* collected from the Baltic Sea: freshwater: *Pithophora varia* Wille, *Vaucheria* sp. (Michalak and Chojnacka, 2008, 2009a, b; 2010a). Studies on kinetics and equilibrium of these processes were undertaken, which were described with mathematical models, universal for all the studied sorbents. The effect of operation conditions (pH, temperature, contact time) on biosorptive properties was investigated. It was proved that for all the seaweeds, the mechanism of biosorption was ion exchange, in which exchange with functional groups (e.g., carboxyl) present on cellular surfaces occurred. This exchange takes place mainly with alkali (Na(I), K(I)) and alkali earth metal cations (Ca(II), Mg(II)), to microelement cations of nutritive significance for plants and animals (Michalak and Chojnacka, 2010b; Chojnacka, 2010).

Enrichment with microelements can occur either by suspending the biomass in the solution containing microelement ions or by spraying, and afterwards drying. Literature widely discusses good biosorptive properties of seaweeds towards metal cations. The content of metal cations after enrichment is on the level 20–50 mg/g (Michalak and Chojnacka, 2008, 2009a, b, 2010a). Enriched biomass can be used as the substitute of inorganic salts in the diet of livestock or in fertilizers.

During biosorption, microelement cations are bound with various functional groups on the surface of cells, mainly with carboxyl (Chojnacka, 2010). The release of bound microelement cations is controlled, similarly as in fertilizers or feed supplements, where soluble chelators (e.g., EDTA or cation-exchange resins) are used. The properties of seaweeds enriched with microelements by biosorption are very similar. The difference of the latter preparation is that is insoluble and biodegradable. However, the chemistry of microelement binding is analogous to that of chelators, whereby microelement cations are chelated also with carboxyl group of for example, the amino acid glycine. The biomass of seaweeds is significantly cheaper

carrier of microelements than amino acids and assures cost-effectiveness.

#### 31.4.4 Seaweeds as biosorbents – carriers of microelements in nutrition of plants and animals – to produce biofortified food

By supplementation of livestock feed and fertilizers with highly bioavailable forms of microelements it is possible to biofortify food of plant and animal origin with microelements (Figure 31.2). Additionally, biologically bound microelements are safer because they resemble the form that naturally occurs in feed or in soil. Therefore, also increase of production yield could be expected (Bhattacharya *et al.*, 2007; Lucena, 2006; McDowell, 1996; Chowdhury *et al.*, 2004).

The preparations with algae were tested on animals – laying hens and fatteners. This enabled to obtain hens' eggs and poultry meat biofortified with microelements (Michalak and Chojnacka, 2008). Studies on fatteners showed enlargement of liver in the control group fed with diet containing inorganic salts. In the experimental group (fed with microelements bound with the biomass of seaweeds), statistically significantly smaller livers were denoted. The difference was statistically significant. This showed that microelements supplemented in the form bound with the biomass of seaweeds did not activate protective mechanisms of an organism. Detoxification properties of the preparation are also probable. The results of these experiments might indicate that by gradual increase of the dose of biologically bound microelements it would be possible to further biofortify food. However, suitable studies are required.

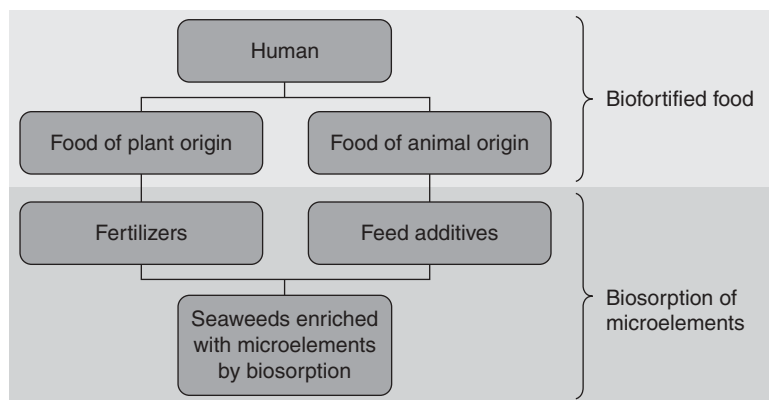
### 31.5 Conclusions

Although every year, 15 million tonnes of products from seaweeds are manufactured, marine seaweeds are still considered an underutilized bioresource (Wajahatullah *et al.*, 2009). Seaweeds in agriculture are used as soil conditioners, fertilizers and green manure (Thirumaran *et al.*, 2009). Using algae as the material for the production of either fertilizers or feeds is consistent with the concept of green agriculture (Castlehouse *et al.*, 2003). Seaweed biomass can be used as manure, animal feed, and food for humans (Sivasankari *et al.*, 2006).

Seaweeds and products derived from them have found an application for centuries as soil amendments due to the presence of biostimulants, which increase plant growth and yield (Wajahatullah *et al.*, 2009). This biomass has been used for a long time in crop protection, as the source of bioactive compounds – plant growth regulators (cytokinins, auxins, gibberellins, indole acetic acid) (Bach *et al.*, 2008).

Algal biomass can be used as slow release nitrogen fertilizer (Mulbry *et al.*, 2005). It is advantageous to use algae rather than manure as the source of nutrients for plants (Mulbry *et al.*, 2005). In coastal regions, it is popular to apply seaweeds to the land as a soil improver and fertilizer (Castlehouse *et al.*, 2003). Seaweeds are rich in nutrients that improve texture and the quality of soil (Castlehouse *et al.*, 2003). Hence, they can be considered as renewable and sustainable resources (Castlehouse *et al.*, 2003). Seaweeds can be applied directly to soil, or can be used as liquid formulations, which are available on the market (Castlehouse *et al.*, 2003).

Algae and their extracts are used as biostimulators (biological preparations which substitute chemical crop protection agents because of the presence of nutrients, trace elements, growth enhancing substances and vitamins).



**Figure 31.2** The production of biofortified food by new seaweed products with microelements.

Some preparations basing on seaweeds are available on the market: Bio-algeen S 90, Plus 2, Kielpak™, Chlorophyll, 3 A 86 and Algan. It is well documented that these products are improve the development of shoots and roots (higher root mass), increased resistance to stress and pathogens attack, as well as stimulation of plants development and increased quality of crops.

Both, seaweed meal and seaweed extracts are used as supplements of livestock diet, eg. pigs. Some seaweeds (*Laminaria japonica*) are harvested to reduce eutrophication and the biomass is used as fodder (Zhou *et al.*, 2006). Advantageous properties such as dietary supplements include: improved feed efficiency, binder effect, nutritional, and nutraceutical properties (Cruz-Suarez *et al.*, 2009). Macroalgae are used rather as supplement than feeding material. While low levels of supplementation have advantageous effect on animal health, higher doses might pose some problems. Supplementation level is usually lower than 80 g/kg feed (Cruz-Suarez *et al.*, 2009). Seaweeds and their extracts are considered to be an alternative to in-feed antibiotics in diets of pigs, and also the source of dietary fiber (laminarin, fucoidan and alginic acids, phlorotannins) (O'Doherty *et al.*, 2010).

If seaweeds are to be included in the diet of livestock, some issues are to be discussed and solved, for example the harvested biomass should be analyzed for the potential presence of toxic elements or sanitary safety. It is also important that the population of harvested seaweeds would not fall below a critical level (O'Doherty *et al.*, 2010).

The mechanisms are still unknown. It is reported that the preparations protect plants from biotic and abiotic stresses of the environment, are environmentally benign, safe for health of animals and human (Wajahatullah *et al.*, 2009). More research is required in order to better understand physiological, biological and chemical mechanisms of seaweeds in fertilizers and feed.

The biomass of seaweeds can serve as biological carrier of microelements for plants and animals. Fresh, composted and extracts of the biomass can be used. Using feeds and fertilizers containing highly bioavailable forms of microelements can be useful in the production of biofortified food of plant (vegetables) and animal origin (eggs, milk and meat) with increased density of microelements. Such biofortified food can be used as a substitute of inorganic dietary supplements.

## Acknowledgments

The work was financially supported by Polish National Centre for Research and Development – project no. NR05-0014-10/2010.

## References

- Alvarado, D., Buitrago, E., Sole, M. and Frontado, K. (2008) Experimental evaluation of a composted seaweed extract as microalgal culture media. *Aquacult. Int.*, **16**, 85–90.
- Bach, S.J., Wang, Y. and McAllister T.A. (2008) Effect of feeding sun-dried seaweed (*Ascophyllum nodosum*) on fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle and on growth performance of lambs. *Anim. Feed Sci. Technol.*, **140**, 110–125.
- Balasse, M., Tresset, A., Dobney, K. and Ambrose, S.H. (2005) The use of isotope ratios to test for seaweed eating in sheep. *J. Zool. (Lond.)*, **266**, 283–291.
- Bateman, A.S. and Simon, K.D. (2007) Fertilizer nitrogen isotope signatures. *Isotopes Env. Health Studies*, **43**, 237–247.
- Beckett, R.P. and Van Staden, J. (1989) The effect of seaweed concentrate on the growth and yield of potassium stressed wheat. *Plant and Soil*, **116**, 29–36.
- Berlyn, G.P. and Russo, R.O. (1990) The use of organic biostimulants in nitrogen fixing trees. *Nitrogen Fixing Trees Research Report*, **81**, 1–2.
- Bhattacharya, I., Bandyopadhyay, S., Varadachari, C. and Ghosh, K. (2007) Development of a novel slow-releasing iron-manganese fertilizer compound. *Industr. Eng. Chem. Res.*, **46**, 2870–2876.
- Bhosle, N.B., Untawale, A.G. and Dhargalkar, V.K. (1975). Effects of seaweed extract on the growth of *Phaseolus vulgaris* L. *Ind. J. Mar. Sci.*, **4**, 208–210.
- Boarder, S. (2001) Demonstration of seaweed nutrient stripping for aquaculture wastewater. Documentation in support of the end of project report for Coasts and Clean Seas Project. Aquaculture Development Unit, Challenger TAFE, Fremantle, Western Australia.
- Braden, K.W., Blanton, J.R., Montgomery, J.L., van Santen, E., Allen, V.G. and Miller, M.F. (2007) Tasco supplementation: Effects on carcass characteristics, sensory attributes and retail display life. *J. Anim. Sci.*, **3**, 85–91.
- Braud, J. (2006) Continuous seaweed tank culture in France: from *Chondrus crispus* to co-culture of macroalgae and diatom *Odontella aurita*. In: *Seaweed Resources of the World* (eds A.T. Critchley, M. Ohno and D.B. Largo). Japan International Cooperation Agency, Yokosuka.
- Cardozo K.H.M., Guaratini, T., Barros, *et al.* (2007) Metabolites from algae with economical impact. *Comp. Biochem. Physiol., Part C*, **146**, 60–78.
- Carrillo, S., López, E., Casas, M.M., *et al.* (2008) Potential use of seaweeds in the laying hen ration to improve the quality of n-3 fatty acid enriched eggs. *Appl. Phycol.*, **20**, 721–728.
- Cassan, L., Jeannin I., Lamaze, T. and Morot Gavdry, J.F. (1992) The effect of the *Ascophyllum nodosum* extract

- GoemarGA 14 on growth of spinach. *Bot. Mar.*, **35**, 437–439.
- Castlehouse, H., Smith, C., Raab, A., Deacon, C., Meharg, A.A. and Feldmann, J. (2003) Biotransformation and accumulation of arsenic in soil amended with seaweed. *Env. Sci. Technol.*, **37**, 951–957.
- Chojnacka K. (2008) Using biosorption to enrich the biomass of seaweeds from the Baltic Sea with microelements to produce mineral feed supplement for livestock. *Biochem. Eng. J.*, **39**, 246–257.
- Chojnacka, K. (2010) Biosorption and bioaccumulation – the prospects for practical applications. *Env. Int.*, **36**, 299–307.
- Chopin, T., Buschmann, A.H., Halling, C., *et al.* (2001) Integrating seaweeds into marine aquaculture systems: A key toward sustainability. *J. Phycol.*, **37**, 975–986.
- Chouliaras, V., Gerasopoulos, D. and Lionakis, S. (1997) Effects of seaweed extract on fruit growth, weight and maturation of ‘Hayward’ kiwifruit. *Acta Hort.*, **444**, 485–492.
- Chouliaras, V., Tasioula M., Chatzissavvidis, C., Therios, I. and Tsalolatidou, E. (2009) The effects of a seaweed extract in addition to nitrogen and boron fertilization on productivity, fruit maturation, leaf nutritional status and oil quality of the olive (*Olea europaea* L.) cultivar Koroneiki. *J. Sci. Food Agric.*, **89**, 984–988.
- Chowdhury, S.D., Paik, I.K., Namkung, H. and Lim, H.S. (2004) Responses of broiler chickens to organic copper fed in the form of copper–methionine chelate. *Anim. Feed Sci. Technol.*, **115**, 281–293.
- Craigie, J.S. (2011) Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.*, doi 10.1007/s10811-010-9560-4.
- Crouch, I.J. and van Staden, J. (1992) Effect of seaweed concentrate on the establishment and yield of greenhouse tomato plants. *J. Appl. Phycol.*, **4**, 291–296.
- Cruz-Suarez, L.E., Tapia-Salazar, M., Nieto-Lopez, M.G., Guajaro-Barbosa, C. and Ricque-Marie, D. (2009) Comparison of *Ulva clathrata* and the kelps *Macrocystis pyrifera* and *Ascophyllum nodosum* as ingredients in shrimp feeds. *Aquacult. Nutr.*, **15**, 421–430.
- Daume, S. (2006) The roles of bacteria and micro and macro algae in abalone aquaculture: a review. *J. Shellfish Res.*, **25**, 151–157.
- de Oliveira, M.N., Freitas, A.L.P., Carvalho, A.F.U., *et al.* (2009) Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. *Food Chem.*, **115**, 254–259.
- Déville, C., Damas, J., Forget, P., Dandrifosse, G. and Peulen, O. (2004) Laminarin in the dietary fibre concept. *J. Sci. Food Agric.*, **84**, 1030–1038.
- Dierick, N., Oryn, A. and De Smet, S. (2009) Effect of feeding intact brown seaweed *Ascophyllum nodosum* on some digestive parameters and on iodine content in edible tissues in pigs. *J. Sci. Food Agric.*, **89**, 584–594.
- Dierick, N., Oryn, A. and de Smet, S. (2010) In vitro assessment of the effect of intact marine brown macro-algae *Ascophyllum nodosum* on the gut flora of piglets. *Livestock Science*, **133**, 154–156.
- Feldmann, J., John, K. and Pengprecha, P. (2000) Arsenic metabolism in seaweed-eating sheep from Northern Scotland. *Fresenius J. Anal. Chem.*, **368**, 116–121.
- Fornes, F., Sanchez-Perales, M. and Guardiola, J.L. (2002) Effect of a seaweed extract on the productivity of Clementine mandarin and Navelina orange. *Bot. Mar.*, **45**, 486–489.
- Franklin, S.T., Martin, K.R., Bare, R.J., Schingoethe, D.J. and Hippen, A.R. (1999) Dietary marine algae (*Schizochytrium* sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. *J. Nutr.*, **129**, 11–16.
- Gahan, D.A., Lynch, M.B., Callan, J.J., O’Sullivan, J.T. and O’Doherty, J.V. (2009) Performance of weanling piglets offered low-, medium- or high-lactose diets supplemented with a seaweed extract from *Laminaria* spp. *Animal*, **3**, 24–31.
- Gardiner, G.E., Campbell, A.J., O’Doherty, J.V., *et al.* (2008) Effect of *Ascophyllum nodosum* extract on growth performance, digestibility, carcass characteristics and selected intestinal microflora populations of grower–finisher pigs. *Anim. Feed Sci. Technol.*, **141**, 259–273.
- Graham, R.D. and Welch R.M. (1996) Breeding for staple food crops with high micronutrient density. International Food Policy Research Institute, Washington, DC.
- Hansen, H.R., Hector, B.L. and Feldmann, J. (2003) A qualitative and quantitative evaluation of the seaweed diet of North Ronaldsay sheep. *Anim. Feed Sci. Technol.*, **105**, 21–28.
- Hong, D.D., Hien, H.M. and Son, P.N. (2007) Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *J. Appl. Phycol.*, **19**, 817–826.
- Immanuel, G., Sivagnanavelmurugan, M., Balasubramanian, V. and Palavesam, A. (2010) Effect of hot water extracts of brown seaweeds *Sargassum* spp. on growth and resistance to white spot syndrome virus in shrimp *Penaeus monodon* postlarvae. *Aquacult. Res.*, **41**, 545–553.
- Ingrid, L. and Mainland, A. (2000) Dental microwear study of seaweed-eating and grazing sheep from Orkney. *Int. J. Osteoarchaeol.*, **10**, 93–107.
- Johns, T. and Pablo, B.E. (2007) Biofortification, biodiversity and diet: A search for complementary applications against poverty and malnutrition. *Food Policy*, **32**, 1–24.
- Kannan, L. and Selvan, C.T. (1990) Effect of seaweed manure on *Vigna radiata* L. (green gram). In: *Perspective*

- in *Phycology*. Prof M.O.P. Iyengar Centenary Celebration Volume, (ed. V.N. Raja Rao). Today and Tomorrow's Printers and Publishers, New Delhi, pp. 427–430.
- Kaufmann, S., Wolfram, G., Delange, F. and Rambeck, W.A. (1998) Iodine supplementation of laying hen feed: A supplementary measure to eliminate iodine deficiency in humans? *Z Ernährungswiss*, **37**, 288–293.
- Kawashima, H., Bazin, M.J. and Lynch, J.M. (1997) A modelling study of world protein supply and nitrogen fertilizer demand in the 21st century. *Env. Cons.*, **24**, 50–56.
- Lee, B. (2008) Seaweed potential as a marine vegetable and other opportunities. RIRDC Publication No 08/009, RIRDC Project No CON-9A, Rural Industries Research and Development Corporation.
- Leonard, S. G., Sweeney, T., Bahar, B., Lynch, B.P. and O'Doherty J.V. (2010) Effect of dietary seaweed extracts and fish oil supplementation in sows on performance, intestinal microflora, intestinal morphology, volatile fatty acid concentrations and immune status of weaned pigs. *Br. J. Nutr.*, **105**, 1–11.
- Lucena, J.J. (2006) Iron fertilizers in correcting iron deficiencies in plants. In *Iron Nutrition in Plants and Rhizospheric Microorganism* (eds L.L. Barton and J. Abadía). Springer-Verlag Academic Publishers, Dordrecht, The Netherlands.
- Lynch, M.B., Sweeney, T., Callan, J.J., O'Sullivan, J.T. and O'Doherty, J.V. (2010) The effect of dietary Laminaria-derived laminarin and fucoidan on nutrient digestibility, nitrogen utilisation, intestinal microflora and volatile fatty acid concentration in pigs. *J. Sci. Food Agric.*, **90**, 430–437.
- McDowell, L.R. (1996) Feeding minerals to cattle on pasture. *Anim. Feed Sci. Technol.*, **60**, 247–271.
- Michalak, I. and Chojnacka K. (2008) The application of macroalga *Pithophora varia* Wille enriched with microelements by biosorption as biological feed supplement for livestock. *J. Sci. Food Agric.*, **88**, 1178–1186.
- Michalak, I. and Chojnacka, K. (2009a) Edible macroalga *Ulva prolifera* as microelemental feed supplement for livestock: the fundamental assumptions of the production method. *World J. Microbiol. Biotechnol.*, **25**, 997–1005.
- Michalak, I. and Chojnacka K. (2009b) Multielemental analysis of the biomass of macroalgae from the Baltic Sea by ICP-OES to monitor environmental pollution and establish potential uses of algae. *Int. J. Env. Anal. Chem.*, **89**, 583–596.
- Michalak, I. and Chojnacka K. (2010a) The new application of biosorption properties of *Enteromorpha prolifera*. *Appl. Biochem. Biotechnol.*, **160**, 1540–1556.
- Michalak, I. and Chojnacka, K. (2010b) The interactions of metal cations with anionic groups on the cell wall of macroalga *Vaucheria* sp. *Eng. Life Sci.*, **10**, 209–217.
- Mulbry, W., Shannon, K. and Pizarro, C. (2007) Biofertilizers from algal treatment of dairy and swine manure effluents. *J. Veg. Sci.*, **12**, 107–125.
- Mulbry, W., Westhead, E.K., Pizarro, C. and Sikora, L. (2005) Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. *Biores. Technol.*, **96**, 451–458.
- Neori, A. and Shpigel, M. (2007) Algae. Key for sustainable mariculture. In: *Seaweed Resources of the World* (eds A.T. Critchley, M. Ohno, and D.B. Largo). Japan International Cooperation Agency, Yokosuka.
- O'Connell, M., Callan, J.J., Byrne, C., Sweeney, T. and O'Doherty, J.V. (2005) The effect of cereal type and exogenous enzyme supplementation in pig diets on nutrient digestibility, intestinal microflora, volatile fatty acid concentration and manure ammonia emissions from pigs. *Anim. Sci.*, **81**, 357–364.
- O'Doherty, J.V., Dillon, S., Figat, S., Callan, J.J. and Sweeney, T. (2010) The effects of lactose inclusion and seaweed extract derived from *Laminaria* spp. on performance, digestibility of diet components and microbial populations in newly weaned pigs. *Anim. Feed Sci. Technol.*, **157**, 173–180.
- O'Sullivan, L., Murphy, B., McLoughlin, P., et al. (2010) Prebiotics from marine macroalgae for human and animal health applications. *Marine Drugs*, **8**, 2038–2064.
- Passam, H.C., Olympios, C.M. and Akoumianakis, C. (1993) The influence of pre and post-harvest applications of seaweed extract on the production and storage quality of cucumber. *Acta Hort.*, **379**, 229–235.
- Rama Rao, K., (1991) Effect of seaweed extract on *Zizyphus mauratiana* Lamk. *J. Ind. Bot. Soc.*, **71**, 19–21.
- Robertson-Andersson, D.V., Leitao, D., Bolton, J.J., Anderson, R.J., Njobeni, A. and Ruck, K. (2006) Can kelp extract (KELPAK) be useful in seaweed mariculture? *J. Appl. Phycol.*, **18**, 315–321.
- Saker, K.E., Fike, J.H., Veit, H. and Ward D.L. (2004) Brown seaweed – (Tasco™) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. *J. Anim. Physiol. Anim. Nutr.*, **88**, 122–130.
- Sivasankari, S., Venkatesalu, V., Anantharaj, M. and Chandrasekaran, M. (2006) Effect of seaweed extracts on the growth and biochemical constituents of *Vigna sinensis*. *Biores. Technol.*, **97**, 1745–1751.
- Strand, A., Herstad, O. and Liaaen-Jensen, S. (1998) Fucocanthin metabolites in egg yolks of laying hens. *Comp. Biochem. Physiol. Part A*, **119**, 963–974.
- Taboada, C., Millan, R. and Miguez, I. (2010) Composition, nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. *J. Sci. Food Agric.*, **90**, 445–449.
- Takashi, K. and Takahiko, I. (2009) Minerals, polysaccharides and antioxidant properties of aqueous solutions

- obtained from macroalgal beach-casts in the Noto Peninsula, Ishikawa, Japan. *Food Chem.*, **112**, 575–581.
- Thirumaran, G., Arumugam, M., Arumugam, R. and Anantharaman, P. (2009) Effect of Seaweed Liquid Fertilizer on Growth and Pigment Concentration of *Abelmoschus esculentus* (l) *medikus*. *American-Eurasian J. Agron.*, **2**, 57–66.
- Troell, M., Robertson-Andersson, D., Anderson, R.J., *et al.* (2006) Abalone farming in South Africa: An overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. *Aquaculture*, **257**, 266–281.
- Tseng, C.K. (2001) Algal biotechnology industries and research activities in China. *J. Appl. Phycol.*, **13**, 375–380.
- Turner, J., Dritz, S.S., Higgins, J.J., and Minton, J.E. (2002) Effects of *Ascophyllum nodosum* extract on growth performance and immune function of young pigs challenged with *Salmonella typhimurium*. *J. Anim. Sci.*, **80**, 1947–1953.
- Wajahatullah, K., Rayirath, U.P., Subramanian, S., *et al.* (2009) Seaweed Extracts as Biostimulants of Plant Growth and Development. *J. Plant Growth Regul.*, **28**, 386–399.
- Wakefield, R.L. and Murray, S.N. (1998) Factors influencing food choice by the seaweed-eating marine snail *Norrisia norrisi* (Trochidae). *Mar. Biol.*, **130**, 631–642.
- Whapham, C.A., Blunder, G., Jenkins, T. and Wankins, S.D. (1993). Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *J. Appl. Phycol.*, **5**, 231–234.
- World Health Organization (1992) *National Strategies for Overcoming Micronutrient Malnutrition*. WHO, Geneva.
- Zhou, Y., Yang, H., Hu, H., *et al.* (2006) Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. *Aquaculture*, **252**, 264–276.
- Zvyagintseva, T.N., Shevchenko, N.M., Chizhov, A.O., Krupnova, T.N., Sundukova, E.V. and Isakov, V.V. (2003) Water-soluble polysaccharides of some far-eastern brown seaweeds. Distribution, structure, and their dependence on the developmental conditions. *J. Exp. Mar. Biol. Ecol.*, **294**, 1–13.

# 32

## Applications of Seaweed in Meat-Based Functional Foods

**Susana Cofrades, Inés López-López and Francisco Jiménez-Colmenero**

*Instituto de Ciencia y Tecnología de Alimentos y Nutrición – ICTAN (Formerly Instituto del Frío) (CSIC), Ciudad Universitaria, Madrid, Spain*

### 32.1 Introduction

Seaweed has been used as human food since ancient times, more commonly in Asian countries, and to a lesser extent in Europe and America. Like other marine organisms, seaweed is an important source of food and bioactive ingredients that can be applied to many aspects of food production. The additional food sources needed for the growing world population may also lead to it being more widely used (Bocanegra *et al.*, 2009).

Because of composition (which varies with species, habitat, maturity, environmental conditions or processing methods), seaweeds are an important source of nutrients. Edible seaweeds contain high quality protein, large amounts of vitamins, and a high proportion of essential unsaturated fatty acids, particularly long chain *n*-3 polyunsaturated fatty acids (LC *n*-3 PUFA) such as eicosapentaenoic acid-EPA (C20:5*n*-3) and docosahexaenoic acid-DHA (C22:6*n*-3). Algae are also an excellent source of minerals (Ca, Na, Mg, P, K, I, Fe, and Zn), and the best natural source of iodine. They also contain compounds with known antioxidant properties (polyphenols, carotenoids and tocopherols) and a good source of dietary fiber (DF) (Fleurence, 1999; Yuan, 2008; Bocanegra *et al.*, 2009). As well as their important nutritional value, marine algae constituents also have technological advantages when used as ingredients in some foods, including processed meats. This is basically due to their

composition, especially to the physicochemical properties of their DF. This fiber is used as a texturing and bulking agent because of its technological properties, particularly for making low calorie foods. The physicochemical properties and technological applications of seaweed polysaccharides have been widely reported (Trius and Sebranek, 1996; Bocanegra *et al.*, 2009).

More recently, there has been growing interest in marine algae and their constituents as functional foods and nutraceuticals with potential beneficial health effects as sources of antioxidants and bioactives to reduce the risk of various diet-related chronic diseases such as atherosclerosis and hyperlipoproteinemia in cardiovascular disease (CVD) as well as breast and colon carcinogenesis (Yuan, 2008). Seaweeds are an important source of bioactive ingredients that can be applied to many aspects of processing healthier foods and developing functional foods (Bocanegra *et al.*, 2009). This chapter describes different aspects of current interest in the opportunities offered by seaweed applications in the development of meat-based functional foods.

### 32.2 Meat-based functional foods

Meat and meat products are one of the main components of our diet. They are an important source of a wide range of nutrients and contribute a considerable proportion of

the dietary intake of various nutrients essential for optimal growth and development. In recent years, however, negative perceptions of meat have emerged based on the link between the consumption of some meat constituents (e.g., fat, saturated fatty acids SFA, cholesterol, sodium, etc.) and the risk of developing some of modern society's most common chronic diseases (e.g., ischaemic heart disease, cancer, hypertension and obesity). Changes in consumer demand and growing market competition have prompted the need to improve the quality and image of meat, not only to prevent the loss of market share resulting from a negative perception of meat, but also to create new market niches, as in the case of products with health-beneficial properties.

Nutrition is coming to the fore as a major modifiable determinant of non-communicable chronic diseases, with scientific evidence increasingly supporting the view that alterations in diet have strong effects, both positive and negative, on health throughout life (WHO/FAO, 2003). It is in this context that the so-called functional foods emerged and have come to represent one of the fastest growing segments of the global food industry. The term 'functional foods' was first introduced in Japan in the 1980s, and from there it spread to the United States and Europe. Although there are currently various different definitions of functional foods, according to the working definition established in Europe "a food can be regarded as a functional food if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to an improved state of health and well-being and/or reduction of risk of disease". Functional foods must remain foods and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet: they are not pills or capsules, but part of a normal food pattern (Diplock *et al.*, 1999). Foods are considered as functional because of their scientifically demonstrated effects, not their origin, and therefore the functional food category may include both natural (unmodified) foods and foods that have been transformed by means of technological or biotechnological processes (Ashwell, 2002).

The meat industry must adapt to the new nutrition/health concepts and functional foods provide an excellent opportunity for this sector to address consumer demands, promoting an intake of functional ingredients without any radical changes in eating habits. To a considerable extent, the development of meat-based functional foods involves modifying the composition of processed meat to optimize the presence of certain components. From farm to table, different strategies can be used effectively to increase the presence of beneficial compounds (*n*-3 PUFA, monounsaturated fatty acids MUFA, antioxidants, dietary fiber, minerals, probiotics, etc.) and to limit those with negative health implications (fat, SFA, *trans* fatty acids, cholesterol, sodium,

allergens, etc.). These strategies are based on animal production practices and meat processing strategies (Jiménez-Colmenero *et al.*, 2001, 2006; Arhiara, 2006). Among them, meat processing plays a major role in the development of functional meat products, especially in relation to modifying the reformulation process. Changing the ingredients (raw meat material and non-meat ingredients) used in the preparation of meat products offers an excellent opportunity to remove, reduce, increase, add and/or replace different components with bioactive compounds.

Many non-meat ingredients (from animal and vegetal sources, terrestrial or marine in origin), have been used in the preparation of meat-based functional foods. Functional components have been used in the specific preparation of individual substances or as constituents of certain non-meat ingredients including extracts, flours, concentrates and homogenates, used for technological, sensory, nutritional, microbiological, economic or even functional purposes in the meat industry. Non-meat ingredients used in meat-based functional food development include soybean, walnut, oats, wheat, sunflower, rosemary, apple, mushroom, plant and marine oils, plant sterols, etc. (Jiménez-Colmenero *et al.*, 2001, 2006; Arhiara, 2006; Jiménez-Colmenero, 2007). In this context, the use of algae because of their chemical composition, nutritional value and bioactive components with potential health-beneficial properties opens up new possibilities in functional food production, including meat products (Fleurence, 1999; Rasmussen and Morrissey, 2007; Yuan, 2008; Bocanegra *et al.*, 2009).

## 32.3 Seaweed as a functional food ingredient in meat products

Seaweed is applied to meat processing at two levels: by adding specific components previously extracted from the algae, or by incorporating the whole dehydrated and powdered algae. At the first level, these components have been used mainly as hydrocolloids while at the second level studies are more recent and limited in scope.

### 32.3.1 Application of specific seaweed components in meat products

Some components of algae, such as polysaccharides (alginate and carrageenans), have been used as additives in the meat industry for decades. In recent years, however, various components of algae such as lipids or carotenoids have been attracting renewed attention because of their potentially beneficial effects on health, and this may increase in the future with the development of new techniques for the

extraction, isolation and characterization of bioactive seaweed components.

### Polysaccharides

Algal carbohydrate content is relatively high (33–75% dry matter), with most of it considered as fiber. The main components of algal fiber, carrageenans, alginates and agar (MacArtain *et al.*, 2007), have been widely used in meat product processing.

Carrageenans have been used by the meat industry for their gelling, thickening, and stabilizing properties, and more recently for healthier meat product formulation. Because of their gel-forming and water retention abilities, carrageenans are used in different technological approaches to low-fat meat product formulations (Trius and Sebranek, 1996). Carrageenans have been tested in meat model systems (Fernández *et al.*, 1998) and incorporated into numerous meat products such as breakfast sausages (Barbut and Mittal, 1992), frankfurters (Bernal *et al.*, 1987; Pietrasik and Duda, 2000; Xiong *et al.*, 1999), cooked beef rolls (Pietrasik and Shand, 2003), burgers (Huffman *et al.*, 1991), roast turkey (Bater *et al.*, 1992), meat balls (Hsu and Chung, 2001). Carrageenan and agar products have been used in canned corned beef (Muller and Stiebing, 1993). Alginates have been widely used in meat processing as binders or fillers. Several alginate systems have recently been used as cold-set binders for meat products. Thermo-irreversible gels are formed when calcium ions are introduced into an alginate solution (usually with an acidulant and a sequestrant to modify the reaction rate) used to form binding comminuted or diced meat pieces and produce restructured meat products (Means *et al.*, 1987; Raharjo *et al.*, 1994; Boles and Shand, 1998; Sun, 2009).

As well as their technological properties, these compounds can be considered as functional ingredients since they also provide various potential beneficial physiological effects (Yuan, 2008; Bocanegra *et al.*, 2009; Draget and Taylor, 2011). In this context seaweed DF has been linked to various health benefits, including those related to digestion, excretion and gastrointestinal functions (Gudiel-Urbano and Goñi, 2002; Bocanegra *et al.*, 2009), and hypocholesterolemic and antihypertensive effects (Jiménez-Escrig and Sánchez-Muniz, 2000; Wong *et al.*, 1999). Some fiber components such as alginates interact with dietary cholesterol facilitating its excretion, while others such as agar are almost inactive (Ito and Tsuchiya, 1972). This effect is also linked to the fermentability of the DF and production of short chain fatty acids (Bocanegra *et al.*, 2003). The important role of some algae polysaccharides in sequestering free radicals and preventing oxidative damage has been demonstrated (Aguilera *et al.*, 2002; Zhang *et al.*, 2004). Carrageenans

have been shown to have a hypoglycaemic effect (Dumelod *et al.*, 1999), anticoagulant activity (Pereira, Mulloy and Mourao, 1999) and anti-human immunodeficiency virus effect (Witvrouw and De Clercq, 1997). In general terms, the physiological effects of seaweed dietary fiber have been linked to its capacity for water absorption and ionic exchange, viscosity and capacity for absorbing organic components such as cholesterol or glucose (Bocanegra *et al.*, 2009).

### Lipids

Marine algae are not particularly rich in lipids (ranging from 0.92–5.2% dry matter), although their lipids are rich in LC *n*-3 PUFA, in particular EPA and DHA, which may constitute up to 20–25% of total fatty acids. These two constituents have been of increasing interest lately due to research revealing their beneficial effects on many aspects of human health such as reducing the risk factors associated with CVD, assisting visual and neuro-development, and ameliorating diseases such as arthritis and hypertension (Bao *et al.*, 1998; Grimm *et al.*, 2002; Horrocks and Yeo, 1999; Hu *et al.*, 2002; Leaf *et al.*, 2003).

Vegetable and marine oils have been used in order to produce *n*-3 PUFA-enriched meat products. In this context, dietary and technological approaches have been reported as a means of improving the *n*-3 PUFA content of meat and meat products using algal oil. This type of marine lipid has been incorporated into the diet of poultry (Rymer *et al.*, 2010) and lamb (Díaz *et al.*, 2011). Reformulation strategies have also been used for the incorporation of algal oil (as a non-meat ingredient) to produce LC *n*-3 PUFA fortified meat products such as patties, fresh pork sausages, restructured ham or low-fat frankfurters (Lee *et al.*, 2005; Valencia *et al.*, 2007; López-López *et al.*, 2009b, c).

### Carotenoids

Carotenoids are one of the most promising biologically active compounds for the food industry present in seaweed. The protective activity of carotenoids, as potent antioxidants and vitamin A precursors, against cancer, aging, ulcers, heart attack, and coronary artery disease has been suggested (Li and Chen, 2001). Lutein, one of the algae carotenoids, has been used in the reformulation of meat products. The xanthophyll carotenoid lutein, abundant in seaweeds and also in green leafy vegetables, has been shown to have anti-mutagenic and anti-clastogenic effects (Wang *et al.*, 2006), as well as chemopreventive (Moreno *et al.*, 2007) and cardioprotective actions (Lidebjer *et al.*, 2007). Lutein is also present in significant amounts in the macular tissue and lens of the eye where it is thought to

function as a shield against the photo oxidative effects of blue light and as an antioxidant (Carpentier *et al.*, 2009; Krinsky, 2002; Palombo *et al.*, 2007). Different meat products such as low-fat frankfurters (Granado-Lorencio *et al.*, 2010), meat patties (Hayes *et al.*, 2009; Daly *et al.*, 2010), comminuted pork products and liver paté (Csapo *et al.*, 2006) have been developed with the addition of lutein, in the first of these products derived from microalgae, and in the others obtained from green leaf sources. These proposals confirm the suitability of meat products as lutein carriers and as a means of increasing the systematic intake of lutein.

### 32.3.2 Incorporation of seaweeds into meat products

Compared to the inclusion of isolated compounds, the use of the whole algae (dehydrated and powdered) has various advantages in the development of meat-based functional foods. The use of a single ingredient favors the simultaneous presence of different health-beneficial components (dietary fiber, protein, minerals, vitamins, carotenoids, polyphenols, etc.), some of which have important technological properties and are widely used in the food industry, including the meat sector. Their inclusion (as a dehydrated powder) does not involve the laborious, expensive and environmentally unfriendly extraction and purification processes which are required when these components are used individually.

The seaweed species used in the development of different meat products are described below. In general terms, although the product design promotes the presence of bioactive seaweed compounds, other strategies have often been considered to optimize the presence of certain components.

#### Frankfurters

Brown (*H. elongata*, sea spaghetti; *U. pinnatifida*, wakame) and red (*P. umbilicalis*, nori) seaweeds (0–5%) have been used to produce low-fat/low-salt gel/emulsion model systems (Cofrades *et al.*, 2008; López-López *et al.* 2009a), while *H. elongata* (5%) has been used for the reformulation of low-fat/low-salt frankfurters (López-López *et al.*, 2009b, c). Seaweed favours the formation of firmer and chewier structures with better water and fat-binding properties, helping to overcome the technological problems associated with low-salt products. The color of gel/emulsion systems was affected by seaweeds although to a different extent depending on the species. No microbiological limitation has been reported in frankfurters with 5% of *H. elongata* during chilled storage (López-López *et al.*, 2009b). Sensory limitations (non-typical flavour) linked to the presence of algae were observed in frankfurters (López-López *et al.*, 2009b). This drawback, which can be reduced through reformula-

tion processes, is less relevant to consumers who normally eat algae and who therefore find their flavor familiar.

#### Breakfast sausages

Breakfast sausages have been formulated with 1–4% *L. japonica* (Kim *et al.*, 2010). The effect produced by this brown seaweed on texture, cooking loss, emulsion stability and color parameters were similar to those described in gel/emulsion systems. Although the inclusion of seaweed had some sensory limitations, the sausages presented high overall acceptability.

#### Restructured steaks

Low-fat, low-salt fresh restructured poultry steaks have been produced with the addition of 3% of *H. elongata* and microbial transglutaminase as cold-set binder (Cofrades *et al.*, 2011). Different effects of *H. elongata* has been observed on water-binding properties and texture in raw and cooked steaks. In the raw state, seaweed decreased the water-binding properties, improving the sample consistency and handle. In cooked products, sea spaghetti improved the water-binding properties, and the texture (with reduced salt content) was similar to the control sample with normal salt content. The influence of *H. elongata* on restructured steak color was similar to that observed in gel/emulsion meat systems (Cofrades *et al.*, 2008; López-López *et al.*, 2009a). Although the addition of seaweed was detected by the sensory panel, adequate overall acceptability scores were obtained.

#### Patties

Different types of algae have been used in the production of patties. Chun *et al.* (1999) evaluated the incorporation of different levels (1–5%) of brown (*S. thunbergii*) or red (*G. amansii*) seaweeds. López-López *et al.* (2010) studied the effect of the addition of 3% of *U. pinnatifida* on the characteristics of low salt (0.5%) and reduced fat content (<10%) beef patties during frozen storage. The inclusion of these seaweeds generally improved the water-binding properties of the patties, helping to solve problems related with low salt content. However, the effect of the seaweeds on the texture of the patties was different to that found in the products described above. Compared with the control product, in both raw and cooked states, *U. pinnatifida* produced a softer texture (López-López *et al.*, 2010). *S. thunbergii* and *G. amansii* decreased or had no effect on texture parameters of patties depending on the concentration added (Chun *et al.*, 1999). These variations seem to be linked to the different particle size of the seaweeds and the different nature of the two types of meat products (restructured products and

frankfurters vs. patties) which lead to different integration levels of the ingredients into the meat matrix (López-López *et al.*, 2010). Lipid oxidation and microbiological counts in reformulated products were not a limiting factor for frozen stability (López-López *et al.*, 2010). In sensory terms, Chun *et al.* (1999) established that 3% of *G. amansii* is the optimal formulation to obtain patties with acceptable sensory properties (similar values to control sample). The same amount of *U. pinnatifida* did not affect any of the sensory parameters evaluated, presenting reasonable overall acceptability scores (López-López *et al.*, 2010).

#### **Nutritional/functional improvement of meat products with added seaweed**

The potential functional effect of the application of seaweed in meat products derives both from its contribution to the presence of bioactive compounds with health implications, and the possibility of using these as strategies to facilitate low-fat and low-salt reformulation processes. The addition of seaweed to meat products produces various changes in their composition. The effect on proximate analyses was determined in restructured poultry steaks with *H. elongata*, in breakfast sausages with *L. japonica* and in patties with *S. thunbergii* and *G. amansii* (Cofrades *et al.*, 2010; Kim *et al.*, 2010; Chun *et al.*, 1999), and an in-depth nutritional evaluation was carried out of frankfurter-type products with *H. elongata* and of patties with added *U. pinnatifida* (López-López *et al.*, 2009c, 2010).

A remarkable change produced by adding seaweed was the increase in mineral (ash) content (Kim *et al.*, 2010; López-López *et al.*, 2010). However, this effect is different when the seaweeds are used as a strategy for reducing NaCl levels in meat products (Cofrades *et al.*, 2008, 2011). Although Na is one of the main minerals present in seaweed, low-salt products reformulated with added seaweed present lower concentrations of Na than traditional products (normal NaCl content) (Cofrades *et al.*, 2010). Reducing people's salt intake worldwide would result in a major improvement in public health, and therefore a gradual, sustained reduction is required in the amount of salt added to food by the food industry (He and MacGregor 2008). The incorporation of seaweed into meat products also increased the concentration of K, Ca, Mg, Fe and Mn, although the magnitude of the increment varied according to the type of seaweed. Products with brown seaweeds (*H. elongata* and *U. pinnatifida*) are a good source of K, Mg, and Ca, while the Fe content of meat products can be increased with red seaweed (*P. umbilicalis*). Since these seaweeds are richer in K than in Na (Cofrades *et al.*, 2010; Rupérez, 2002), the addition of seaweed reduces the Na/K ratios ( $\sim 1$ ) in the reformulated meat products, as compared with conventional ones ( $\sim 3$ )

(López-López *et al.*, 2009a,c, 2010). Since high sodium intake and diets with high Na/K ratios have been linked to the incidence of hypertension (Rupérez, 2002), the recommended Na/K ratio is  $\sim 1$  (WHO/FAO, 2003). In relation to the contribution of seaweeds to the heavy metal (toxic minerals) content in meat products, arsenic concentrations close to 0.200 mg/100 g were found in the meat systems with added (5%) *P. umbilicalis*, *H. elongata* and *U. pinnatifida* (López-López *et al.*, 2009a). Less than 1% of the total As content in nori and sea spaghetti is inorganic As, and the proportion is lower than 3% in the case of wakame, so that there is no risk involved in consuming these meat products, as the amount ingested is below the tolerable daily intake. Appreciable amounts ( $< 0.40 \mu\text{g/g}$  dry matter) of other minerals such as Cd or Pb were not found in the meat systems containing seaweeds (López-López *et al.*, 2009a).

The addition of seaweed caused some changes in the amino acid profile of meat products. The addition of nori produced an increase in levels of serine, glycine, alanine, valine, tyrosine, phenylalanine and arginine in the meat products; wakame or sea spaghetti produced no significant changes in the amino acid profiles of meat emulsions (López-López *et al.*, 2009a). No effect was observed on the lysine/arginine ratio (range 1.32–1.38) in the products, even though this ratio is generally lower in seaweed protein than in meat protein (Pellet and Young, 1990; Dawczynski, Schubert and Jarheis, 2007). Adding seaweed to meat products favours the presence of DF; for example the consumption of 100 g of frankfurters with 5% of *H. elongata* would supply around 10% of the recommended daily DF intake, helping to correct DF deficiency in the European diet (López-López *et al.*, 2009a,c). Similarly, adding seaweeds increased the polyphenolic content (López-López *et al.*, 2009a). On the other hand, their low lipid concentration means that the incorporation of seaweeds into meat products is not a good strategy for improving fatty acids content in meat products. Complementary strategies are required to obtain healthier lipid levels in meat product formulation (López-López *et al.*, 2009a,c).

According to current EU legislation (Regulation 1924/2006, EC, 2007; Regulation 116/2010, EC, 2010), which regulates claims made for the nutritional and health properties of foods, meat products with added seaweed as the main reformulation strategy can be labelled as shown in Table 32.1.

## **32.4 Conclusions**

The use of seaweed or its components as ingredients allows reformulation strategies to be designed to develop potentially functional meat products with a lower Na, fat

**Table 32.1** Possible nutritional claims (X) for meat products with whole added seaweeds

Claim/Component	General conditions	Frankfurters <sup>a</sup>	Restructured steaks <sup>b</sup>	Patties <sup>c</sup>
Reduced content:				
Sodium	Reduction > 25%	X	X	
Fat	Reduction > 30%	X		X
Saturated fat	Reduction > 30%	X		X
Low fat content	<3 g fat/100 g		X	
Source of:				
Mg	> 15% RDI <sup>d</sup>	X		X
K	> 15% RDI			X
Dietary fiber	> 10% RDI/serving <sup>e</sup>	X		
High K content	> 30% RDI	X		
High protein content	> 20% energy	X	X	X
High fat content:				
unsaturated	> 70% of total FA <sup>f</sup>	X		
monounsaturated	> 45% of total FA	X		
<i>n</i> -3	> 80 mg EPA <sup>g</sup> + DHA <sup>h</sup> /100 g	X		X

<sup>a</sup>López-López *et al.*, 2009c; <sup>b</sup>Cofrades *et al.*, 2011; <sup>c</sup>López-López *et al.*, 2010; <sup>d</sup>Recommended daily intake; <sup>e</sup>For 100 g serving. Condition currently set by FAO/WHO (2009); <sup>f</sup>Fatty acids; <sup>g</sup>Eicosapentaenoic acid; <sup>h</sup>Docosahexaenoic acid.

and SFA content and a relevant contribution to the intake level of DE, polyphenols, minerals and LC *n*-3 PUFA. In technological terms the presence of seaweeds (whole, dehydrated and powdered) overcomes the negative consequences linked to the reduction of NaCl in different types of meat products at the level of water and fat-binding properties. How they affect the texture is conditioned by the type of product and the structural breakdown level of the algae. Their presence does not affect the product stability during chilled or frozen storage. The main limitations on the use of whole algae in meat products are the sensory properties of the final product. While the inclusion of 3% of brown algae in products with a lower structural breakdown level produces meat products which are sensorially acceptable, in the case of finely comminuted systems the quantity of algae must be reduced to obtain products with acceptable organoleptic properties.

In view of all described above, further research into the use of algae as an ingredient in meat products is clearly indicated. This will open up new horizons for the meat industry offering nutritional/functional and technological benefits based on the use of an abundant natural resource currently little used in the Western society. The approach to the technological and nutritional challenges used in this research may be fully applicable to the development of other functional food products.

## Acknowledgment

This research was supported under projects AGL2005-07204-CO2-O2, AGL2008-04892-CO3-01 and the Consolider Ingenio CSD2007-00016, Ministerio de Ciencia y Tecnología.

## References

- Aguilera, J., Dummermuth, A., Karsten, U. *et al.* (2002) Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae. *Polar Biol.*, **25**, 432–441.
- Arihara, K. (2006) Strategies for designing novel functional meat products. *Meat Sci.*, **74**, 219–229.
- Ashwell, M. (2002). Concepts of functional foods. *International Life Science Institute. ILSI Europe Concise Monograph Series*. Bruselas, Bélgica.
- Bao, D.Q., Mori, T.A., Burke, V. *et al.* (1998) Effects of dietary fish and weight reduction on ambulatory blood pressure in overweight hypertensives. *Hypertension*, **32**, 710–717.
- Barbut, S. and Mittal, G.S. (1992) Use of carrageenans and xanthan gum in reduced fat breakfast sausages. *Food Sci. Technol.*, **25**, 509–513.

- Bater, B., Descamps, O. and Maurer, A.J. (1992) Quality characteristics of hydrocolloid-added oven-roasted turkey breasts. *J. Food Sci.*, **57**, 1068–1070.
- Bernal, V.M., Smajda, C.H., Smith, J.L. and Stanley, D.W. (1987) Interactions in protein polysaccharide systems. *Can. Inst. Food Sci. Technol. J.*, **20**, 316–316.
- Bocanegra, A., Nieto, A., Blas, B. and Sánchez-Muniz, F.J. (2003) Diets containing a high percentage of Nori or Kombu algae are well-accepted and efficiently utilized by growing rats but induce different degrees of histological changes in the liver and bowel. *Food Chem. Toxicol.*, **41**, 1473–1480.
- Bocanegra, A., Bastida, S., Benedí, J. *et al.* (2009) Characteristics and nutritional and cardiovascular-health properties of seaweeds. *J. Med. Food*, **12** 236–258.
- Boles, J.A. and Shand, P.J. (1998) Effect of comminution method and raw binder system in restructured beef. *Meat Sci.*, **49**(3), 297–307.
- Carpentier S., Knaus M. and Suh M.Y. (2009) Associations between lutein, zeaxanthin, and age-related macular degeneration: An overview. *Crit. Rev. Food Sci. Nutr.*, **49**(4), 313–326.
- Chun, S.S., Park, J.R., Park, J.C. *et al.* (1999) Quality characteristics of hamburger patties added with seaweed powder. *J. Korean Soc. Food Sci. Nutr.*, **28**, 140–144.
- Cofrades, S., López-López, I., Solas, M. T. *et al.* (2008) Influence of different types and proportions of added edible seaweeds on characteristics of low-salt gel/emulsion meat systems. *Meat Sci.*, **79**, 767–776.
- Cofrades, S., López-López, I., Bravo, L. *et al.* (2010) Nutritional and antioxidant properties of different brown and red Spanish edible seaweeds. *Food Sci. Technol. Int.*, **16**, 361–370.
- Cofrades S., López-López, I., Ruiz-Capillas, C. *et al.* (2011) Quality characteristics of low-salt restructured poultry with microbial transglutaminase and seaweed. *Meat Sci.*, **87**, 373–380.
- Csapo, I., Incze, K., Kovacks, A. *et al.* (2006) Development of meatproducts with lutein for eye health. In: *52nd International Congress of Meat Science and Technology* (eds D. Troy, R. Pearce, B. Byrne and J. Kerry). Wageningen Academic Publishers, The Netherlands, pp. 687–688.
- Daly T., Ryan E., Aherne S.A. *et al.* (2010) Bioactivity of ellagic acid-, lutein- or sesamol-enriched meat patties assessed using an in vitro digestion and Caco-2 cell model system. *Food Res. Int.*, **43**, 753–760.
- Dawczynski C., Schubert R. and Jarheis G. (2007). Amino acids, fatty acids and dietary fibre in edible seaweed products. *Food Chem.*, **103**, 891–899.
- Díaz M.T., Cañeque V., Sánchez C.I., Lauzurica S. *et al.* (2011) Nutritional and sensory aspects of light lamb meat enriched in n-3 fatty acids during refrigerated storage. *Food Chem.*, **124**, 147–151.
- Diplock, A.T., Aggett, P.J., Ashwell, M. *et al.* (1999) Scientific concepts of functional foods in Europe consensus document. *Br. J. Nutr.*, **81**, S1–S27.
- Dragnet, K.I. and Taylor, C. (2011) Chemical, physical and biological properties of alginates and their biomedical implications. *Food Hydrocolloids*, **25**, 251–256.
- Dumelod, B.D., Ramirez, R.P.B., Tiangson, C.L.P. *et al.* (1999) Carbohydrate availability of arroz caldo with lambda-carrageenan. *Int. J. Food Sci. Nutr.*, **50**, 283–289.
- EC (2007) Commission Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Corrigendum to Regulation (EC) No 1924/2006. *Official Journal of the European Union*, **L12**, 3–17.
- EC (2010) Commission Regulation (EU) No 116/2010 of 9 February 2010 amending Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims. *Official Journal of the European Union*, **L3**, 7–16.
- FAO/WHO (2009) Codex Alimentarius Commission. Report of the 30th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (ALINORM 09/32/23). Joint FAO/WHO Food Standard Programme, Geneva.
- Fernández, P., Cofrades, S., Solas M. T. *et al.* (1998) High pressure-cooking of chicken meat batters with starch, egg white and iota carrageenan. *J. Food Sci.*, **63**, 267–271.
- Fleurence, J. (1999) Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.*, **10**, 25–28.
- Granado-Lorencio, F., López-López, I., Herrero-Barbudo, C. *et al.* (2010) Lutein-enriched frankfurter-type products: physicochemical characteristics and lutein *in vitro* bioaccessibility. *Food Chem.*, **120**, 741–748.
- Grimm, H., Mayer, K., Mayser, P., and Eigenbrodt, E. (2002) Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. *Br. J. Nutr.*, **87**(Suppl. 1), S59–S67.
- Gudiél-Urbano, M. and Goñi, I. (2002). Effect of edible seaweeds (*Undaria pinnatifida* and *Porphyra tenera*) on the metabolic activities of intestinal microflora in rats. *Nutr. Res.*, **22**, 323–331.
- Hayes, J.E., Stepanyan, V., Allen P. *et al.* (2010) Effect of lutein, sesamol, ellagic acid and olive leaf extract on the quality and shelf-life stability of packaged raw minced beef patties. *Meat Sci.*, **84**, 613–620.
- He, F.J. and Mac Gregor, G.A. (2008) Beneficial effects of potassium on human health. *Plant Physiol.*, **133**, 725–735.
- Horrocks, L.A. and Yeo, Y.K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacol. Res.*, **40**, 211–225.

- Hsu, S.Y. and Chung, H.Y. (2001) Effects of  $\kappa$ -carrageenan, salt, phosphates and fat on qualities of low fat emulsified meatballs. *J. Food Eng.*, **47**, 115–121.
- Hu, F.G.B., Bronner, L., Willett, W.C. and Stampfer, M.J. (2002) Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA*, **287**, 1815–1821.
- Huffman, D.L., Egbert, W.R., Chen, C.M. and Dyleski, D.P. (1991) Technology for low-fat ground beef. *Reciprocal Meat Conference Proceedings*, **44**, 73–78.
- Ito, K., and Tsuchiya, Y. (1972) The effect of algal polysaccharides on the depressing of plasma cholesterol level in rats. In: *Proceedings of the Seventh International Seaweed Symposium*. Tokyo University Press, Tokyo, pp. 451–454.
- Jiménez-Colmenero, F. (2007) Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends Food Sci. Technol.*, **18**, 567–578.
- Jiménez-Colmenero, F., Carballo and J. and Cofrades, S. (2001) Healthier meat and meat products: Their role as functional foods. *Meat Sci.*, **59**, 5–13.
- Jiménez-Colmenero, F., Reig, M. and Toldrá, F. (2006). New approaches for the development of functional meat products. In: *Advanced Technologies for Meat Processing* (eds L.M. L. Nollet and F. Toldrá). Taylor & Francis Group, London, New York, pp. 275–308.
- Jiménez-Escrig, A. and Sánchez-Muniz, F.J. (2000) Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res.*, **20**, 585–598.
- Kim H.W., Choi J.H., Choi Y.S., Han D.J. *et al.* (2010) Effects of sea tangle (*Laminaria japonica*) powder on quality characteristics of breakfast sausages. *Korean J. Food Sci. Anim. Res.*, **30**, 55–61.
- Krinsky N.I. (2002) Possible biologic mechanisms for a protective role of xanthophylls. *J. Nutr.*, **132**, 540S–542S.
- Leaf, A., Kang, J.X., Xiao, Y.F., and Billman, G.E. (2003) Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. *Circulation*, **107**, 2646–2652.
- Lee, S., Decker, E.A., Faustman, C. and Mancini, R.A. (2005) The effects of antioxidant combinations on color and lipid oxidation in n-3 oil fortified ground beef patties. *Meat Sci.*, **70**, 683–689.
- Li, H.B. and Chen, F. (2001) Preparative isolation and purification of astaxanthin from the green microalga *Chlorococcum* sp. by highspeed counter-current chromatography. In: *Algae and their Biotechnological Potential* (eds F. Chen and Y. Jiang). Kluwer, Dordrecht, pp. 127–134.
- Lidebjer, C., Leanderson, P., Ernerudh, J. and Jonasson, L. (2007) Low plasma levels of oxygenated carotenoids in patients with coronary artery disease. *Nutr. Metab. Cardiovasc. Dis.*, **17**, 448–456.
- López-López, I., Bastida, S., Ruiz-Capillas, C. *et al.* (2009a) Composition and antioxidant capacity of low-salt meat emulsion model systems containing edible seaweeds. *Meat Sci.*, **83**, 492–498.
- López-López, I., Cofrades, S. and Jiménez-Colmenero, F. (2009b) Low-fat frankfurters enriched with n-3 PUFA and edible seaweed: Effects of olive oil and chilled storage on physicochemical, sensory and microbial characteristics. *Meat Sci.*, **83**, 148–154.
- López-López, I., Cofrades, S., Ruiz-Capillas, C. and Jiménez-Colmenero, F. (2009c). Design and nutritional properties of potential functional frankfurters based on lipid formulation, added seaweed and low salt content. *Meat Sci.*, **83**, 255–262.
- López-López, I., Cofrades, S., Yakan, A. *et al.* (2010) Frozen storage characteristics of low-salt and low-fat beef patties as affected by Wakame addition and replacing pork backfat with olive oil-in-water emulsion. *Food Res. Int.*, **43**, 1244–1254.
- MacArtain, P., Gill, C.I.R., Brooks, M. and Campbell, R. (2007) Nutritional value of edible seaweeds. *Nutr. Rev.*, **65**, 535–543.
- Means W.J., Clarke A.D., Sofos J.N. and Schmidt G.R. (1987) Binding, sensory and storage properties of algin calcium structured beef steaks. *J. Food Sci.*, **52**, 252–257.
- Moreno F.S., Toledo L.P., de Conti A. *et al.* (2007) Lutein presents suppressing but not blocking chernopreventive activity during diethylnitrosamine-induced hepatocarcinogenesis and this involves inhibition of DNA damage. *Chem.-Biol. Interact.*, **168**, 221–228.
- Muller, W.D. and Stiebing, A. (1993) Suitability of animal and plant gelling for the production of canned corned beef. *Fleischwirtschaft*, **73**, 1307–1311.
- Palombo, P., Fabrizi G., Ruocco V. *et al.* (2007) Beneficial long-term effects of combined oral/topical antioxidant treatment with the carotenoids lutein and zeaxanthin on human skin: A double-blind, placebo-controlled study. *Skin Pharmacol. Physiol.*, **20**, 199–210.
- Pellet, P. and Young, V.R. (1990) Role of meat as source of protein and essential amino acids in human protein nutrition. In: *Meat and Health. Advances in Meat Research*, Vol. 6 (eds A.M. Pearson and T.R. Dutson). Elsevier Applied Science, London, pp. 329–370.
- Pereira, M.S., Mulloy, B. and Mourao, P.A.S. (1999) Structure and anticoagulant activity of sulfated fucans – comparison between the regular, repetitive, and linear fucans from echinoderms with the more heterogeneous and branched polymers from brown algae. *J. Biol. Chem.*, **274**, 7656–7667.

- Pietrasik, Z. and Shand, P.J. (2003) The effect of quantity and timing of brine addition on water binding and textural characteristics of cooked beef rolls. *Meat Sci.*, **65**, 771–778.
- Pietrasik, Z. and Duda, Z. (2000) Effect of fat content and soy protein/carrageenan mix on the quality characteristics of comminuted, scalded sausages. *Meat Sci.*, **56**, 181–188.
- Raharjo S., Dexter D.R., Worfel R.C. *et al.* (1994) Restructuring veal steaks with salt/phosphate and sodium alginate calcium lactate. *J. Food Sci.*, **59**, 471–473.
- Rasmussen, R. and Morrissey, M.T. (2007) Marine biotechnology for production of food ingredients. *Adv. Food Nutr. Res.*, **52**, 237–292.
- Rupérez, P. (2002) Mineral content of edible marine seaweeds. *Food Chem.*, **79**, 23–26.
- Rymer, C., Gibbs, R.A., Givens, D.I. (2010) Comparison of algal and fish sources on the oxidative stability of poultry meat and its enrichment with omega-3 polyunsaturated fatty acids. *Poultry Sci.*, **89**, 150–159.
- Sun, X.D. (2009) Utilization of restructuring technology in the production of meat products: a review. *Cyta-Journal of Food*, **7**, 153–162.
- Trius, A. and Sebranek, J.G. (1996) Carrageenans and their use in meat products. *Crit. Rev. Food Sci. Nutr.*, **36**, 69–85.
- Valencia, I. Ansorena, D. and Astiasaran, I. (2007) Development of dry fermented sausages rich in docosahexaenoic acid with oil from the microalgae *Schizochytrium* sp.: Influence on nutritional properties, sensorial quality and oxidation stability. *Food Chem.*, **104**, 1087–1096.
- Wang, M.C., Tsao, R., Zhang, S.F. *et al.* (2006) Antioxidant activity, mutagenicity/anti-mutagenicity, and clastogenicity/anti-clastogenicity of lutein from marigold flowers. *Food Chem. Toxicol.*, **44**, 1522–1529.
- WHO/FAO Expert Consultation (2003) Diet, nutrition and the prevention of chronic diseases. WHO Technical Report Series, 916. WHO, Geneva. Available at [http://www.who.int/hpr/NPH/docs/who\\_fao\\_expert\\_report.pdf](http://www.who.int/hpr/NPH/docs/who_fao_expert_report.pdf) (accessed 18 April 2011).
- Witvrouw, M. and De Clercq, E. (1997) Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *Gen. Pharmacol.-Vasc. Syst.*, **29**, 497–511.
- Wong, K.H., Sam, S.W., Cheung, P.C.K. and Ang, P.O. (1999) Changes in lipid profiles of rats fed with seaweed-based diets. *Nutr. Res.*, **19**, 1519–1527.
- Xiong, Y.L. Noel, D.C. and Moody, W.G. (1999) Textural and sensory properties of low-fat beef sausages with added water and polysaccharides as affected by pH and salt. *J. Food Sci.*, **64**, 550–554.
- Yuan, Y.V. (2008) Marine algal constituents. In: *Marine Nutraceuticals and Functional Foods* (eds C. Barrow and F. Shahidi). CRC Press, Taylor & Francis Group, Boca Raton, pp. 259–296.
- Zhang, Q.B., Li, N., Liu, X.G. *et al.* (2004) The structure of a sulfated galactan from *Porphyra haitanensis* and its in vivo antioxidant activity. *Carbohydr. Res.*, **339**, 105–11.

# Industrial Applications of Macroalgae

**A. Malshani Samaraweera, Janak K. Vidanarachchi and Maheshika S. Kurukulasuriya**

*Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka*

## 33.1 Introduction

The unpredictable weather causes the supply of terrestrial crops and animal products to fluctuate keeping part of the world in hunger. Moreover, with an increasing world population the necessity of food, both quantity and quality wise, is also increasing. Emerging novel unidentified diseases, energy crises, and environmental problems are threats to human wellbeing. In search of solutions for these problems people tend to exploit untapped natural resources, thus marine-derived organisms and compounds are being identified as promising for many applications.

Seaweeds play a major role as primary producers in the sea, where about 150 macroalgal species are consumed as human food and 250 are commercially utilized worldwide (Kumari *et al.*, 2010). The marine macroalgae can be classified into three major classes as brown algae (Phaeophyceae), red algae (Rhodophyceae), and green algae (Chlorophyceae), based on the presence of specific phytopigments other than chlorophyll for photosynthesis (Bocanegra *et al.*, 2009). Depending on their varying capacity to perform photosynthesis, green algae inhabit shallow coastal regions while brown and red algae found in deep waters.

Fresh or dried forms of seaweeds have been used by East Asian countries like Japan, Korea, China, Vietnam, Indonesia, and Taiwan (Besada *et al.*, 2009) for their daily cuisines as a condiment or a vegetable in soups, stews, or with rice or noodles. Popularity among Westerners is as agar, alginate, and carrageenan (phycocolloids) in industrial

applications as emulsifiers or stabilizers in the food industry, as dental impression materials, and in the paper industry, etc. Traditionally, seaweeds have been used in therapeutics, as a soil conditioner, and as animal feed.

Macroalgae usually grow abundantly, thus they can be harvested to control excessive proliferation, specially the invasive seaweed species (Zubia *et al.*, 2008) or can be cultivated to meet demand. Applications of this renewable resource and derived compounds (polysaccharides, phenols, alkaloids) are being explored by various scientists worldwide, in the fields of food, feed, biomedical, agricultural, environmental, and other industrial applications. Thus, the objective of this chapter is to summarize the present and probable future applications of macroalgal-derived compounds.

## 33.2 Composition of seaweeds

Seaweeds are important primary producers in the food chains; especially in marine and freshwater habitats. They have been well adapted to various harsh environmental conditions found in the diverse habitats that they live. Certain secondary metabolites such as carotenoids, polyphenols and mycosporine like amino acids (MAAs) produced by these macroalgae, make it possible for them to thrive under these stressful environmental conditions. Since these bioactive compounds, as well as certain other structural compounds such as polysaccharides, have exhibited many important biological properties, they have been widely applied

as well as having the potential to be used in various important industries.

### 33.2.1 Seaweed polysaccharides

Polysaccharides are a major group of substances that has been extracted from many species of macroalgae. Usually seaweeds possess three functionally different polysaccharides, structural cell wall polysaccharides, intercellular mucilage polysaccharides, and storage polysaccharides (Bocanegra *et al.*, 2009). The structural cell wall polysaccharides are distinctive to seaweeds, where they provide the mechanical strength and flexibility to avoid ripping during water currents and tidal fluctuations. Among the structural polysaccharides, including alginates, carrageenans, agar, and fucans, and certain storage polysaccharides such as laminarans have been widely used in the current industrial world.

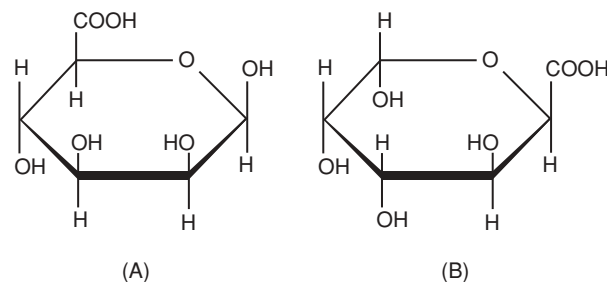
#### Alginates

Alginates, also known as alginic acid or algin, are found in brown marine algae as the most abundant structural polysaccharide (Davis *et al.*, 2003). They are unbranched polymers consisting of (1→4)-linked  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G) rich regions (Figure 33.1) with alternate G- and M-rich regions (Draget *et al.*, 2005). Therefore, the linear alginate polymer is composed of homopolymorphic M or G regions, interspersed with alternate M and G regions, where a regular repeating pattern is absent (Draget *et al.*, 2005).

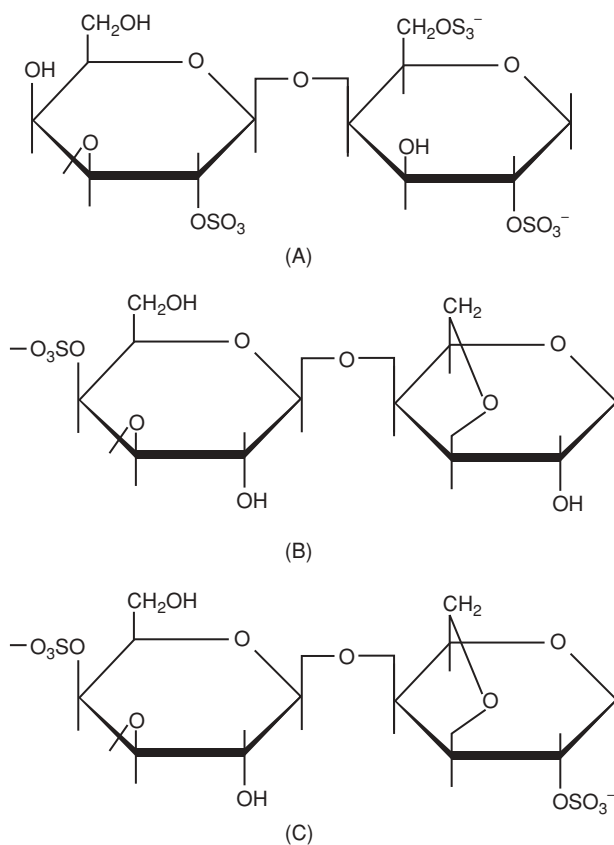
The free hydroxyl and carboxyl groups of alginate, which are distributed along the polymer chain backbone make the chemical modification, which can alter the characteristics of native alginate (Yang *et al.*, 2011).

#### Carrageenans

Carrageenans are sulfated polysaccharides extracted from certain species of red algae. They have alternate monomers



**Figure 33.1** Structure of alginate monomers,  $\beta$ -D-mannuronic acid (A),  $\alpha$ -L-guluronic acid (B).



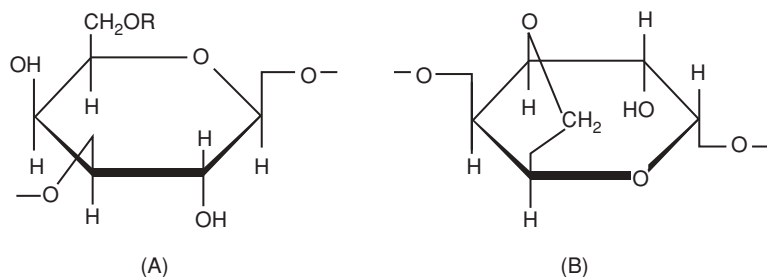
**Figure 33.2** Basic structures of lambda (A), kappa (B) and iota (C) carrageenan.

of  $\beta$ -D-galactose and  $\alpha$ -D-galactose (O'Sullivan *et al.*, 2010). Commercially, carrageenans are classified as lambda ( $\lambda$ ), kappa ( $\kappa$ ) and iota ( $\iota$ ) carrageenans (Figure 33.2) depending on the gel-forming ability.

#### Agar

Similar to carrageenan agar is extracted from certain families of Rhodophyta. Agar is a linear polymer with altering 3-linked  $\beta$ -D-galactopyranosyl and 4-linked 3,6-anhydro- $\alpha$ -L-galactopyranosyl units (Glicksman, 1987; O'Sullivan *et al.*, 2010). The structure of monomers of agar liner polymer is given in Figure 33.3.

Agar can be divided into two types – agarose and agaropectin – where agarose is the fraction having the greatest gelling capacity. It is an altering copolymer of 3-linked  $\beta$ -D-galactopyranose and 4-linked 3,6-anhydro- $\alpha$ -L-galactopyranose units (Glicksman, 1987). Agaropectin is structurally similar to agar, except that some units in the copolymer are substituted by pyruvic acid ketal,



**Figure 33.3** Structure of agar constituents  $\beta$ -D-galactose (A),  $\alpha$ -L-galactose (B).

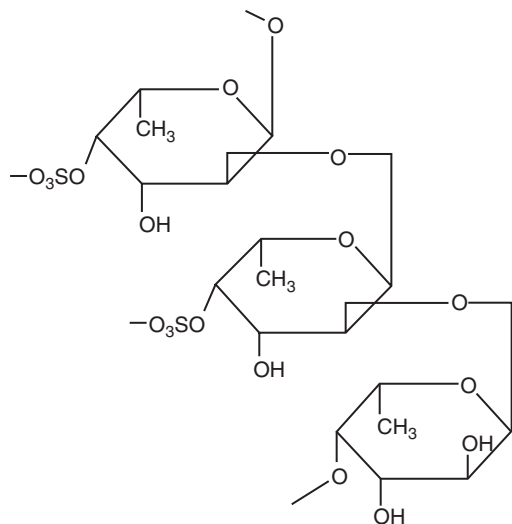
4,6-*O*-(1-carboxyethylidene)-D-galactopyranose or by sulfated or methylated sugar residues (O'Sullivan *et al.*, 2010).

### Fucoidans

Fucoidans, also known as fucan, fucosan, and sulfated fucan, are heterogeneous sulfated polysaccharides found in brown algae (Li *et al.*, 2008). They are primarily composed of (1 $\rightarrow$ 2)- $\alpha$ -L-fucose-4-sulfate with branching or a sulfate ester group on C3 (Figure 33.4), and contain very small quantities of D-xylose, D-galactose, D-mannose and uronic acids (Jiménez-Escrig and Sánchez-Muniz, 2000).

### Ulvan

Ulvan is one of the complex sulfated hetero-polysaccharides from the green algal cell wall. It is composed of  $\beta$ -(1 $\rightarrow$ 4)-xyloglucan, glucuronan, and cellulose in a linear arrangement (O'Sullivan *et al.*, 2010).



**Figure 33.4** The structure of fucoidan with  $\alpha$ -(1 $\rightarrow$ 2) linkages in L-fucose monomer.

### Laminarans

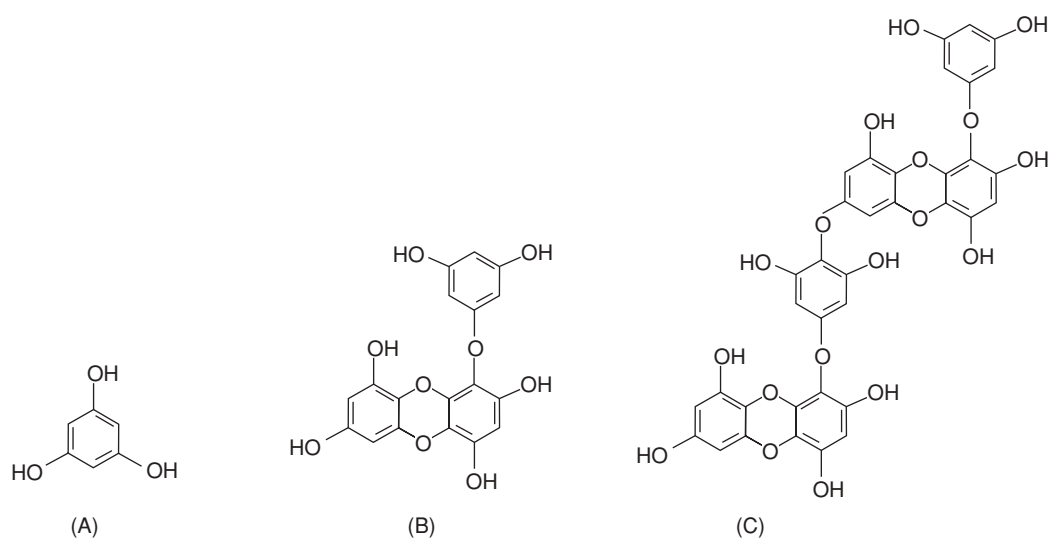
Apart from these structural polysaccharides, macroalgae consists of certain other storage polysaccharides which have many industrial applications. Laminarans are storage polysaccharides commonly found in brown algae, and the structure of this polysaccharide composed of (1 $\rightarrow$ 3)- $\beta$ -D-glucopyranose residues in which some 6-*O*-branching in the main chain and some  $\beta$ -(1 $\rightarrow$ 6)-intrachain linkages (Chizhov *et al.*, 1998).

### 33.2.2 Polyphenols

Polyphenols found in the algae have been identified as photoprotective compounds that can be found in the natural environment. Among the three algal groups brown algae generally contain higher amount of polyphenols than red and green algae (Wang *et al.*, 2009a). A group of phenolic compounds known as phlorotannins (1,3,5-trihydroxybenzene), are the only phenolic compounds found within brown algae, and are formed by polymerization of phloroglucinol. Polyphenolic compounds have been identified from brown algal families Alariaceae, Fucales, and Sargassaceae (Wang *et al.*, 2009a). Shibata *et al.* (2008) isolated the phlorotannins eckol (a phloroglucinol trimer), phlorofucofuroeckol A (a pentamer), dieckol and 8,8'-bieckol (hexamers) from brown algae *Eisenia bicyclis*, *Ecklonia cava*, and *Ecklonia kurome*. The structures of monomer phloroglucinol (Figure 33.5a), eckol (Figure 33.5b) and dieckol (Figure 33.5c) are shown in Figure 33.5.

### 33.2.3 Mycosporine-like amino acids (MAAs)

The MAAs are unique to red algae and found in some other marine organisms as a response to stressful environmental conditions. These are intracellular, low molecular weight (<400 Da), polar compounds that act as an important source for absorption of solar radiation. The MAAs are characterized by a cyclohexenone or cyclohexenimine core



**Figure 33.5** Structures of the major phlorotannins in algae, phloroglucinol (A), eckol (B) and dieckol (C).

conjugated with the nitrogen moiety of an amino acid (Yuan *et al.*, 2009). The same authors have isolated MAAs such as palythine, shinorine, palythanol, porphyra-334, asterina-330 and usujirene (the *cis*-isomer of palythene) from the methanol extract of lyophilized dulse *Palmaria palmata*. Among them shinorine, porphyra-334 (Figure 33.6) is the most abundant MAA found in the seaweeds, which have a high industrial importance.

### 33.3 Seaweeds as vegetables: their nutritive value

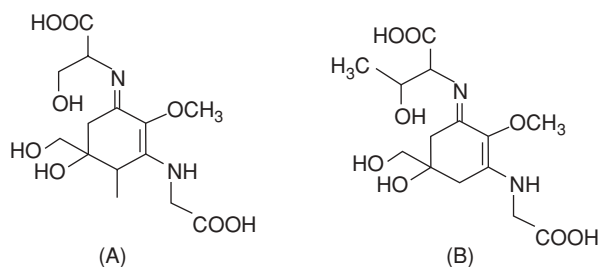
Fresh or dried pieces of seaweed (Table 33.1) in cooked products or in salads as a vegetable is popular among the East Asian countries. Nori is a primary constituent of the Japanese well known dish sushi. Direct consumption of seaweed has becoming increasingly popular among Western countries. Hence, the nutritive value of seaweed is impor-

tant as a source of amino acids, essential fatty acids, minerals and vitamins.

#### 33.3.1 Fatty acids

The total fat content of macroalgae ranges from 0.3 to 4.3 g/100 g dry weight (Ortiz *et al.*, 2006) where in most seaweeds palmitic acid (C 16:0) is the most abundant saturated fatty acid (Colombo *et al.*, 2006; Kumari *et al.*, 2010). The highest palmitic acid content is found in green algae which accounts 41–53% of the total fatty acid, followed by brown algae and red algae (Kumari *et al.*, 2010).

Although algae have a low lipid content, they are a good source of long chain *n*-3 and *n*-6 polyunsaturated fatty acids (PUFA) of (Table 33.2), compared with traditional vegetables (Kumari *et al.*, 2010). These fatty acids cannot be synthesized by humans and animals, hence they are essential



**Figure 33.6** Structures of some MAAs, shinorine (A) and porphyra-334 (B).

**Table 33.1** Some popular seaweeds as vegetables

Class	Common name	Scientific name
Red	Nori	<i>Porphyra tenera</i>
Brown	Wakame	<i>Undaria pinnatifida</i>
Brown	Kombu	<i>Laminaria japonica</i>
Red	Dulse	<i>Palmaria palmate</i>
Green	Hiziki	<i>Hizikia fusiforme</i>
Green	Sea lettuce	<i>Ulva</i> and <i>Enteromorpha</i> spp

Source: Fitton, 2003; Yuan *et al.*, 2009.

**Table 33.2** Fatty acid composition of several edible seaweeds

	Seaweed	SFAs	MUFAs	PUFAs	PUFA <i>n</i> -6	PUFA <i>n</i> -3	Ratio <i>n</i> -6/ <i>n</i> -3
Brown	<i>Laminaria ochroleuca</i> <sup>b</sup>	33.82 ± 2.21	19.23 ± 1.99	46.94 ± 4.58	20.99 ± 1.27	25.08 ± 3.21	0.83
	<i>Undaria pinnatifida</i> <sup>b</sup>	20.39 ± 1.73	10.50 ± 1.78	69.11 ± 9.01	22.10 ± 2.00	44.70 ± 5.05	0.49
	<i>Hizikia fusiforme</i> <sup>a</sup>	28.1 ± 4.3	13.4 ± 6.4	57.0 ± 11.6	13.5 ± 0.4	42.9 ± 11.7	0.3:1 ± 0.1
Red	<i>Palmaria</i> sp. <sup>b</sup>	60.48 ± 2.58	10.67 ± 1.55	28.86 ± 3.94	2.14 ± 0.45	25.52 ± 3.34	0.13
	<i>Porphyra</i> sp. <sup>b</sup>	64.95 ± 2.24	18.91 ± 2.81	16.10 ± 3.31	7.97 ± 1.31	7.20 ± 1.48	1.21
Green	<i>Codium</i> sp. <sup>c</sup>	30.81–33.84	5.20–20.39	18.82–42.90	NA	NA	NA

Source: <sup>a</sup>Dawczynski *et al.*, 2007, <sup>b</sup>Machado *et al.*, 2004 and <sup>c</sup>Goecke *et al.*, 2010  
NA = not available.

in the diet. For example, the higher unsaturated fatty acid content of more than >74% of total fatty acid is reported from seaweeds *Gracilaria changgi* and *Kappaphycus* sp. (Norziah and Ching, 2000; Rajasulochana *et al.*, 2010).

Among the PUFA green algae showed higher C:18 PUFA content than C:20, while for red algae the trend is usually opposite (Kumari *et al.*, 2010; Khotimchenko *et al.*, 2002) as illustrated in Table 33.2. In brown algae, C:18 and C:20 PUFA content is 39–49% of total fatty acid (Khotimchenko *et al.*, 2001; Kumari *et al.*, 2010).

Moreover, cold water algae showed a higher PUFA content and lower saturated fatty acid content than that of warm water algae (Colombo *et al.*, 2006). Thus, consumption of seaweeds may increase the dietary supply of *n*-3 fatty acids which deficient in Western diets and thereby reduce the *n*-6/*n*-3 ratio (Table 33.2), where the *n*-6/*n*-3 ratio recommended by the World Health Organization (WHO) for adult humans should be less than 10 as a whole in the diet. Therefore, seaweeds are an alternative source of PUFA to fish and fish oils (Colombo *et al.*, 2006). Moreover, the consumption of these seaweeds may beneficial for the prevention of cardiovascular diseases and other chronic diseases, such as diabetes, hypertension, and autoimmune diseases in humans (Dawczynski *et al.*, 2007).

### 33.3.2 Amino acids

Aspartic acid and glutamic acids are the most abundant amino acids, representing about 22–44% of the total amino acids (Munda, 1977; Dawczynski *et al.*, 2007). Wong and Cheung (2000) reported that red seaweeds (*Hypnea charoides* and *Hypnea japonica*) and green seaweed (*Ulva lactuca*) contained all the essential amino acids (excluding tryptophan), which accounts for 42–48% of the total amino acid content. Thus, all red and green seaweeds are able to contribute to adequate levels of total essential

amino acids with respect to Food and Agriculture Organization (FAO)/WHO requirements (Wong and Cheung, 2000; Fleurence *et al.*, 1999).

### 33.3.3 Minerals

Mineral content of seaweeds ranges from 8 to 40% of algal dry weight, where macrominerals and trace minerals levels vary from 8083 to 17 875 mg/100 g and 5 to 15 mg/100 g, respectively (Rupérez, 2002). This mineral content is generally higher than terrestrial plants except for spinach. Considering the brown and red seaweeds, high amounts of minerals are found in brown algae (30–39% algal dry weight) than red algae (21% of algal dry weight) (Rupérez, 2002). Therefore, intake of algal products would supplement the daily requirement of all the essential minerals and trace elements for adults (Rupérez, 2002). Furthermore, algal extracts contain balanced levels of certain minerals, which bring many health benefits to consumers. As an example, the presence of balanced Na and K levels in a seaweed diet prevents hypertension caused by the presence of a high Na/K ratio in the diet (Rupérez, 2002; Rajasulochana *et al.*, 2010).

### 33.3.4 Antinutrients and toxic factors

Even though the macroalgae are important as a nutrient-rich vegetable, antinutritive factors such as lectins, tannins, phytic acid, trypsin inhibitors, and amylase inhibitors have been identified from washed-up seaweeds in Brazil by Oliveira *et al.* (2009). Furthermore, heavy metals were detected from seaweed samples, due to their ability to accumulate elements present in water (Besada *et al.*, 2009; Oliveira *et al.*, 2009).

Other than heavy metals, some seaweeds contain higher levels of iodine than the tolerable upper intake level of 1100 µg/day, which may cause hypertension in

some individuals (Teas *et al.*, 2004). For example, an iodine content of 8165 µg/g is recorded in kelp granules made from *Laminaria digitata* (Teas *et al.*, 2004). Thus, seaweeds should be constantly monitored for heavy metal and mineral contents and further studies are required to guarantee their safe utilization.

### 33.4 Applications as functional foods

#### 33.4.1 Dietary fiber as prebiotics

Prebiotics are non-digestible selectively fermented compounds that stimulate the growth and/or activity of beneficial gut microbiota, which in turn confers health benefits to the host (O'Sullivan *et al.*, 2010). Since seaweed polysaccharides are not digested by the human alimentary tract, they can be considered as dietary fiber (Bocanegra *et al.*, 2009; Gomez-Ordóñez *et al.*, 2010). Prebiotic activity of seaweed-derived polysaccharides was reviewed by O'Sullivan *et al.* (2010), using evidence from both *in vitro* and *in vivo* studies on animals. Other than complex polysaccharides, this component may consist of oligosaccharides and resistant starches, resistant proteins, and associated compounds such as polyphenols (Jimenez-Escrig *et al.*, 2000; Rupérez and Toledano, 2003).

The dietary fiber content in edible seaweeds ranges from 25–75% of the dry weight (Jiménez-Escrig *et al.*, 2000), where soluble fiber content varies from 50% to 85% of total dietary fiber (Gómez-Ordóñez *et al.*, 2010). Soluble fiber forms viscous gels in water, while the other fraction is insoluble. Despite their solubility, dietary fiber gives satiety and numerous health benefits upon consumption.

Dietary fiber confers health benefits such as increased fecal bulk; increased digestion and absorption in the human small intestine due to slow rate of passage; stimulation of colonic fermentation; reduction of the glycemic response; and reduction in preprandial cholesterol levels (Elleuch *et al.*, 2011) and thereby reduces the risk of coronary heart diseases, diabetics, obesity, and colon cancer. Thus, seaweed-incorporated functional foods can be produced with low calories, and low density cholesterol and fat.

Considering the nutritive value and prebiotic effects, seaweeds can be used to replace part of the expensive diet, alleviating nutritional deficiencies in under developed countries and dietary problems associated with Western diets. Seaweeds can be incorporated into foods that can be used daily and add value as functional foods.

In this context, Prabashanker *et al.* (2009) developed seaweed-incorporated pasta, adding up to 10% seaweed (wakame; *Undaria pinnatifida*) which was accepted sen-

sorily. Interestingly, the pasta had a mild seaweed flavor. Addition of seaweeds improved the antioxidant activity, fatty acid and amino acid profile. The *n-3/n-6* fatty acid ratio was 1:3.4 as compared to 1:15.2 in the control. Recently, a bread incorporated with commercial *Ascophyllum nodosum* product (Seagreens; Seagreens® Ltd, Plummers Plain, UK) reported by Hall *et al.* (2010). Adding seaweed (*Enteromorpha compressa*) up to 7.5% to “pakoda”, a traditional snack food in India, increased the nutritional quality and functional properties (Mamatha *et al.*, 2007). Moreover, at gastric pH bioavailability of iron in seaweed added pakoda was slightly higher (27.1%) than that of the seaweed. Herber and van Elswyk (1996) used marine algae as a poultry ration supplement to produce shelled eggs rich in *n-3* fatty acids.

The nutritive value of seaweeds may vary since there is a strong relationship between biogeographical conditions and composition of mainly fatty acids, amino acids and minerals (Dawczynski *et al.*, 2007; Goecke *et al.*, 2010). The composition of the same seaweed may differ depending on the season of the year, growth stage, part of the seaweed harvested, geographic location etc. Thus, in order to assure the nutritional value of seaweeds, they need to be evaluated before used them as supplements.

#### 33.4.2 Microencapsulation of bacteria as probiotics

Seaweed incorporated diets exert their health benefits not only as prebiotics, but also as probiotics, where seaweed polysaccharides are used for microbial entrapment. Calcium alginate is the most commonly used capsule material (Kermanshahi *et al.*, 2010; Yabur *et al.*, 2007), where the monomers of alginate (M and G) can be linked by calcium ions through binding consecutive blocks of guluronic acid to form polymer networks. Microencapsulated bacteria in food should tolerate the strong acidic and alkaline conditions of the stomach and small intestine and should bypass these to the large intestine to exert their beneficial effects as probiotics. The indigestible nature of seaweed polysaccharides provides better protection for bacteria against enzymatic digestion in the human digestive tract.

Bacteria-encapsulated probiotic dairy products are in high demand as functional foods. Microencapsulation of *Lactobacillus reuteri* for dairy products using alginate, alginate plus starch,  $\kappa$ -carrageenan with locust bean gum or xanthan with gellan by extrusion and two phase (water/oil) emulsion method have been studied by Muthukumaraswamy *et al.* (2006) in order to select the best material for microencapsulation. In contrast, alginate and alginate plus starch provided better protection for bacteria *in vitro*,

yielding 38  $\mu\text{m}$  and 43  $\mu\text{m}$  microcapsules for alginate and alginate plus starch, respectively. However, sensory qualities of foods need to be assessed before they used in food applications (Muthukumaraswamy *et al.*, 2006).

### 33.5 Application of seaweeds as antioxidants in the food industry

Antioxidants are substances that delay or prevent the oxidation of cellular substrates by inhibiting the initiation or propagation of oxidizing chain reactions (Song *et al.*, 2010; O'Sullivan *et al.*, 2011).

Usually these substances are produced within organisms to maintain homeostasis against oxidative stress. Oxidation of cellular substrates is initiated with the formation of oxygen-derived free radicals also known as reactive oxygen species (ROS), such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), hydroxyl radical ( $\text{OH}^\cdot$ ), superoxide anion ( $\text{O}_2^{\cdot-}$ ) and nitric oxide (NO); they are synthesized in biological systems due to various cellular stresses. Excessive generation of ROS may shift the pro-oxidant–antioxidant balance towards a more oxidative state, which may damage important molecules including proteins, lipids, and DNA, through chain reactions. In animals this may ultimately result in cellular dysfunction and cytotoxicity (O'Sullivan *et al.*, 2011). Hence, dietary intake of antioxidants may help to maintain homeostasis within the organisms. Furthermore, formation of ROS in food systems leads to oxidation of lipids and food spoilage. Synthetic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) are used in food products to maintain nutritional and sensory qualities, to extend the shelf life and thereby to assure food safety (Ngo *et al.*, 2011). However, these synthetic compounds suspected of being carcinogens (Song *et al.*, 2010). BHT and  $\alpha$ -tocopherol are ineffective as antioxidants in complex food systems as fish muscle (He and Shahidi, 1997). Therefore, effective and safe alternative antioxidants, especially with natural origin are of prior importance. Marine algae are one of the richest sources of natural antioxidants (Wijesekara *et al.*, 2011). Antioxidant potential of seaweed-derived compounds such as sulfated polysaccharides, phenolic compounds, MAAs and carotenoids have been investigated recently. Seaweed extracts with proven antioxidant activities are summarized in Table 33.3.

Antioxidant activity of sulfated polysaccharides of green, brown, and red algal polysaccharides has investigated in the last decade employing various assays. Several sulfated polysaccharides showed even better antioxidant activity

than commercially available synthetic antioxidants. For example, a group of sulfated heteropolysaccharides extracted from the green algae *Bryopsis plumose* had stronger superoxide radical scavenging activity compared to vitamin C (Song *et al.*, 2010).

The antioxidant activity of these sulfated polysaccharides vary depending on the sulfate content, sulfation position, molecular weight, and the type of solvent used for extraction (Wang *et al.*, 2009a; Ye *et al.*, 2008; Ngo *et al.*, 2011). The polysaccharides with high sulfate content (Souza *et al.*, 2007) and low molecular weight (Song *et al.*, 2010) showed the highest antioxidant activity. The reason may be that polysaccharides with low molecular weight may be incorporated into the cells and donate protons more efficiently compared to high molecular weight polysaccharides (Wijesekara *et al.*, 2011). However, molecular weight may associate with the type of the major sugar and glycosidic branching.

Phenolic compounds of land plants and their antioxidant activity have been studied extensively. Similarly, the antioxidant potential of seaweed derived polyphenols has been proven in many studies, with the observation of a statistically significant correlation between the total phenolic content and antioxidant activity (Jiménez-Escrig *et al.*, 2001; Kumar *et al.*, 2008; Zubia *et al.*, 2008). Antioxidant capacities of these compounds are related to their phenol rings. Wang *et al.* (2009a) observed that polyphenols of brown algae with up to eight interconnected phenol rings are more potent antioxidants with multifunctional antioxidant activity, than analogous polyphenols derived from terrestrial plants. Some other investigators observed that the total phenolic content is not accordance with the antioxidant activity (Lim *et al.*, 2002). This may be due to presence of other compounds that exert antioxidant activity, such as ascorbic acid and vitamin A ( $\beta$ -carotene) in crude extracts. Thus, the method of extraction or the solvent used may also determines the antioxidant activity.

Kumar *et al.* (2008) reported that phenol content was maximum when a mixture of chloroform and methanol (2:1) was used, followed by ethanol, methanol, *n*-propanol and ethyl alcohol. Extracts obtained using other solvents, namely acetone, *n*-hexane, and chloroform showed less than 1% total phenolic content.

A group of phenolic compounds known as phlorotannins have shown antioxidant capacity (Table 33.3). A phlorotannin-incorporated soybean food showed pronounced DPPH radical scavenging activity over control, which was composed solely of soybean proteins (Shibata *et al.*, 2008). Soybean proteins are rich in isoflavonols, which have antioxidant activity. Moreover, phlorotannins of high molecular weight (pentamers and hexamers) of phloroglucinol showed pronounced affinity for soybean

**Table 33.3** Sulfated polysaccharides, polyphenols and mycosporine like amino acids in algae as potential sources with antioxidant activity

Class	Name	Active compound	Reference
Brown	<i>Fucus vesiculosus</i>	Fucoidan	Souza <i>et al.</i> , 2007
Red	<i>Gigartina acicularis</i>	Lambda carrageenan	
Red	<i>Gigartina pisillata</i>	Lambda	
Red	<i>Eucheuma cottonii</i>	Kappa carrageenan	
Red	<i>Eucheumia spinosa</i>	Iota carrageenan	
Brown	<i>Padina gymnospora</i>	Fucans	
Brown	<i>Sargassum pallidum</i>	Polysaccharides	Ye <i>et al.</i> , 2008
Green	<i>Bryopsis plumosa</i>	Polysaccharides	Song <i>et al.</i> , 2010
Brown	<i>Dictyota cervicornis</i>	Sulfated polysaccharides	Costa <i>et al.</i> , 2010
Brown	<i>Dictyopteris delicatula</i>		
Brown	<i>Dictyota menstrualis</i>		
Brown	<i>Dictyota mertensii</i>		
Brown	<i>Sargassum filipendula</i>		
Brown	<i>Spatoglossum schroederi</i>		
Red	<i>Gracilaria caudata</i>		
Green	<i>Caulerpa cupressoides</i>		
Green	<i>Caulerpa prolifera</i>		
Green	<i>Caulerpa sertularioides</i>		
Green	<i>Codium isthmocladum</i>		
Brown	<i>Laminaria japonica</i>	Sulfated polysaccharides	Zhang <i>et al.</i> , 2010
Red	<i>Porpyra haitanensis</i>		
Green	<i>Ulva pertusa</i>		
Green	<i>Enteromorpha linza</i>		
Green	<i>Bryopsis plumose</i>		
Brown	<i>Sargassum mangarevense</i>	Phenols	Zubia <i>et al.</i> , 2008
	<i>Turbinaria ornata</i>		
Red	<i>Kappaphycus alvarezii</i>	Phenols	Kumar <i>et al.</i> , 2008
Brown	<i>Ascophyllum nodosum</i>	Phenols	O'Sullivan <i>et al.</i> , 2011
	<i>Pelvetia canaliculata</i>		
	<i>Fucus serratus</i>		
	<i>Fucus vesiculosus</i>		
Red	<i>Rhodomela confervoides</i>	Phenols	Wang <i>et al.</i> , 2009b
Brown	<i>Eisenia bicyclis</i>	Phlorotannins	Shibata <i>et al.</i> , 2008
	<i>Ecklonia cava</i>		
	<i>Ecklonia kurome</i>		
Brown	<i>Fucus vesiculosus</i>	Total phenols	Wang <i>et al.</i> , 2009a
Brown	<i>Fucus serratus</i>		
Brown	<i>Laminaria hyperborea</i>		
Brown	<i>Laminaria digitata</i>		
Brown	<i>Saccharina latissima</i>		
Brown	<i>Alaria esculenta</i>		
Red	<i>Palmaria palmata</i>		
Red	<i>Chondrus crispus</i>		
Green	<i>Ulva lactuca</i>		
Red	<i>Gelidella acerosa</i>	Polyphenols	Devi <i>et al.</i> , 2008
Red	<i>Palmaria palmate</i>	Phenols	Yuan <i>et al.</i> , 2005
Brown	<i>Sargassum siliquastrum</i>	Phenols	Lim <i>et al.</i> , 2002
Brown	<i>Ecklonia cava</i>	Phenols	Senevirathne <i>et al.</i> , 2006

(Continued)

Table 33.3 (Continued)

Class	Name	Active compound	Reference
Brown	<i>Ecklonia cava</i>	Phorotannins (phloroglucinol, eckol, dieckol)	Ahn <i>et al.</i> , 2007
Brown	<i>Fucus vesiculosus</i>	Polyphenols	Zaragoza <i>et al.</i> , 2008
Brown	<i>Fucus vesiculosus</i>	Phenols	Keyrouz <i>et al.</i> , 2011
	<i>Fucus serratus</i>		
	<i>Ascophyllum nodosum</i>		
Brown Red	Processed seaweeds of <i>Laminaria Porphyra</i>	Polyphenols	Jiménez-Escrig <i>et al.</i> , 2001
Brown	<i>Fucus vesiculosus</i>		
Brown	<i>Laminaria ochroleuca</i>		
Brown	<i>Undaria pinnatifida</i>		
Red	<i>Chondrus crispus</i>		
Red	<i>Porphyra umbilicalis</i>		
Red	<i>Porphyra rosengurttii</i>	Porphyra-334, Shinorine	Coba <i>et al.</i> , 2009
	<i>Gelidium corneum</i>	Asterina-330, Palythine	
	<i>Ahnfeltiopsis devoniensis</i>	Shinorine	
Red	<i>Palmaria palmata</i>	Palythine, Shinorine, Palythinol, Asterina-330, Porphyra-334	Yuan <i>et al.</i> , 2009

protein (Shibata *et al.*, 2008), which can be effectively utilized for development of new functional material with antioxidant capacity for the food industry.

In addition to sulfated polysaccharides and polyphenols, antioxidant activity has been observed in MAAs, mannitol, alkaloids, terpenes, ascorbic acid, tocopherols and carotenoids, including astaxanthin and fucoxanthin (Zubia *et al.*, 2008; Yuan *et al.*, 2009; Wang *et al.*, 2009a; O'Sullivan *et al.*, 2011). Antioxidant activity of carotenoid pigments is associated with their highly unsaturated nature (Ngo *et al.*, 2011). Mannitol is a sugar alcohol found in brown algae produced by photosynthesis and this also exhibited hydrating and antioxidant properties (Zubia *et al.*, 2008).

Usually, fresh seaweeds showed better antioxidant activity than processed seaweeds (Jiménez-Escrig *et al.*, 2001). Hence, in order to preserve the antioxidant activity processing may play an important role. Moreover, alginate residues of the commercial alginate recovery industries can be effectively recovered for the production of natural antioxidants (Ngo *et al.*, 2011).

These compounds with potential antioxidant activities with natural origin can be incorporated in foods in order to delay the oxidative deterioration and enhance food safety. However, several investigators have suggested that optimization of the extraction, fractionation, purification,

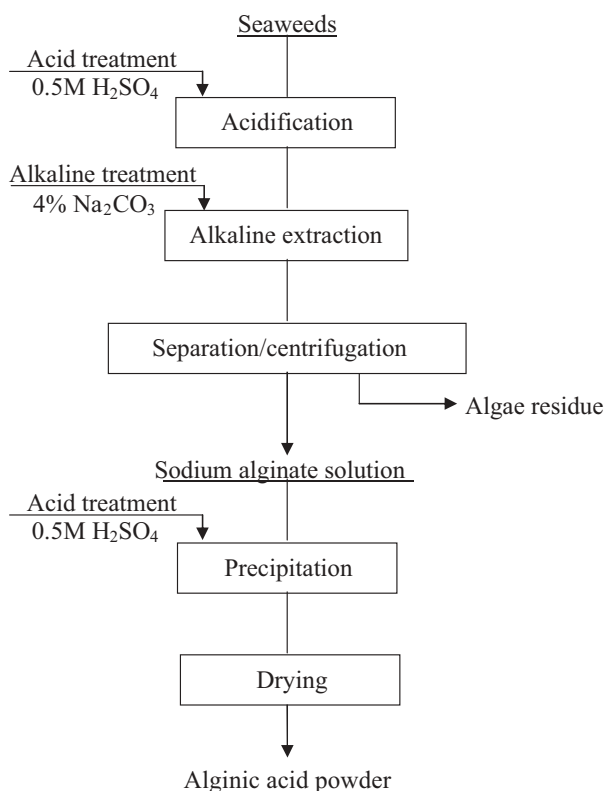
and characterization of the active components and identification of mechanism of action *in vivo* using animal or food models is required for further investigation (Wang *et al.*, 2010).

## 33.6 Industrial applications of phycocolloids

### 33.6.1 Extraction of seaweed phycocolloids

Carrageenan and agar are obtained from different species of red algae, while alginates are obtained from brown algae (Radmer, 1996). These substances are considered as widely using phycocolloids in the industrial scenario.

Insoluble alginic acid from washed macerated brown seaweed is industrially extracted using hot alkali (usually sodium carbonate) followed by addition of sodium/calcium chloride to the filtrate, which forms a fibrous precipitation of sodium/calcium alginate. This formed alginate salt is transformed to alginic acid by treatment with hydrochloric acid, then the alginate is purified, dried and powdered (O'Sullivan *et al.*, 2010; Radmer, 1996). However, the protocol for industrial-scale extraction of alginate is given in



**Figure 33.7** Protocol for industrial scale extraction of alginate. Adapted from Vauchel *et al.* (2008).

Figure 33.7 and the study of Vauchel *et al.* (2008) has found that the modification of this process by introducing reactive extrusion increased the efficiency of alginate extraction from the seaweeds.

The other two industrially important seaweed phycocolloids, carrageenan and agar, have a similar extraction method. Usually, both these substances are extracted with hot water, the concentrated filtrate is allowed to gel, and the gel is then treated, dehydrated, and milled (Armisen and Galatas, 1987).

### 33.6.2 Phycocolloids in food preparation

Alginates and carrageenan are widely used as phycocolloids in various industries, including the food industry, due to their properties such as viscosity enhancement, gel forming ability, stabilization of aqueous mixtures, dispersions, and emulsions. Their usages are wide in food. They have been used in beverages (fruit drinks, coffee, cocoa, tea, soy milk, alcoholic beverages), milk products (fermented milk, instant powders, powdered milk, yoghurt and ice cream), desserts (puddings and sherbets), table spreads (jellies,

jams, marmalades, honey products), confectionary (candy, cookies, biscuits, chocolate, sweets), and bakery products (bread, pastries).

Alginates available for industrial uses include sodium, potassium, ammonium and mixed salts of alginic acid (Glicksman, 1987). Among the esterified derivatives of alginate, the only alginate having a commercial value is propylene glycol alginate (PGA), produced by partial esterification of the carboxyl groups on uronic acid residues by reaction with propylene oxide (Draget *et al.*, 2005; Yang *et al.*, 2011). Native alginate precipitates in acidic conditions, but PGA stabilizes acid emulsions, such as fruit drinks and juices and also it stabilize beer foam (Abbot, 1996). Furthermore, alginates are able to interact with components in the food such as proteins fat or fibers (Draget *et al.*, 2005), increasing their industrial applications. For example, alginates have been used in meat industry in order to interact with amino acids in meat proteins (Draget *et al.*, 2005). However, some of these characteristics of native alginate can be altered by chemical modification of the free hydroxyl and carboxyl groups of alginate which distributed along the polymer chain backbone. Research carried out so far for chemical modification of alginate has been reviewed by Yang *et al.* (2011) and the favorable characters that have been achieved by these chemical modifications can be applied to the industrial development.

Kappa ( $\kappa$ ), lambda ( $\lambda$ ) and iota ( $\iota$ ) carrageenan are the main commercially utilized carrageenans; *Kappaphycus alvarezii* is considered to produce pure kappa (<10% iota) and iota carrageenan is produced from *Eucheuma denticulatum* (<15% kappa) (Bixler *et al.*, 2001). These carrageenans have many industrial applications including the dairy industry. Kappa-2 carrageenans, which are also known as “kappa iota hybrids” or “weak gelling kappas”, are used in dairy applications (Bixler *et al.*, 2001). However, among the three types,  $\lambda$ -carrageenan has been widely applied in the dairy industry.  $\lambda$ -Carrageenan does not form gels and this non-gelling carrageenan reacts with certain proteins, especially caseins, and produces highly viscous solutions (Glicksman, 1987). Thus, carrageenans are used widely in the dairy industry as a viscosity enhancer in non-fat milk products and as a stabilizer for cocoa in chocolate drinks (Bixler *et al.*, 2001). Apart from usage in the dairy industry,  $\lambda$ -carrageenan has been also used as a stabilizer mainly in beverages, syrups, and sauces (Glicksman, 1987). On the other hand, the gelling portion of the carrageenan has been widely used in dessert gels, jellies, jams, pet foods, frozen desserts, etc. (Glicksman, 1987).

Even though the seaweed phycocolloid, agar is not as widely used as carrageenan and alginate in the food industry, some food applications such as usage in bakery products, canned meat and confectionary have been reported.

### 33.6.3 Edible food coatings

The environmental pollution associated with disposal of synthetic non-degradable food packaging materials led to the development of less hazardous edible or biodegradable food coatings. These edible coatings enhance the shelf life of the products by preventing water loss and acting as a barrier to gases and other volatile compounds. These food coatings are mostly prepared from hydrocolloids, lipids or proteins, which form a thin layer on the surface of the product. Among the seaweed hydrocolloids alginate and carrageenan are commonly used for edible coating preparation (González-Aguilar *et al.*, 2010).

In the production of food coatings for different types of foods such as fresh fruits and vegetables, meat and meat products and dairy products like cheese, the coating materials should possess adequate strength and elasticity. Calcium cross-linked alginate forms strong food coatings. However, they are relatively brittle and less water resistant (Campos *et al.*, 2010). On the other hand the degree of water resistance depends on the proportion of G blocks in the polymer chain, due to formations of calcium cross-links with the G blocks (Olivas and Barbosa-Cánovas, 2008). Considering the three types of commercial carrageenans of red algae, kappa, lambda and iota, the highest tensile strength have been recorded in kappa carrageenan (Seol *et al.*, 2009). Other than the commonly used alginate and carrageenan, agar has been used for preparation of coatings. Sousa *et al.* (2010) used agar extracted from the red seaweed *Gracilaria vermiculophylla* to produce a biodegradable agar film and an agar/glycerol solution as a food coating for fresh vegetables.

Furthermore, active food coatings can be prepared by incorporating antimicrobial and antioxidant substances, to prevent food spoilage. Seaweeds with potent antioxidant activity are summarized in Table 33.3. Some other seaweed extracts have also shown potent antimicrobial activity. For example, *Haligra* spp. exhibits potent antimicrobial activity against the food born pathogen *Staphylococcus aureus* (Devi *et al.*, 2008) and methanol extracts of *Himanthalia elongata* on food spoiling (*Pseudomonas aeruginosa* and *Enterococcus faecalis*) and food pathogenic (*Listeria monocytogenes* and *Salmonella abony*) microorganisms (Gupta *et al.*, 2010). Thus, these authors suggested that a combination of antimicrobial and antioxidant substances can be used as potent food preservation agents.

### 33.6.4 Other applications of phycocolloids

Apart from the food applications, seaweed phycocolloids have been widely used in many other industries. Since carrageenan and alginate have special biological properties such as viscosity enhancement, gel-forming ability, stabi-

lization of aqueous mixtures, dispersions, and emulsions, these compounds have been applied as thickening agents in the cosmeceutical industry. Furthermore, carrageenan has been used as a stabilizer in toothpaste both in gel and non-gel forms (Abbot, 1996). These phycocolloids have involved in the maintenance of proper texture of the cosmeceutical product, which is needed for the even distribution of active ingredients in the final product. Therefore, carrageenan and alginate have been identified as important substances in the cosmeceutical industry.

Alginate has been also used as an impression material in dental mold preparation. Recently, use of an extended storage alginate impression material which exhibited dimensional stability up to 100 h (Walker *et al.*, 2010).

The other industrially important phycocolloid agar consists of agarose and agarpectin, where agarose have the greatest gel formation ability (Glicksman, 1987). Agarose is produced by excessive separation and purification of agar (Radmer, 1996) and used extensively in gel electrophoresis in molecular biology. Furthermore, agar has been widely used in microbiological culture media preparation, which is important for medical diagnostic activities, food safety and quality regulatory activities.

## 33.7 Biomedical applications

Algal derived compounds have long been used in Chinese, Japanese, and Ayurvedic medicines (Fitton, 2003). These compounds have been used for the treatment of wounds, dental impression materials and as potential anticancer drugs at present.

### 33.7.1 Antioxidant activity

ROS-induced damage to cellular molecules including DNA, protein and lipids are associated with number of pathological conditions including atherosclerosis, arthritis, diabetes, pulmonary dysfunction, inflammatory disorders, and neurological disorders such as Alzheimer's disease (Kumar *et al.*, 2008).

Chronic exposure of the skin to ultraviolet radiation damages DNA in skin cells directly by producing photo-products or indirectly increasing level of ROS, which may resulted in various skin related abnormalities such as sunburns, photoaging and skin cancer (Hwang *et al.*, 2006; Coba *et al.*, 2009). Phenolic compounds present in algal thalli known to protect the alga from UV radiation (Roleda *et al.*, 2006). These compounds extracted from brown algae exert a highly protective effect against UV-B induced skin carcinogenesis (Hwang *et al.*, 2006). Thus, algal polyphenols can be used as chemopreventive agents for UV-B

induced skin carcinogenesis. Therefore, dietary intake of antioxidants may help to overcome some of these free radical induced diseases in the long run.

### 33.7.2 Antitumor and immunomodulatory activity

Cancer is still an unresolved problem for the human health worldwide. Currently used anticancer drugs are cytotoxic both to cancer as well as to healthy cells. Therefore, naturally derived, orally consumable anticancer drugs with minimal or no side effects and toxicities are of prior importance (Kim *et al.*, 2010). Different seaweeds and their isolates studied in anti-tumor studies are given in Table 33.4. Among them the use of fucoidan has been studied extensively.

Fucoidan is a sulfated polysaccharide found in brown seaweeds, which is known to exert anticancer activity. Fucoidan has shown to induced apoptosis in number of cancer cells including human lymphoma HS-Sultan cells (Aisa *et al.*, 2005), MCF 7 human breast cancer cells (Yamasaki-Miyamoto *et al.*, 2009), HT 29 (Kim *et al.*, 2010), and HCT 15 (Hyun *et al.*, 2009) human colon cancer cells, human leukemia U937 cells (Teruya *et al.*, 2007) and enzyme digested fucoidan extract on human leukemic HL60 cells (Matsuda *et al.*, 2010). A fucoidan isolated from *Undaria pinnatifida* showed antitumor activity against PC-3 (prostate cancer), HeLa (cervical cancer), A549 (alveolar carcinoma), and HepG2 (hepatocellular carcinoma) cells (Synytsya *et al.*, 2010).

Anticancer activity of fucoidan is influenced by degree of sulfation, substituting position of sulfate group and molecular weight (Teruya *et al.*, 2007; You *et al.*, 2010; Cho *et al.*, 2011), where oversulfated and low molecular weight fucoidan derivatives significantly increased the inhibition of

cell growth. Therefore, anticancer activity of fucoidans can be enhanced by lowering their molecular weight by hydrolysis (by copper acetate as described by You *et al.*, 2010) and production of a less compact confirmation to improve binding of sulphate groups (Cho *et al.*, 2011).

Efficacy of fucoidan-induced apoptosis varies among different types of cancer cells as well as depending on the dose. For example, the degree of response to fucoidan-induced apoptosis in HCT 116 cells is smaller compared to HT 29 human colon cancer cells at concentrations between 5–20 µg/ml *in vitro* (Kim *et al.*, 2010). However, Hyun *et al.* (2009) have found high concentrations of fucoidan (such as 100 µg/ml) induced apoptosis in HCT 15 cells. In the former study, the same concentration had no effect on the growth of FHC human normal colon epithelial cells, indicating non-toxicity of fucoidan as a potential anticancer drug. Moreover, anticancer drugs against human colon cancer are facilitated by the indigestible nature of seaweed sulfated polysaccharides in the human digestive tract. The increased concentrations of indigested polysaccharides may beneficially exert their anticancer activity in the colon.

Other than polysaccharides, polyphenolic compounds and MAAs also have potential applications, especially against UV-B induced skin carcinogenesis. According to the findings of Hwang *et al.* (2006), dietary feeding and topical application of polyphenols from brown algae significantly reduced UV-B-induced skin carcinogenesis on a SKH-1 hairless mouse skin model. Dietary feeding of 0.5% and topical application 3 mg of polyphenols significantly reduced tumor multiplicity by 56% and 60% and tumor volume by 65% and 66%, respectively, per tumor-bearing mouse. MAAs isolated from *Palmaria palmata* also inhibited B16-F1 murine skin melanoma cell proliferation up to 92% (Yuan *et al.*, 2009). However, Yuan *et al.* (2009) have

**Table 33.4** Some seaweeds and their extracts used in antitumor studies

Class	Seaweed	Extract	Reference
Red	<i>Chondrus ocellatus</i>	Low molecular weight $\lambda$ -carrageenan	Zhou <i>et al.</i> , 2005
Red	<i>Kappaphycus striatum</i>	$\kappa$ -carrageenan	Yuan <i>et al.</i> , 2006
Red	<i>Champia feldmannii</i>	Sulfated polysaccharide	Lins <i>et al.</i> , 2008
Green	<i>Monostroma nitidum</i>	Water-soluble sulfated polysaccharide	Karnjanapratum and You, 2011
Brown	<i>Colpomenia sinuosa</i>	Ethyl acetate extracts	Huang <i>et al.</i> , 2005
Green	<i>Halimeda discoidae</i>		
Red	<i>Galaxaura oblongata</i>		
Brown	<i>Hydroclathrus clathratus</i>	Sulfated polysaccharide (sulfur content 8.2%)	Wang <i>et al.</i> , 2010
Red	<i>Gracilaria corticata</i>	Crude extract	Zandi <i>et al.</i> , 2010
Brown	<i>Cladosiphon okamuranus</i>	Oversulfated fucoidan	Teruya <i>et al.</i> , 2007
Brown	<i>Undaria pinnatifida</i>	Fucoidans	You <i>et al.</i> , 2010; Synytsya <i>et al.</i> , 2010
Brown	<i>Cladosiphon novae-caledoniae</i>	Enzyme digested fucoidan	Matsuda <i>et al.</i> , 2010

suggested that studies with purified MAA and combinations may require studying the synergistic effects. Therefore, these compounds are potential compounds as antiproliferative against UV induced skin cell proliferation.

### 33.7.3 Anti-inflammatory activity

Fucoidans and polyphloroglucinols are two major seaweed extracts associated with anti-inflammatory activity.

In osteoarthritis, progressive deterioration of articular cartilage results in pain, stiffness, and difficulty with physical activities. These overall symptoms of osteoarthritis have been significantly reduced by 52% in response to a 1000 mg dose of a seaweed extract from brown algae including zinc, manganese and vitamin B6 using an open label design (Myers *et al.*, 2010).

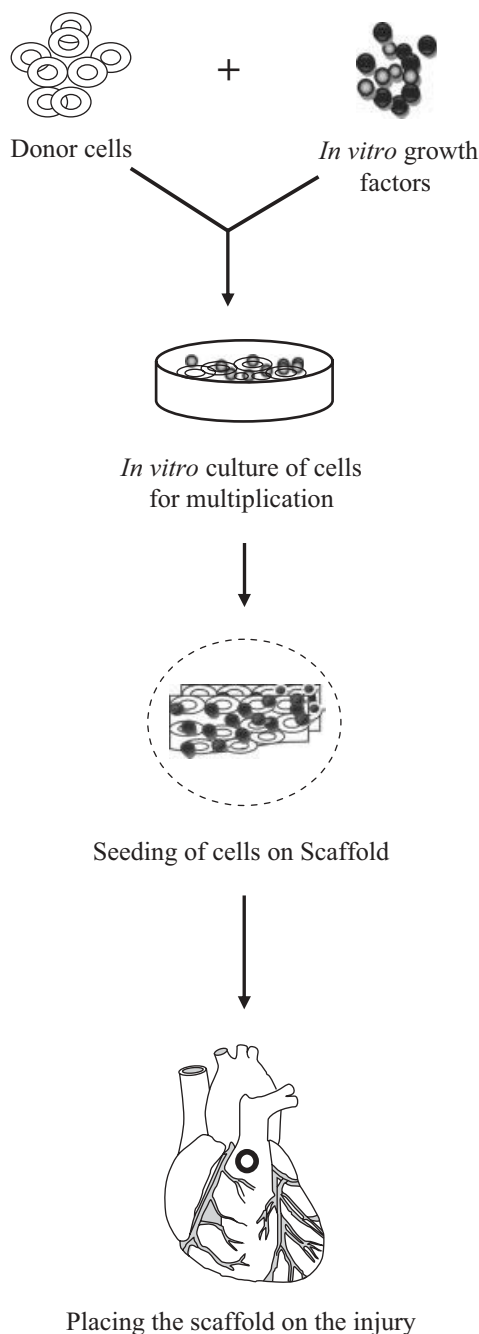
### 33.7.4 Anticoagulant activity

At present heparin is extracted from mucosal tissue of slaughter meat animals from porcine intestine and from bovine lung and, therefore, availability of heparin is limited. Seaweed-derived compounds that have shown anticoagulant activity can be utilized as alternative sources and to overcome the side effects associated with existing heparin.

A highly sulfated (21.76% as  $\text{SO}_4^{2-}$ ) proteoglycan mainly consists of galactose and a small amount of glucose with 9.42% of protein has been purified from red algae *Lomentaria catenata* by Pushpamali *et al.* (2008), which has showed higher anticoagulant activity than heparin in activated partial thromboplastin time and thrombin time assays showing anticoagulation possibilities through intrinsic and common pathways of blood coagulation. Furthermore, Shanmugan and Mody (2000) reported that carrageenans with higher molecular weight and higher sulfur content showed better anticoagulant activity than those with low molecular weight and low sulfur content.

### 33.7.5 Applications in tissue engineering

Tissue repair and regeneration technology, and the development of tissue engineering, has emerged as a solution for the limited availability of tissue and organ transplants. Tissue regeneration is aided by a scaffold, which is used as the substrate for tissue adhesion and proliferation. The scaffold is a porous, degradable material, which provides volume and mechanical strength to the damaged tissue being repaired. The red seaweed extract alginate has been used in wound healing treatments, where hydrogel-regenerated tissues resulted in less marks after healing (Barnett, 1987).



**Figure 33.8** Use of the scaffold in tissue repair.

At present, alginate hydrogels are widely used as scaffolds for tissue regeneration (Figure 33.8).

Alginate hydrogels have the ability to form polymer networks in the presence of calcium, by the formation of calcium ion cross-links. Calcium ion cross-linked sodium alginate gel has been used as a biodegradable scaffold in

cartilage tissue engineering by Wang *et al.* (2003) on rat bone marrow cells. Bone marrow cell proliferation was faster and increased steadily with time on G-rich alginate gels than on M-rich gels. This cell proliferation on G-rich alginate gels may relate to cell adhesion and subsequent colonization on the alginate scaffold. Furthermore, Ueyama *et al.* (2002) found that alginate membrane derived from 3% calcium chloride and 1% sodium alginate functions well as a GBR membrane.

Besides, the scaffolds should have adequate porosity and strength to ensure both tissue regeneration and subsequent degradation (Hollister, 2005). Furthermore, Wang *et al.* (2003) reported that highly purified G-rich alginate scaffolds retained its initial strength by 27% for 12 days in culture.

### 33.8 Macroalgal-derived cosmeceuticals

Antitumor therapy, as well as the prevention of tumor development, has a similar importance in reducing the incidence of cancers among the human population. Thus, the greatest attention of the scientists has been focused on the elimination of possible carcinogenetic factors such as UV radiation. Both UV-A and UV-B radiation have detrimental effects on human skin, leading to photoaging of skin and, more seriously, development of skin cancers. Therefore, the minimization of skin contact with UV radiation is favorable for the prevention of UV-induced harmful effects on human skin. In this regard, certain macroalgal-derived substances such as MAAs have a wide role, as these compounds are able to absorb wide range of UV radiation including the both UV-A and UV-B radiation (Coba *et al.*, 2009; Yuan *et al.*, 2009). Hence the importance of having these natural UV-absorbing compounds in everyday use cosmeceutical products has received the attention of the cosmeceutical industry.

Among the different MAAs, porphyra-334 and shinorine are the most abundant MAAs found in macroalgae; especially in the marine red algae of the genus *Porphyra*. The ratio of the porphyra-334 and shinorine in the extract of *Porphyra umbilicalis* is 2:1, with major contribution from porphyra-334. The pH, temperature, and the polarity of the porphyra-334 solvents have an influence on the UV absorption property of this molecule (Zhang *et al.*, 2005a,b). Furthermore, porphyra-334 molecules are highly heat stable. Sinha *et al.* (2000) have observed unaffected absorption property of porphyra-334 and shinorine under heat treatment at 75 °C for about 6 h. Since the absorbed UV radiation is excreted as thermal energy, the heat stability of porphyra-334 is a greater advantage for them to be applied as cosmeceuticals. However, the efficacy of the

porphyra-334- and shinorine-incorporated cosmeceuticals have been studied by Schmid *et al.* (2004), and these authors have found efficient neutralization of UV-A effect from the cream produced by liposome encapsulated with 0.005% MAA, which was similar to the effects of cream with 1% synthetic UV-A filters and 4% UV-B filters. Therefore, these macroalgal-derived MAAs have the potential to be applied as sunscreens cosmeceuticals, which helps to prevent the premature photoaging of skin as well as human skin cancers.

Apart from the usage of macroalgal MAAs as sunscreens cosmeceuticals, these are also important as antioxidant compounds in the cosmeceutical preparations. Furthermore, algal-derived carotenoids (such as  $\beta$ -carotene, astaxanthin and fucoxanthin), polyphenols, and fucoidans have a greater importance as cosmeceutical antioxidant compounds, which are involved in the neutralization of UV-induced ROS such as superoxide anion and hydrogen peroxide produced in the skin. However, these compounds have different mechanisms in the neutralization of ROS. Usually carotenoids exert their antioxidant activity by absorbing the excited energy of singlet oxygen on to their chains (Guerin *et al.*, 2003) whereas the electron-rich polyphenols act as electron donors to the free radicals such as superoxide anion and hydrogen peroxide produced in the skin (Ahn *et al.*, 2007). High antioxidant capacity has been reported from the carotenoids extracted from different algal species including *Sargassum dentifolium*, *Laurencia papillosa*, *Jania corniculata*, *S. polycystum* and *L. obtuse* (Anggadiredja *et al.*, 1997; Shanab, 2007) and phenolic compounds extracted from many macroalgae (Zubia *et al.*, 2007; Matanjan *et al.*, 2008; Ye *et al.*, 2009; Cho *et al.*, 2010). Furthermore, high superoxide radical scavenging activity, which is important for the prevention of photodamage to the skin, has been reported from fucoidans extracted from certain brown and red algae, including *Laminaria japonica* and *Fucus vesiculosus* (So *et al.*, 2007; Souza *et al.*, 2007; Wang *et al.*, 2008). However, the mode of action of fucoidans that is involved in their antioxidant activity is unclear due to the complexity in their structure.

Apart from their antioxidant activity, fucoidans are important algal-derived matrix metalloproteinase (MMP) inhibitors, which have a high potential to be applied as antiaging cosmeceuticals. MMPs found in the fibroblasts and keratinocytes of skin are matrix-degrading enzyme systems, which get stimulated by the excess production of ROS in the skin. These enzymes ultimately cause the destruction of collagen in the skin resulting in premature photoaging of skin. Disturbing this photoaging pathway, fucoidans inhibit the MMP 1 enzyme by preventing the expression of mRNA promoter activity (Moon *et al.*, 2008a, b, 2009) or by preventing the expression of MMP-1 by inhibiting

**Table 33.5** Plant growth regulators extracted from macroalgae

Class	Name	Growth regulator	Reference
Brown	<i>Kappaphychus alvarezii</i>	IAA Gibberellins GA <sub>3</sub> Kinetin Zeatin	Prasad <i>et al.</i> , 2010
Red	<i>Prionitis lanceolata</i>	3-(Hydroxylacetyl) indole	Bernart and Gerwick, 1990
Red	<i>Porphyra perforate</i>	IAA	Zhang <i>et al.</i> , 1993
Brown	<i>Ascophyllum nodosum</i>	ABA	Boyer and Dougherty, 1988
Brown	<i>Ascophyllum nodosum</i> <i>Fucus vesiculosus</i>	IAA	Buggeln and Craigie, 1971
Brown	<i>Laminaria digitata</i> <i>Ascophyllum nodosum</i>	Similar to ABA	Hussain and Boney, 1973
Brown	<i>Sargassum heterophyllum</i>	Cytokinins	Mooney and van Staden, 1984
Green	<i>Ulva intestinalis</i>	Ethene (ethylene)	Plettner <i>et al.</i> , 2005

ABA, abscissic acid; IAA, indole-3-acetic acid.

the extracellular signal regulated kinase (ERK) pathway in a dose-dependent manner (Moon *et al.*, 2008a; Lee and Ku, 2010), thereby preventing the destruction of structural collagen in the skin. Moreover, fucoidan treatments have increased type I procollagen synthesis, which improves the health status of the skin (Moon *et al.*, 2009).

Therefore, these algal-derived fucoidans have multiple roles such as antioxidant agents, MMP inhibitors and procollagen synthesis enhancers in the prevention of UV-induced photoaging of skin, increasing the potential application of fucoidans as antiphotaging cosmeceuticals. Furthermore, certain other substances such as carotenoids, MAAs and polyphenols, which are produced as secondary metabolites in macroalgae, can be applied as UV-absorbing agents and antioxidant agents in the cosmeceutical industry.

### 33.9 Applications in agriculture

Seaweed extract applications on crops have exhibited beneficial effects, such as early seed germination and establishment, improved yield, increased resistance to biotic and abiotic stress, and enhanced postharvest shelf life of perishable products (Khan, 2009).

Seaweeds have also been added to the soil as fertilizer and soil amendments to improve physical and biological properties of soil, especially in coastal regions, where the soil is sandy (Haslam and Hopkins, 1996). Addition of seaweeds to soil has increased the water-holding capacity by increasing the total pore volume (Haslam and Hopkins,

1996). Increased availability of soil organic matter also beneficially affects the growth of soil bacteria and fungi, which aid in soil aggregate formation and thereby increase the soil porosity. Increased soil aggregate formation and its stability may be influenced not only by the exudates of soil microbes but also due to the binding effects of algal-soluble carbohydrates.

Moreover, considering the mineral composition of the seaweeds they can be effectively utilized to fulfill the macro- and micronutrient requirements of the plants. Washed up seaweeds, residues of phycocolloid extraction industries, and algal biochar can be effectively used as a soil ameliorant and fertilizer to increase crop productivity (Bird *et al.*, 2011).

Beneficial effects of seaweeds extend even toward plant growth regulation, by the presence of plant growth regulatory substances in seaweeds. Several studies on identification of these biostimulants are given in Table 33.5. At present seaweeds are used in commercial products as plant growth stimulants, and as biofertilizers in agriculture and horticultural industries.

Furthermore, antioxidant properties of seaweeds can be used to overcome soil biotic and abiotic stress. Root treatment of spinach with commercial extracts of brown algae *Ascophyllum nodosum* significantly increased the total phenolic and flavanoid content and total antioxidant activity (Fan *et al.*, 2011), which may enhance the shelf life. This exogenous application of seaweed extracts is also known to increase endogenous antioxidant enzymatic activities including ascorbate peroxidase, glutathione reductase and superoxide dismutase (Allen *et al.*, 2001).

### 33.10 Applications in pollution detection and control

Seaweeds have been used as biomonitors or pollution detectors in different studies (Muse *et al.*, 1999; Filho *et al.*, 1999; Karez *et al.*, 1994), due to their ability to assimilate nutrients, especially nitrogenous and phosphorus waste and other elements such as heavy metals (Bird *et al.*, 2010). Using this ability of seaweeds they can be utilized for waste water treatment plants and integrated aquacultural systems (Rodrigueza and Montano, 2007).

Algal biomass itself is able to chelate or absorb metal ions, where high molecular weight, non-dialyzable polyphloroglucinols from marine brown algae *Ascophyllum nodosum* and *Fucus vesiculosus* chelate the metal ions  $\text{Sr}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Be}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$  in weakly acidic aqueous solutions (Ragan *et al.*, 1979). Addition of 100–200  $\mu\text{g}$  of polyphenols extracted from the same above brown algae had reduced the toxicity of  $\text{Zn}^{2+}$  (0.5–2.0 mg) against the growth of several species of marine phytoplankton as well (Ragan *et al.*, 1980).

Metal biosorption capacity of seaweed polysaccharides have observed in several studies. In brown alga *Sargassum* cell wall alginate is the main component responsible for metal absorption (Fourest and Volesky, 1995). Modification of alginate by methanolic hydrochloride or propylene oxide demonstrated an uptake of Ca and Pb. At low pH, sulfonate groups also contributed to heavy metal binding at a lower extent, compared to alginate. Binding of Cd to carboxyl groups of alginate arises by bridging or bidentate complex formation.

### 33.11 Utilization of macroalgae for energy production

The energy crisis has led to finding alternative, renewable resources in place of fossil fuels for sustainable development. Hence, production of biofuel from plants is of major concern, where global liquid biofuel production in the year 2007 accounts for about 16 billion gallons (Jegannathan *et al.*, 2009). However, utilization of terrestrial energy crops or any other terrestrial plants has brought controversy against ethical and sustainability issues. Thus, use of both micro- and macroalgal biomass is of great interest due to their higher availability and growing potentials. However, microalgal biomass, as a potential source for energy, has been studied extensively, with recent studies of macroalgae as potential sources for biofuel, bioethanol, and biochar production (Table 33.6).

Considering the biofuel properties of macroalgae, Ross *et al.* (2008) stated that higher sodium, potassium and alkali content present in seaweeds, especially brown algae may create severe problems in combustion or gasification, encountering problems in component failure. Thus, these authors recommended that further washing may be needed to reduce the alkali levels. Furthermore, considering the widely used methods of biodiesel extraction, thermochemical liquefaction has been identified as more efficient than with supercritical  $\text{CO}_2$  extraction from macroalgae considering the yield (Aresta *et al.*, 2005). However, decomposition of fatty acids under the former extraction method may result. Moreover, the authors pointed out that biodiesel can be used in the existing diesel engines

**Table 33.6** Some seaweeds utilized for bioenergy production

Purpose	Class	Seaweeds	Reference
Biofuel	Brown	<i>Fucus vesiculosus</i> <i>Fucus serratus</i> <i>Chorda filum</i> <i>Laminaria digitata</i> <i>Laminaria hyperborea</i> <i>Macrocystis pyrifera</i>	Ross <i>et al.</i> , 2008
Biodiesel	Green	<i>Chaetomorpha linum</i>	Aresta <i>et al.</i> , 2005
Bioethanol	Brown	<i>Laminaria hyperborea</i>	Horn <i>et al.</i> , 2000
Biochar	Green	<i>Cladophora coelothrix</i> <i>Cladophora vagabunda</i> <i>Cladophora patentiramea</i> <i>Chaetomorpha indica</i> <i>Chaetomorpha linum</i> <i>Cladophoropsis sp.</i> <i>Ulva flexuosa</i>	Bird <i>et al.</i> , 2011

without modification, differing that from the hydrogen energy sources.

### 33.12 Conclusions

Owing to the unique physicochemical properties of seaweed-derived compounds, there are enormous potential applications in the fields of food, pharmaceutical, and agriculture. Consumption of seaweeds is associated with numerous health benefits ranging from nutritional perspective to functional foods. Their probable uses in biomedical applications have led to the development of new potential drugs. There are ways in which seaweeds can help to detect environmental pollution and to control it. The residues of the seaweed-processing industries can be used to amend the agricultural lands to enhance fertility. The vast potential uses of seaweeds are still being discovered, showing that threats to the human wellbeing can be solved from the same place that life originated.

### References

- Abbott, I.A. (1996) Ethnobotany of seaweeds: clues to uses of seaweeds. *Hydrobiologia*, **326/327**, 15–20.
- Ahn, G., Kim, K., Cha, S. et al. (2007) Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H<sub>2</sub>O<sub>2</sub>-mediated DNA damage. *Eur. Food Res. Technol.*, **226**, 71–79.
- Aisa, Y., Miyakawa Y., Nakazato, T. et al. (2005) Fucoidan induces apoptosis of human HS-sultan cells accompanied by activation of caspase-3 and down-regulation of ERK pathways. *Am. J. Hematol.*, **78**(1), 7–14.
- Allen, V.G., Pond, K.R., Saker, K.E. et al. (2001) Tasco-Forage: III. Influence of a seaweed extract on performance, monocyte immune cell response, and carcass characteristics in feedlot-finished steers. *J. Anim. Sci.*, **79**, 1032–1040.
- Anggadiredja, J., Andyani, R., Hayati, et al. (1997) Antioxidant activity of *Sargassum polycystum* (Phaeophyta) and *Laurencia obtuse* (Rhodophyta) from Seribu islands. *J. Appl. Phycol.*, **9**, 477–479.
- Aresta, M., Dibenedetto, A., Carone, M. et al. (2005) Production of biodiesel from macroalgae by supercritical CO<sub>2</sub> extraction and thermochemical liquefaction. *Env. Chem. Lett.*, **3**, 136–139.
- Armisen, R., Galatas, F. (1987) Production, properties and uses of agar. In: *Production and utilization of products from commercial seaweeds* (ed. D.J. McHugh). FAO Fisheries Technical Paper nr.288. Food and Agriculture Organization of the United Nations, Geneva, pp. 1–57
- Barnett, S.E. (1987) The effects of calcium alginate on wound healing. *Ann. Roy. Coll. Surg. Engl.*, **69**, 153–155.
- Bernart, M. and Gerwick, W.H. (1990) 3-(Hydroxyacetyl)indole, a plant growth regulator from the Oregon red alga *Prionitis lanceolata*. *Phytochemistry*, **29**(12), 3697–3698.
- Besada, V., Andrade, J.M. and Schultze, F. (2009) Heavy metals in edible seaweeds commercialized for human consumption. *J. Mar. Syst.*, **75**, 305–313.
- Bird, M.I., Wurster, C.M. and de Paula Silva, P.H. (2011) Algal biochar – production and properties. *Biores. Technol.*, **102**(2), 1886–1891.
- Bixler, H.J., Johndro, K. and Falshaw, R. (2001) Kappa-2 carrageenan: structure and performance of commercial extracts II. Performance in two simulated dairy applications. *Food Hydrocolloids*, **15**, 619–630.
- Bocanegra, A., Bastida, S., Benedí, J. et al. (2009) Characteristics and nutritional and cardiovascular-health properties of seaweeds. *J. Med. Food*, **12**(2), 236–258.
- Boyer, G.L. and Dougherty, S.S. (1980) Identification of the abscisic acid in the seaweed *Ascophyllum nodosum*. *Phytochemistry*, **27**(5), 1521–1522.
- Buggeln, R.G. and Craigie, J.S. (1971) Evaluation of evidence for the presence of indole-3-acetic acid in marine algae. *Planta*, **97**, 173–178.
- Campos, C.A., Gerschenson, L.N. and Flores, S.K. (2010) Development of edible films and coatings with antimicrobial activity. *Food Bioproc. Technol.*, doi:10.1007/s11947-010-0434-1
- Coba, F., Aguilera, J., Figueroa, F.L. et al. (2009) Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen. *J. Appl. Phycol.*, **21**, 161–169.
- Chizhov, A.O., Dell, A., Morris, R. et al. (1998) Structural analysis of laminarans by MALDI and FAB mass spectrometry. *Carbohydr. Res.*, **310**, 203–210.
- Cho, M. L., Kang, I., Won, M. H. et al. (2010) The antioxidant properties of ethanol extracts and their solvent-partitioned fractions from various green sea weeds. *J. Med. Food*, **13**, 1232–1239.
- Cho, M. L., Lee, B. and You, S. (2011) Relationship between oversulfation and conformation of low and high molecular weight fucoidans and evaluation of their in vitro anticancer activity. *Molecules*, **16**, 291–297.
- Colombo, M.L., Rise, P., Giavarini, F. et al. (2006) Marine macroalgae as sources of polyunsaturated fatty acids. *Plants Foods Human Nutrition*, **61**, 67–72.
- Costa, L.S., Fidelis, G.P., Cordeiro, S.L. et al. (2010) Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomed. Pharmacother.*, **64**, 21–28.

- Craigie, J. S. (2010) Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.*, doi 10.1007/s10811-010-9560-4.
- Davis, T.A., Volesky, B., Mucci, A. (2003) A review of the biochemistry of heavy metal absorption by brown algae. *Wat. Res.*, **37**, 4311–4330.
- Dawczynski, C., Schubert, R. and Jahreis, G. (2007) Amino acids, fatty acids, and dietary fiber in edible seaweed products. *Food Chem.*, **103**, 891–899.
- Devi, K.P., Suganthi, N. and Kesika, P. (2008) Bioprotective properties of seaweeds: In vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. *BMC Compl. Altern. Med.*, **8**(38), 1472–6882.
- Draget, K. I., Smidsrød, O., Skjåk-Bræk, G. (2005) Alginates from algae. In: *Polysaccharides and Polyamides in the Food Industry: properties, production and patents* (eds A. Steinbuchel and S.K. Rhee). Wiley-VCH, Weinheim, Germany, pp. 2–24.
- Elleuch, M., Bedigian, D., Roiseux, O. *et al.* (2011) Dietary fiber and fiber-rich by-products of food processing: characterization, technological functionality and commercial applications: a review. *Food Chem.*, **124**, 411–421.
- Fan, D., Hodges, D.M., Zhang, J. *et al.* (2011) Commercial extract of the brown seaweed *Ascophyllum nodosum* enhances phenolic antioxidant content of spinach (*Spinacia oleracea* L.) which protects *Caenorhabditis elegans* against oxidative and thermal stress. *Food Chem.*, **124**, 195–202.
- Filho, G.M.A., Andrade, L.R., Karez, C.S., *et al.* (1999) Brown algae species as biomonitors of Zn and Cd at Sepetiba Bay, Rio de Janeiro, Brazil. *Mar. Env. Res.*, **48**(3), 213–224.
- Fitton, J.H. (2003) Brown marine algae: a survey of therapeutic potentials. *Altern. Complem. Ther.*, **9**(1), 39–41.
- Fleurence, J. (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.*, **10**, 25–28.
- Fourest, E. and Volesky, B. (1995) Contribution of sulfonate groups and alginate to heavy metal biosorption by the dry biomass of *Sargassum fluitans*. *Env. Sci. Technol.* **30**, 277–282.
- Glicksman, M. (1987) Utilization of seaweed hydrocolloids in the food industry. *Hydrobiologia*, **151/152**, 31–47.
- Goecke, F., Hernández, V., Bittner, M. *et al.* (2010) Fatty acid composition of three species of *Codium* (Bryopsidales, Chlorophyta) in Chile. *Revista de Biología Marina y Oceanografía*, **45**(2), 325–330.
- Gómez-Ordóñez, E., Jiménez-Escrig, A. and Rupérez, P. (2010) Dietary fiber and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. *Food Res. Int.*, **43**, 2289–2294.
- González-Aguilar, G.A., Ayala-Zavala, J.F., Olivas, G.I. *et al.* (2010) Preserving quality of fresh-cut products using safe technologies. *J. Consum. Protect. Food Safety*, **5**, 65–72.
- Guerin, M., Huntley, M. L., and Olaizola, M. 2003. *Haematococcus astaxanthin*: applications for human health and nutrition. *Trends Biotechnol.*, **21**: 210–216.
- Gupta, S., Cox, S., Rajauria, G. *et al.* (2010) Growth inhibition of common food spoilage and pathogenic microorganisms in the presence of brown algal seaweed extracts. *Food Bioproc. Technol.*, doi:10.1007/s11947-010-0502-6.
- Hall, A.C., Fairclough, A.C., Mahadevan, K. *et al.* (2010) Seaweeds (*Ascophyllum nodosum*) enriched bread is acceptable to consumers. *Proc. Nutr. Soc., Nutr.*, 16–18.
- Haslam, S.F.I. and Hopkins, D.W. (1996) Physical and biological effects of kelp (seaweed) added to soil. *Appl. Soil Ecol.*, **3**, 257–261.
- He, Y.H. and Shahidi, F. (1997) Antioxidant activity of green tea and its catechins in a fish meat model system. *J. Agric. Food Chem.*, **45**(11), 4262–4266.
- Herber, S.M. and van Elswyk, M.E. (1996). Dietary marine algae promotes efficient deposition of n-3 fatty acids for the production of enriched shell eggs. *Poultry Sci.*, **75**, 1501–1507.
- Hollister, S. J. (2005) Porous scaffold design for tissue engineering. *Nat. Mater.*, **4**, 518–590.
- Horn, S.J., Aasen, I.M., Ostgaard, K. (2000) Ethanol production from seaweed extract. *J. Indust. Microbiol. Biotechnol.*, **25**, 249–254.
- Huang, H., Wu, S., Liao, H. *et al.* (2005). Induction of apoptosis by three marine algae through generation of reactive oxygen species in human leukemic cell lines. *J. Agric. Food Chem.*, **53**(5), 1776–1781.
- Hussain, A. and Boney, A.D. (1973) Hydrophilic growth inhibitors from *Laminaria* and *Ascophyllum*. *New Phytol.*, **72**, 403–410.
- Hwang, H., Chen, T., Nines, R.G. *et al.* (2006) Photochemoprevention of UVB-induced skin carcinogenesis in SKH-1 mice by brown algae polyphenols. *Int. J. Cancer*, **110**, 2742–2749.
- Hyun J.H., Kim S.C., Kang J.I., *et al.* (2009) Apoptosis inducing activity of fucoidan in HCT-15 Colon carcinoma cells. *Biol. Pharm. Bull.*, **32**(10), 1760–1764.
- Jegannathan, K.R., Chan, E. and Ravindra, P. (2009) Harnessing biofuels: a global renaissance in energy production? *Renewable and Sustainable Energy Reviews*, **13**(8), 2163–2168.
- Jensen, A. (1993) Present and future needs for algae and algal products. *Hydrobiologia*, **260/261**, 15–23.
- Jiménez-Escrig, A. and Sánchez-Muniz, F.J. (2000) Dietary fiber from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res.*, **4**, 585–598.

- Jimenez-Escrig, A., Jiménez-Jiménez, I., Pulido, R. *et al.* (2001) Antioxidant activity of fresh and processes edible seaweeds. *J. Sci. Food Agric.*, **81**, 530–534.
- Karez, C.S., Magalhaes, V.F., Pfeiffer, W.C. *et al.* (1994) Trace metal accumulation by algae in Sepetiba Bay, Brazil. *Env. Poll.*, **83**(3), 351–356.
- Karnajanapratum, S. and You, S. (2011). Molecular characteristics of sulfated polysaccharides from *Monostroma nitidum* and their in vitro anticancer and immunomodulatory activities. *Int. J. Molec. Macromol.*, doi:10.1016/j.ijbiomac.2010.12.002.
- Kermanshahi, R.S., Fooladi, J. and Peymanfar, S. (2010) Isolation and microencapsulation of *Lactobacillus* spp. from corn silage for probiotic application. *Iranian J. Microbiol.*, **2**(2), 98–102.
- Keyrouz, R., Abasq, M.L., Bourvellec, C.L. *et al.* (2011) Total phenolic contents, radical scavenging and cyclic voltammetry of seaweeds from Brittany. *Food Chem.*, **126**, 831–836.
- Khan, W., Rayirath, U. P., Subramanian, S. *et al.* (2009) Seaweeds extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.*, **28**, 386–399.
- Khotimchenko, S.V., Vaskovskv, V.E. and Titlvanova, T.V. (2002) Fatty acids of marine algae from the Pacific coast of North California. *Bot. Mar.*, **45**, 17–22.
- Kim, E. J., Park, S.Y., Lee, J.Y. *et al.* (2010) Fucoidan present in brown algae induces apoptosis of human colon cancer cells. *BMC Gastroenterol.*, **10**, 96.
- Kumar, K.S., Ganesan, K. and Rao, P.V.S. (2008) Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty – An edible seaweed. *Food Chem.*, **107**, 289–295.
- Kumari, P., Kumar, M., Gupta, V. *et al.* (2010) Tropical marine microalgae as potential sources of nutritionally important PUFAs. *Food Chem.*, **120**, 749–757.
- Lee, Y. H. and Ku, M. J. (2010) Effect of *Fucus evanescens* fucoidan on expression of matrix mettaloproteinase-1 promoter, mRNA, protein and signal pathway. *FASEB J. (Meeting Abstract Supplement)*, **484**, 3.
- Li, B., Lu, F., Wei, X., *et al.* (2008) Fucoidan: Structure and bioactivity. *Molecules*, **13**, 1671–1675.
- Lim, S.N., Cheung, P.C.K., Ooi, V.E.C. *et al.* (2002) Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum*. *J. Agric. Food Chem.*, **50**, 3862–3866.
- Lins, K.O.A.L., Bezerra, D. P., Alves, A. P. N.N., *et al.* (2008) Antitumor properties of a sulfated polysaccharide from the red seaweed *Champia feldmannii* (Diaz-Pifferer). *J. Appl. Toxicol.*, **29**, 20–26.
- Machado, D.I.S., Cervantes, J.L., Hernández, J.L. *et al.* (2004) Fatty acids, total lipid and ash contents of processed edible seaweeds. *Food Chem.*, **85**, 439–444.
- Mamatha, B.S., Namitha, K.K., Senthil, A., *et al.* (2007) Studies on use of *Enteromorpha* in snack food. *Food Chem.*, **101**, 1707–1713.
- Matanjun, P., Mohamed, S., Mustapha, N.M. *et al.* (2008) Antioxidant activities and phenolics content of eight species of seaweeds from North Borneo. *J.Appl. Phycol.*, **20**, 367–373.
- Matsuda, Y., Teruya, K., Matsuda, S. *et al.* (2010) Anti-cancer effects of enzyme digested fucoidan extract from seaweed Mozuku. *Anim. Cell Technol.: Basic Appl. Aspects*, **16**, 295–300.
- Moon, H.J., Lee, S.R., Shim, S.N. *et al.* (2008a) Fucoidan inhibits UVB-induced MMP-1 expression in human skin fibroblasts. *Biol. Pharm. Bull.*, **31**, 284–289.
- Moon, H.J., Shim, S.N., Ku, M.J. *et al.* (2008b) Effect of fucoidan on mRNA and promoter activity of matrix metalloproteinases (MMPs) in skin fibroblasts. *J. Fed. Am. Soc. Exp. Biol.*, **31**, 284–289.
- Moon, H.J., Lee, S.H., Ku, M. J. *et al.* (2009) Fucoidan inhibits UVB-induced MMP-1 promoter expression and down regulation of type I procollagen synthesis in human skin fibroblasts. *Eur. J. Dermatol.*, **19**, 129–134.
- Mooney, P.A. and van Staden, J. (1984) Seasonal changes in the levels of endogenous cytokinins in *Sargassum heterophyllum* (Phaeophyceae). *Bot. Mar.*, **27**(9), 437–442.
- Munda, I.M. (1977) Differences in amino acid composition of estuarine and marine fucoids. *Aquat. Bot.*, **3**, 273–280.
- Muse, J.O., Stripeikis, J.D., Fernández, F.M. *et al.* (1999) Seaweeds in the assessment of heavy metal pollution in the Gulf San Jorge, Argentina. *Env. Poll.*, **104**(2), 315–322.
- Myers, S.P., O'Connor, J., Fitton, J.H. *et al.* (2010) A combined phase I and II open label study on the effects of a seaweed extract nutrient complex on osteoarthritis. *Biologics: Targets and Therapy*, **4**, 33–44.
- Muthukumarasamy, P., Allan-Wajtas, P. and Holley, R.A. (2006) Stability of *Lactobacillus reuteri* in different types of microcapsules. *J. Food Sci.* **71**(1): M20–M24.
- Ngo, D., Wijesekara, I., Vo, T. *et al.* (2011) Marine food-derived functional ingredients as potential antioxidants in the food industry: An overview. *Food Res. Int.*, doi:10.1016/j.foodres.2010.12.030.
- Norziah, M. and Ching, C.Y. (2000) Nutritional compositional of edible seaweeds *Gracilaria changgi*. *Food Chem.*, **68**, 69–76.
- Olivas, G.I. and Barbosa-Cánovas, G.V. (2008) Alginate-calcium films: water vapour permeability and mechanical properties as affected by plasticizer and relative humidity. *Lebensmittel-Wissenschaft und Technologie*, **41**, 359–366.
- Oliveira, M.N.D., Freitas, A.L.P., Carvalho, A.F.U. *et al.* (2009) Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. *Food Chem.*, **115**, 254–259.

- Ortiz, J., Romero, N., Robert, P. *et al.* (2006) Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chem.*, **99**, 98–104.
- O'Sullivan, L., Murphy, B., McLoughlin, P. *et al.* (2010) Prebiotics from marine macroalgae for human and animal health applications. *Marine Drugs*, **8**, 2038–2064.
- O'Sullivan, A.M., O'Callaghan, Y.C., O'Grady, M.N. *et al.* (2011) In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland. *Food Chem.*, **126**, 1064–1070.
- Prabhasanker, P., Ganeshan, P., Bhaskar, N. *et al.* (2009) Edible Japanese seaweed, Wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chem.*, **115**, 501–508.
- Prasad, K., Das, A.K., Oza, M.D. *et al.* (2010) Detection and quantification of some plant growth regulators in a seaweed-based foliar spray employing a mass spectrometric technique and chromatographic separation. *J. Agric. Food Chem.*, **58**(8), 4594–4601.
- Plettner, I., Steinke, M. and Malin, G. (2005) Ethene (ethylene) production in the marine macroalgae *Ulva* (*Enteromorpha*) *intestinalis* L. (*Chlorophyta*, *Ulvo-phyceae*): effect of light stress and co-production with dimethyl sulphide. *Plant, Cell and Environment*, **28**, 1136–1145.
- Pushpamali, W. A., Nikapitiya, C., Zoysa, M.D. *et al.* (2008) Isolation and purification of an anticoagulant from fermented red seaweed *Lomentaria catenata*. *Carbohydr. Polym.*, **73**, 274–279.
- Radmer, R.J. (1996) Algal diversity and commercial algal products. *Bioscience*, **46**(4), 263–270.
- Ragan, M.A., Smidsrød, O. and Larsen, B. (1979) Chelation of divalent metal ions by brown algal polyphenols. *Mar. Chem.*, **7**(3), 265–271.
- Ragan, M.A., Ragan, C.M. and Jensen, A. (1980) Natural chelators in seawater: Detoxification of Zn<sup>2+</sup> by brown algal polyphenols. *J. Exp. Mar. Biol. Ecol.* **44**(2), 261–267.
- Rajasulochana, P., Krishnamoorthy, P. and Dhamotharan, R. (2010) Amino acids, fatty acids and minerals in *Kappaphycus* spp. *ARPN J. Agric. Biol. Sci.*, **5**(5), 1–12.
- Regine H.S.F. and Vieira, B.V. (2000) Biosorption: a solution to pollution? *Int. Microbiol.*, **3**(1), 17–24.
- Rodrigueza, M.R.C. and Montano, M.N.E. (2007) Bioremediation potential of three carrageenophytes cultivated in tanks with seawater from fish farms. *J. Appl. Phycol.*, **19**, 755–762.
- Roleda, M.Y., Clayton, M.N. and Wiencke, C. (2006) Screening capacity of UV-absorbing compounds in spores of Arctic Laminariales. *J. Exp. Mar. Biol. Ecol.*, **338**, 123–133.
- Ross, A.B., Jones, J.M., Kubacki, M.L. *et al.* (2008) Classification of macroalgae as fuel and its thermochemical behavior. *Biores. Technol.*, **99**, 6494–6504.
- Rupérez, P. (2002) Mineral content of edible marine seaweeds. *Food Chem.*, **79**, 23–26.
- Rupérez, P. and Toledano, G. (2003) Indigestible fraction of edible marine seaweeds. *J. Sci. Food Agric.*, **83**, 1267–1272.
- Senevirathne, M., Kim, S., Siriwardhana, N. *et al.* (2006) Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Sci. Technol., Int.*, **12**(1), 27–28.
- Seol, K., Lim, D., Jang, A. *et al.* (2009). Antimicrobial effect of  $\kappa$ -carrageenan-based edible film containing ovotransferrin in fresh chicken breast stored at 5°C. *Meat Sci.*, **83**, 479–483.
- Schmid, D., Schurch, C., and Zulli, F. (2004) UVA-screening compounds from red algae protect against photoageing. *Personal Care*, 29–31.
- Shanab, S.M.M. (2007) Antioxidant and antibiotic activities of some seaweeds (Egyptian isolates). *Int. J. Agric. Biol.*, **9**, 220–225.
- Shanmugan, M. and Mody, K.H. (2000) Heparinoid-active sulfated polysaccharides from marine algae as potential blood anticoagulant agents. *Curr. Sci.*, **79**, 1672–1683.
- Shibata, T., Ishimaru, K., Kawaguchi, S., *et al.* (2008) Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *J. Appl. Phycol.*, **20**, 705–711.
- Sinha, R.P., Klisch, M., Groniger, A. *et al.* (2000) Mycosporine-like amino acid in marine red alga *Gracilaria cornea* – effects of UV and heat. *Env. Exp. Bot.*, **43**, 33–43.
- Smith, A.J. (2004) Medicinal and pharmaceutical uses of seaweed natural products: A review. *J. Appl. Phycol.*, **16**(4), 245–262.
- So, M.J., Kim, B.K., Choi, M.J. *et al.* (2007) Protective activity of fucoidan and alginic acid against free radical-induced oxidative stress under in vitro and cellular system. *J. Food Sci. Nutr.*, **12**, 191–196.
- Song, H., Zhang, Q., Zhang, Z. *et al.* (2010) In vitro antioxidant activity of polysaccharides extracted from *Bryopsis plumose*. *Carbohydr. Polym.*, **80**, 1057–1061.
- Sousa, A.M.M., Sereno, A.M., Hilliou, L. *et al.* (2010) Biodegradable agar extracted from *Gracilaria vermiculophylla*: Film properties and application to edible coating. *Materials Science Forum*, **636–637**, 739–744.
- Souza, M.C.R.D., Marques, C.T., Dore, C.M.G. *et al.* (2007) Antioxidant activities of sulphated polysaccharides from brown and red seaweeds. *J. Appl. Phycol.*, **19**, 153–160.
- Synnysya, A., Kim, W., Kim, S. *et al.* (2010) Structure and antitumor activity of fucoidan isolated from sporophyll of

- Korean brown seaweed *Undaria pinnatifida*. *Carbohydr. Polym.*, **81**(1), 41–48.
- Teas, J., Pino, S., Critchley, A. *et al.* (2004) Variability in iodine content in common edible seaweeds. *Thyroid*, **14**(10), 836–841.
- Teruya, T., Konishi, T., Uechi, S. *et al.* (2007) Anti-proliferative activity of oversulfated fucoidan from commercially cultured *Cladosiphon okamuranus* TOKIDA in U937 cells. *Int. J. Biol. Macromol.*, **41**(3), 221–226.
- Ueyama, Y., Ishikawa, K., Mano, T. *et al.* (2002) Usefulness as guided bone regeneration membrane of the alginate membrane. *Biomaterials*, **23**(9), 2027–2033.
- Vauchel, P., Kaas, R., Arhalias, A. *et al.* (2008) A new process for extracting alginates from *Laminaria digitata*: Reactive extrusion. *Food Bioproc. Technol.*, **1**, 297–300.
- Walker, M.P., Burckhard, J., Mitts, D. A. *et al.* (2010) Dimensional change over time of extended-storage alginate impression materials. *Angle Orthodontist*, **80**(6), 1110–1115.
- Wang, L., Shelton, R.M., Cooper, P.R. *et al.* (2003) Evaluation sodium alginate for bone marrow cell tissue engineering. *Biomaterials*, **24**, 3475–3481.
- Wang, J., Zhang, Q., Zhang, Z. *et al.* (2008) Antioxidant activity of sulphated polysaccharide fractions extracted from *Laminaria japonica*. *Int. J. Biol. Macromol.*, **42**, 127–132.
- Wang, T., Jónsdóttir, R. and Ólafsdóttir, G. (2009a) Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem.*, **116**, 240–248.
- Wang, B., Zhang, W., Duan, X. *et al.* (2009b) In vitro antioxidative activities of extract and semi-purified fractions of the marine red alga, *Rhodomela confervoides* (Rhodomelaceae). *Food Chem.*, **113**, 1101–1105.
- Wang, H., Chiu, L.C.M., Ooi, V.E.C. *et al.* (2010) A potent antitumor polysaccharide from the edible brown seaweed *Hydroclathrus clathratus*. *Bot. Mar.*, **53**(3), 265–274.
- Wijesekara, I., Pangestuti, R. and Kim, S.K. (2011) Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydr. Polym.*, **84**, 14–21.
- Wong, K.H. and Cheung, P.C.K. (2000) Nutritional evaluation of some subtropical red and green seaweeds: Part I-proximate composition, amino acid profiles and some physico-chemical properties. *Food Chem.*, **71**, 475–482.
- Yabur, R., Bashan, Y. and Hernández-Carmona, G. (2007) Alginate from the macroalgae *Sargassum sinicola* as a novel source for microbial immobilization material in wastewater treatment and plant growth promotion. *J. Appl. Phycol.*, **19**, 43–53.
- Yamasaki-Miyamoto Y., Yamasaki M., Tachibana H. *et al.* (2009) Fucoidan induces apoptosis through activation of caspase-8 on human breast cancer MCF-7 cells. *J. Agric. Food Chem.*, **57**(18), 8677–8682.
- Yang, J., Xie, Y. and He, W. (2011) Research progress on chemical modification of alginate: A review. *Carbohydr. Polym.*, **84**, 33–39.
- Ye, H., Wang, K., Zhou, C. *et al.* (2008) Purification, antitumor and antioxidant activities in vitro of polysaccharides from the brown seaweed *Sargassum pallidum*. *Food Chem.*, **111**, 428–432.
- Ye, H., Zhou, C., Sun, Y. *et al.* (2009) Antioxidant activities in vitro of ethanol extract from brown seaweed *Sargassum pallidum*. *Eur. Food Res. Technol.*, **230**, 101–109.
- You, S., Yang, C., Lee, H.Y. *et al.* (2010) Molecular characteristics of partially hydrolyzed fucoidans from sporophyll of *Undaria pinnatifida* and their in vitro anticancer activity. *Food Chem.*, **119**(2), 554–559.
- Yuan, Y.V., Bone, D.E. and Carrington, M.F. (2005) Antioxidant activity of dulce (*Palmaria palmata*) evaluated in vitro. *Food Chem.*, **91**, 485–494.
- Yuan, H., Song, J., Lia, X. *et al.* (2006) Immunomodulation and antitumor activity of  $\kappa$ -carrageenan oligosaccharides. *Cancer Lett.*, **243**(2), 228–234.
- Yuan, Y.V., Westcott, N. D., Hu, C. *et al.* (2009) Mycosporine-like amino acid composition of the edible red alga, *Palmaria palmata* (dulce) harvested from the west and east coasts of Grand Manan Island, New Brunswick. *Food Chem.*, **112**, 321–328.
- Zandi, K., Tajbakhsh, S., Nabipour, I. *et al.* (2010) In vitro antitumor activity of *Gracilaria corticata* (a red alga) against Jurkat molt-4 human cancer cell lines. *Afr. J. Biotechnol.*, **9**(40), 6787–6790.
- Zaragoza, M.C., Lopez, D., Saiz, M.P. *et al.* (2008) Toxicity and antioxidant activity in vitro and in vivo of two *Fucus vesiculosus* extracts. *J. Agric. Food Chem.*, **56**, 7773–7780.
- Zhang, W., Yamane, H. and Chapman, D.J. (1993) The phytohormone profile of the red alga *Porphyra perforata*. *Bot. Mar.*, **36**, 257–266.
- Zhang, Z., Gao, X., Tashiro, Y. *et al.* (2005a) The isolation of porphyra-334 from marine algae and its UV-absorption behavior. *Chin. J. Oceanol. Limnol.*, **23**, 400–405.
- Zhang, Z., Tashiro, Y., Matsukawa, S. *et al.* (2005b) Influence of pH and temperature on the ultraviolet-absorbing properties of porphyra-334. *Fish. Sci.*, **71**, 1382–1384.
- Zhang, Z., Wang, F., Wang, X. *et al.* (2010) Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro. *Carbohydr. Polym.*, **82**, 118–121.
- Zhou, G., Xin, H., Sheng, W. *et al.* (2005) In vivo growth-inhibition of S180 tumor by mixture of 5-Fu and

- low molecular  $\lambda$ -carrageenan from *Chondrus ocellatus*. *Pharmacol. Res.*, **51**(2), 153–157.
- Zubia, M., Robledo, D., and Pelegrin, Y. F. (2007) Antioxidant activities in tropical marine macroalgae from the Yucatan, Mexico. *J. Appl. Phycol.*, **19**, 449–458.
- Zubia, M., Payri, C. and Deslandes, E. (2008) Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornate* (Phaeophyta: Fucales), from Tahiti (French Polynesia). *J. Appl. Phycol.*, **20**, 1033–1043.

# 34

## Application of Seaweeds in the Food Industry

**Cristina García Sartal, María Carmen Barciela Alonso and Pilar Bermejo Barrera**

*Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Santiago de Compostela, Spain*

### 34.1 Introduction

Around 221 species of macroalgae (seaweed) are harvested worldwide for different purposes, resulting in approximately 66% for use as foodstuffs, and 101 species suitable for phycocolloid production (Lindsey Zemke-White and Ohno, 1999). Seaweeds have been catalogued as a high nutrition value food due to their valuable composition. They are rich in minerals such as Ca, K, P, and Na, and also approximately 54 trace elements that are required for human physiological processes. Their high content of proteins, essential amino acids, vitamins and lipids (good sources of unsaturated fatty acids) and their low caloric content make them very appealing for human consumption.

Although many people do not ingest algae directly in salads, stews or other dishes, indirectly, they consume seaweeds through by-products present in the daily diet. Another indirect way to consume algae is through a diet based on certain animals and plants, as some seaweed and microalgae are used in the preparation process of animal feed and fertilizers.

This chapter deals with the different uses of seaweed and microalgae in the food industry.

### 34.2 Compounds extracted from algae of interest to the human nutrition industry

#### 34.2.1 Macroalgae-extracted compounds

Seaweeds are a source of phycocolloids such as agar, carrageenan, and alginate. These compounds are used as gelling, stabilizing, and thickening agents in the pharmaceutical, food, textile, paint, and varnish industries. The different applications of these compounds in the food industry and their use will be treated in this chapter.

#### *Alginate*

Alginate is a polysaccharide widely present in the cell walls of brown seaweed. This compound is extracted from different types of brown seaweed such as *Ecklonia*, *Macrocystis*, *Undaria*, *Laminaria*, *Durvillea*, *Turbinaria*, and *Sargassum* (Kaliaperumal, 2003). Alginate consists of 1,4-linked  $\alpha$ -L-guluronic acid (G) and  $\beta$ -D-mannuronic acid (M) subunits in GG, MM and MG domains (Renn, 1997). It is present in these seaweeds as sodium, magnesium, and calcium salts of alginic acid. Alginate is extracted in the form of sodium

alginate following the calcium alginate process or the alginic acid process (McHugh, 2003).

The use of alginate in the food industry is based on its ability to increase the viscosity of aqueous solutions and to form gels that do not melt when heated. This latter property differences these gels from the agar gels. Due to its capacity to form flavorless gum, it is used for increasing viscosity and also as an emulsifier in the food industry. Alginate is used as a stabilizer in ice-cream and other dairy products, in beer to produce froth and in soft drinks. It is used as a thickener in drinks and soap, as a clarifying agent in wines and cider, and to increase the viscosity of fruit juices and mustard (González *et al.*, 1998). The food additive code depends on the salt: E-401 for sodium alginate, E-402 for potassium alginate, E-403 for ammonium alginate, E-404 for calcium alginate and E-405 for propylene glycol alginate.

### Agar

Agar is the major cell-wall constituent of a group of red seaweed, especially the members of the Gelidiaceae, Gelidiales, and Gracilariaceae families. Agar is extracted mainly from *Gracilaria*, *Gelidium*, *Pterocladia*, and *Ahnfeltia*, out of which *Gracilaria* and *Gelidium* are the predominant species used in industry. Extraction from *Gelidium* and *Pterocladia* species provides the best quality agar (taking into account the gel strength). The *Gelidium* used in industry to produce agar comes principally from Spain, Portugal, and Morocco, with lesser amounts coming from South Korea, Mexico, and Japan (McHugh, 2003; Bixler and Porse, 2010; Dhargalkar and Verlecar, 2009).

Agar consists of alternating 1,4-linked  $\alpha$ -D-galactose and 3,6-anhydro- $\alpha$ -L-galactose backbone (agarobiose) substituted with varying percentages of methoxyl, ester sulphate and ketal pyruvate groups (Renn, 1997). Agar is extracted from the seaweeds using hot water (90–110 °C), but the treatment varies depending on the type of seaweed used. *Gelidium* is simply washed and placed in tanks for extraction with hot water, while *Gracilaria* is washed and treated with alkali (sodium hydroxide at 85–90 °C for 1 h), before the extraction with hot water. Without this alkaline pretreatment, the agar obtained has very low gel strength and does not have commercial applications.

The use of agar in the food industry is due to its ability to dissolve in boiling water and form gels. It forms gel at a temperature between 32–43 °C, depending on the source of the agar. The melting point of the formed gel is more than 85 °C. The advantage of agar with respect to other gelling compounds (gelatin gels), is that the food products obtained using agar can be stored at room temperature in hot climates (McHugh, 2003). Agar is used in the food industry in different products because it acts as a stabilizer

and thickener in pie filling, ice-creams, dairy products, etc. It is used to clarify wines and in the preparation of low fat products; for example, as a substitute of fats in sausages due to its binding properties. Agar's food additive code is E-406 (González *et al.*, 1998).

### Carrageenan

Carrageenan is extracted from red seaweeds, mainly *Kappaphycus alvarezii* and *Eucheuma denticulatum*. The principal producers of carrageenan are Denmark, Ireland, New Zealand, Canada, China, Japan, and Mozambique (Dhargalkar and Verlecar, 2009). The structure of carrageenan is composed of D-galactose units with alternate  $\alpha$ -(1–3) and  $\beta$ -(1–4) linkages. Carrageenans are composed of different copolymers, classified according to the presence of 3,6-anhydro-D-galactose on the 1,4-linked residue and the number and the position of sulphated groups (Popescu *et al.*, 2007). The main copolymers are designated as iota ( $\iota$ ), kappa ( $\kappa$ ) and lambda ( $\lambda$ ). Carrageenan presents different properties depending on the copolymer used, thus kappa forms strong rigid gels, iota forms soft elastic gels and lambda does not form a gel but produces the highest viscosities in water (O'Sullivan *et al.*, 2010; McLachlan, 1985).

Carrageenan is obtained industrially by treating the seaweed with alkali and water to eliminate the soluble compounds. The insoluble residue consists mainly of carrageenan and cellulose. This dry residue is called semirefined carrageenan.

Refined carrageenan is produced by heating the seaweed for several hours with water mixed with an alkali solution (generally sodium hydroxide). The carrageenan extracted with this process is then precipitated with potassium chloride or with alcohol. The precipitate is separated, dried and milled, obtaining in this way the refined carrageenan (McHugh, 2003).

Carrageenans are used in a wide variety of applications in the food industry, for example, as gelling, thickening, and stabilizing agents in the preparation of products such as ice-cream, cheese, syrups, chocolate milk, puddings, meat products, etc. This compound is also used for clarifying beer, fruit juices, and other beverages. The food additive code for carrageenan is E-407, and for semirefined carrageenan E-407a (Pereira *et al.*, 2009).

### Furcellaran

Furcellaran or Danish agar is extracted from red seaweed called *Furcellaria fastigiata*. It has intermediate properties between agar and carrageenan (Wood, 1974). The powder of furcellaran extracted from seaweed is soluble in hot water

and it is used in the food industry as a gelling, stabilizing and thickening agent, with similar applications as carrageenan in the preparation of jellies, food preserves, fish or meat preparations and baby food (Naylor, 1976).

### *Iridophycan*

The use of this compound is not very widespread; although its properties, similar to those found in carrageenans, would make it ideal for adding to chocolate and beverages as a stabilizer. California is the predominant area to harvest the genus *Iridea*, which is used in the extraction of this compound (Naylor, 1976).

### *Mannitol*

Mannitol is an important sugar alcohol present in seaweed, especially in brown seaweed. This compound is extracted mainly from *Fucus vesiculosus*, *Laminaria hyperborean*, *Ecklonia radiata*, *Bifurcaria brassiformis*, *Sargassum* spp. and *Turbinaria* spp. (Kaliaperumal, 2003).

Mannitol is extracted from seaweed using 10–15% hydrochloric acid solution. The extract obtained is evaporated, dried and treated with boiling methanol for 5 h to extract the mannitol. The separated solution is allowed to stand for 24 h at 5 °C to allow the precipitation of crystalline mannitol. Finally, the precipitate is separated, dried and prepared for use (Kaliaperumal, 2003).

Mannitol can replace sugar as a bulking agent in food, thus it is used in the food industry to make diabetic food or sugar-free products (Kaliaperumal, 2003).

## 34.2.2 Microalgae-extracted compounds

Microalgae are a source of natural products such as pigments, fatty acids, enzymes, fibers, proteins, polysaccharides, and vitamins. New food products containing natural ingredients from algae are designed every day with the idea of incorporating nutritional supplements with health benefits into our diet, such as *Chlorella vulgaris* biomass as a coloring ingredient in butter cookies (Gouveia *et al.*, 2007). The rate of growth of microalgae organisms and the use of cost-effective natural resources for their development, makes them appealing for exploitation and marketing. However, the market value of a particular bioproduct determines the use of a given microalgae among others in the field of microalgal biotechnology, carotenoids being the major high-value products extracted from microalgae for commercial applications (EonSeon and Anastasios, 2003). Microalgae like *Spirulina* are traditionally sold as a food coloring or as a human food supplement in tablets or capsules, based on its dry biomass due to the presence of high con-

tent of proteins, polyunsaturated fatty acids, edible fiber, microelements, and vitamins (Belay *et al.*, 1996; Metting, 1996; Liang *et al.*, 2004). On a smaller production scale, *Spirulina* products are sold in Chinese markets as liquid extracts, conveniently suitable for drinking, or mixed with other natural plants or microorganisms as ingredients of a product called *Spirulina* Cosmetic Cake. The Chinese food industry also adds microalgae to traditional foods to obtain higher nutritive noodles, bread, biscuits, drinks, beer, candy and tea (Liang *et al.*, 2004; Li and Qi, 1997).

### *Pigments*

Often the appearance of a food determines its acceptance by consumers who reject food that does not satisfy their expectations. Appearance of any food is often related to its condition, but the healthier and more appealing aspect of foods can be reinforced by adding color.

The food industry uses synthetic coloring materials to add color to food that lacks it, as well as to improve color loss suffered by the food after industrial processing. Nowadays, natural pigments are becoming more valuable than synthetic colorants in this industry due to increasing consumer demand for natural, functional and healthy products (Batista *et al.*, 2006). In addition, natural dyes have an isomer ratio in nature that cannot be provided by synthetic forms (Spolaore *et al.*, 2006).

One of the main problems occurring with pigments in the food industry is the low stability of natural colorants under different conditions of temperature, light or pH after extraction from their media (Wissgott and Bortlik, 1996). The difficulty in stabilizing pigments by natural methods as well as the small number of existing natural pigments commercialized makes it necessary to search for alternative natural colorants.

Another drawback is the cost-effective production of natural pigments as compared to their synthetic counterparts (Olaizola, 2003). The objective is to lower the production costs following different strategies such as improving the technology to produce a given algae biomass, or by diminishing land, labor or energy costs. Ultimately, price is one of the most important factors for a product to be successful in the market.

Most of the algal pigments used in the food industry come from microalgae such as species of *Chlorella*, *Spirulina*, *Dunaliella*, and *Haematococcus* (Borowitzka, 1997)

### *Chlorophylls*

Some green microalgal species such as *Chlorella* sp. and *Haematococcus pluvialis* contain large amounts of photosynthetic pigments like chlorophyll, specifically chlorophylls a

and *b* (Plaza *et al.*, 2009). Both chlorophylls *a* and *b* are becoming important in the food industry as food additives due to their health effects such as incrementing hemoglobin in blood, cell reparation capacity and improvement of cell growth (Harun *et al.*, 2010). Chlorophylls are widely used in the manufacture of jam, jelly, candy, and ice cream among other foods, being allowed in the European Union under an E-140 food additive code (Chattopadhyay *et al.*, 2008).

### Carotenoids

Carotenoids are yellow, orange, or red lipophilic compounds composed of isoprene units. They are divided into two main groups according to their oxygen composition. Carotenoids composed of oxygen-free hydrocarbons are called carotenes, and those containing oxygen molecules are classified as xanthophylls. Among the latter, we find lutein, zeaxanthin, canthaxanthin or astaxanthin, which are useful as dye in the food industry (Becker, 1994; Perez-Garcia *et al.*, 2011).

Free oxygen radicals are normally produced in the body and some conditions such as air pollution, smoking, and UV light can increase their formation. Such agents in excess quantities can cause oxidative stress with damage to DNA, proteins, and lipid membranes. This type of damage has been related to carcinogenesis, aging and macular degeneration, among other ailments. Carotenoids inhibit the destruction of molecules and tissues by absorbing the excited energy of the singlet oxygen onto their structures, acting as antioxidants (Guerin *et al.*, 2003; Mata *et al.*, 2010). Their antioxidant activity and anti-inflammatory properties classify them as potential functional ingredients (Spolaore *et al.*, 2006; Plaza *et al.*, 2009). In addition to the positive health effects of antioxidant compounds, they are useful in food technology due to their capacity to avoid lipid peroxidation. During food processing and storage, endogenous antioxidants contained in a given food can be lost leaving the foodstuff unprotected against lipid peroxidation (Herrero *et al.*, 2006).

The value of carotenoids in the worldwide market has been estimated to 1 billion US dollars in 2010 (Campo *et al.*, 2007), being  $\beta$ -carotene, astaxanthin, canthaxanthin and lutein, the main biocolorants are commercialized.

**$\beta$ -Carotene** This compound is in high demand in the food industry as an orange–yellow coloring agent, as a health food and also as a source of retinol or provitamin A (Oren, 2005). Lack of vitamin A can cause eye diseases such as xerophthalmia and blindness and even premature death.  $\beta$ -Carotene supplementation in food can control vitamin A deficiency (Muntean *et al.*, 2007).  $\beta$ -Carotene is found in microalgae under different stereogeometries. The most

common are all-*trans* and 9-*cis* forms which confer them higher bioavailability and antioxidant properties than synthesized all-*trans*  $\beta$ -carotene (Campo *et al.*, 2007; Raja *et al.*, 2007). Furthermore, unlike the artificial  $\beta$ -carotene, the natural compound extracted from microalgae is accompanied by other carotenoids and essential nutrients in a lower concentration which enrich their value as a functional food (Dufossé *et al.*, 2005). The food industry uses this additive in the preparation of butter, margarine, cheese, fruit juices, baked goods, canned goods, dairy products, pastries, soft drinks, etc (Dufossé *et al.*, 2005; Baker and Günther, 2004). It is mainly found concentrated in microalgae such as *Haematococcus pluvialis* and *Dunaliella salina*. The latter can provide up to 14% of its dried base in  $\beta$ -carotene (Spolaore *et al.*, 2006; Plaza *et al.*, 2009).  $\beta$ -Carotene from *Dunaliella* is currently marketed for human use under three formats:  $\beta$ -Carotene extracts consist of a mixture of carotenoids (commercially named “carotenoids mix”), *Dunaliella* powder and dried *Dunaliella* (Dufossé *et al.*, 2005). There is evidence in the literature that suggests a lower incidence of cancer and degenerative diseases relating to increased human consumption of  $\beta$ -carotenes (Campo *et al.*, 2007). The production of this carotenoid has reached a worldwide market estimated value of US \$250 million in 2009, being the major production companies Betatene, Western Biotechnology, AquaCarotene (Australia), Cyanotech (Hawaii), Inner Mongolia Biological Eng (China), Nature Beta Technologies (Israel), among others (Campo *et al.*, 2007). The Food and Drug Administration (FDA) labels it as GRAS (substances generally recognized as safe in foods) being exempt from certification. In the European Union it is commercialized under food additive code E-160a (Chattopadhyay *et al.*, 2008).

**Astaxanthin** Astaxanthin is a hydro- and liposoluble red colorant used mainly for animal feed, although in human nutrition it is applied to enrich the pinkish-red color of some foods (Ferraro *et al.*, 2010). The optimal production of this compound is performed by subjecting the microalgae to stressful conditions; for example, by adding glucose to the growth medium (Perez-Garcia *et al.*, 2011), or cultivating the microalgae under intense light conditions with low nutrient content in the media (Spolaore *et al.*, 2006). Nowadays, synthetic astaxanthin is more economically accessible than the natural form; however, there are some applications for which natural astaxanthin is preferred, such as captive rearing of carp, red sea bream or chicken, because the natural pigment has a better deposition in the animal tissues (Spolaore *et al.*, 2006). Astaxanthin is believed to protect against neurodegenerative diseases, inflammation and cancer. It is also beneficial in maintaining eye, skin, cellular, and heart health (Guerin *et al.*, 2003). It has been

reported that astaxanthin antioxidant activity is ten times higher than that found in zeaxanthin, lutein, canthaxanthin and  $\beta$ -carotene and has better dyeing properties than other carotenoids (Dufossé *et al.*, 2005; Higuera-Ciapara *et al.*, 2006). *Haematococcus pluvialis* and *Chorella vulgaris* are the main microalgae from which these types of pigments are extracted (Plaza *et al.*, 2009). Astaxanthin production reached a rough market value of US \$257 million in 2009 (Campo *et al.*, 2007). The synthetically derived form is the most commonly marketed. BioReal (Sweden), Algatechnologies (Israel), Cyanotech, Mera Pharmaceuticals and Fuji Health Science (Hawaii) are the major commercial producers of astaxanthin (Campo *et al.*, 2007). Its beneficial antioxidant and nutraceutical properties have led to the distribution of these compounds as dietary supplements currently sold in health food stores (Higuera-Ciapara *et al.*, 2006). *Haematococcus* astaxanthin-rich algae meal has been approved for consumption in different countries such as Japan, the United States, Canada and several parts of Europe as a natural red pigment for human consumption and for farming (Lorenz and Cysewski, 2000).

**Canthaxanthin** Canthaxanthin pigments are mainly extracted from *Chlorella vulgaris* and *Haematococcus pluvialis* microalgae (Plaza *et al.*, 2009). Although canthaxanthin is mainly used to enhance the color of egg yolks, it is also used in dairy products, pastries, beverages, snacks, beer and wine, because it is less photosensitive than  $\beta$ -carotene. In the European Union it is commercialized under food additive code E-161g (Chattopadhyay *et al.*, 2008).

**Lutein** This food coloring is permitted in the European Union and is marketed under the food additive code E-161b (Ceron *et al.*, 2008). Various types of animal tissues such as chicken skin and egg yolks have been naturally colored by using lutein, which has a high nutritional value in the food industry (Perez-Garcia *et al.*, 2011). It is found naturally in the eye retina and eye lens together with zeaxanthin (another carotenoid) as an essential component. A lutein-rich diet may protect against eye ailments, such as cataracts and retina degeneration (Campo *et al.*, 2007; Jin *et al.*, 2003). *Dunaliella* sp. contains up to 14% dry basis of lutein. Together with some *Chlorella* species, *Dunaliella* sp. is the microalga strains richest in lutein (Plaza *et al.*, 2009; Perez-Garcia *et al.*, 2011). Marigold flowers (containing up to 0.03% dry basis of lutein), are the current source of this coloring used in the food industry. Lutein in marigold flowers is mostly esterified, thus a chemical saponification is needed during pigment processing which would suggest that microalgae containing non-esterified lutein are a good alternative for extracting this pigment (Campo *et al.*, 2007;

Ceron *et al.*, 2008). The market value of lutein was approximately US \$187 million in 2009 (Campo *et al.*, 2007).

### Phycobiliproteins

Phycobiliproteins are a group of proteins present in some types of algae that carry out photosynthesis processes. These compounds are covalently bonded to a phycobilin center consisting of a tetrapyrrolic pigment (Plaza *et al.*, 2009). As in the case of carotenoids, phycobiliproteins have antioxidant, immunomodulation, anticancer, hepatoprotective, and anti-inflammatory properties (Plaza *et al.*, 2009).

### Phycocyanin

This is a blue dye used in the food industry and is extracted mainly from *Spirulina platensis* (Li and Qi, 1997; Perez-Garcia *et al.*, 2011). A company called Dainippon Ink & Chemicals (Sakura) commercializes a product named Lina Blue, based on phycocyanin to prepare chewing gum, ice sherbets, popsicles, candies, soft drinks, wasabi and dairy products (Spolaore *et al.*, 2006). Small doses of phycocyanin have proven to prevent or inhibit tumour formation in rats (Belay *et al.*, 1993).

### Phycoerythrin

Phycoerythrin is a water soluble red phycobiliprotein that could be used in the food industry. B-phycoerythrin and b-phycoerythrin extracted from *Porphyridium* sp. is a fluorescent pink color that could be used to dye pastry, gelatin desserts and dairy products in the food industry. Between 50–100 mg/kg of this pigment is necessary to dye 1 kg of food. Despite different patents having been accepted to market this natural coloring extracted from *Porphyridium*, its use in food has not yet been approved (Dufossé *et al.*, 2005).

### Fatty acids

Higher plants and animals are not able to synthesize long chain polyunsaturated fatty acids (PUFAs), thus they must acquire these essential compounds by way of their diet. Currently, fish and fish oil are the main natural sources of PUFAs commercialized, but their consumption has some drawbacks; for example, possible accumulation of toxins, ease of oxidation, mixing of different types of PUFAs, as well as an unpleasant fishy odor and flavor that makes them unappealing for consumption (Spolaore *et al.*, 2006; Sijtsma and Swaaf, 2004). Microalgae are a good alternative because they are primary producers of essential PUFAs in the marine food chain (Osbourn and Lanzotti, 2009; Kumari *et al.*, 2010). These microorganisms contain linoleic acid

(LA, 18:2,  $\omega$ -6) and  $\alpha$ -linolenic (ALA, 18:3,  $\omega$ -3) that act as precursors of some  $\omega$ -3 and  $\omega$ -6 families of PUFAs essential to human functions, including eicosapentaenoic acid (EPA, 20:5,  $\omega$ -3) and docosahexaenoic acid (DHA, 22:6,  $\omega$ 3) widely used in the food and pharmaceutical industries (Chen and Jiang, 2001).

In higher animals and humans, EPA acts as the forerunner of several substances such as prostaglandins, thromboxanes, and leukotrienes, which play an important role in regulating developmental and regulatory physiology (Cardozo *et al.*, 2007). Deficiency of EPA and DHA in the diet seems to be related to higher cardiovascular disease risk (Herrero, Cifuentes and Ibanez, 2006; Osbourn and Lanzotti, 2009; Chen and Jiang, 2001). DHA is involved in brain and retina development in infants, causing learning disabilities in the case of DHA-low diets (Chen and Jiang, 2001). Breast-fed infants generally acquire the required amount of DHA through the mother's milk; however, bottle-fed babies need to have DHA-supplemented infant formulas to prevent neuronal and visual diseases (Sijtsma and Swaaf, 2004; Wroble *et al.*, 2002). Fortunately, it is believed that infants acquire most of the necessary PUFAs intake through the placenta when they are yet unborn (Wroble *et al.*, 2002). The  $\omega$ -3-PUFAs have aroused wide attention because they have been recognized to reduce the risk of cancer, arthritis, and mental health disorders such as dementia, depression, schizophrenia, Alzheimer's, and Parkinson's diseases (Harwood and Guschina, 2009; Trautwein, 2001; Barrow *et al.*, 2007).

The main gap in the application of EPA and DHA in the food industry is related to different oxidative processes induced by light or free radical oxygen. During the autoxidation, EPA and DHA are destabilized, and aldehydes might be generated leading to rancid tastes and smells. To avoid these kinds of problems microencapsulation of these  $\omega$ -3-PUFAs by complex coacervation has been suggested (Barrow *et al.*, 2007). Microencapsulation has the advantage of avoiding the addition of antioxidants to the food and making possible the release of EPA and DHA only in the intestine (Ferraro *et al.*, 2010).

A need for  $\omega$ -3-fortified food has arisen because humans have mainly incorporated  $\omega$ -6-rich cereals and vegetable oils into their diets together with other saturated fat foods, decreasing the intake of  $\omega$ -3-PUFAs. The current  $\omega$ -6/ $\omega$ -3 ratio is 15:1. Historically, this ratio was approximately 1:1 (Fredriksson *et al.*, 2006; Simopoulos, 1999).

DHA is the only commercialized algal PUFA product (Hallmann, 2007). Martek Biosciences (USA) sells the bulk to infant formula manufacturers and also capsules containing the product (Spolaore *et al.*, 2006; Olaizola, 2003). Some of the algal DHA products are DHASCO-T oil (triglyceride containing 40% DHA) and DHASCO-S (triglyceride con-

taining 40% DHA, 15% docosapentaenoic acid  $\omega$ -6-PUFA, DPA, and 2.5% EPA). Both Martek capsules are catalogued as GRAS by the FDA (Trautwein, 2001; Arterburn *et al.*, 2007). OmegaTech (USA), recently acquired by Martek, produces low-cost oil called DHA Gold used for dietary supplements, beverages, animal feed, and also some foods such as cheese, yogurt, spreads, foods for pregnant and nursing women, and breakfast cereals. In addition, Nutrinova Process (Germany) commercializes an oil called DHActive (Spolaore *et al.*, 2006).

## 34.3 Animal feeding

Although the main objective of supplementing animal feed with algae is the improvement of the appearance of certain animals through their diet, it is worth mentioning that those animals are the beginning of our food chain; thus, it is important to consider the positive or adverse effects of the fodder-added compounds that could affect our health.

The use of color additives is regulated by different laws depending on the country where they are applied. Canthaxanthin, lutein, astaxanthin, zeaxanthin and  $\beta$ -carotene natural pigments are listed as authorized additives in the European Union for animals such as poultry, laying hens and salmon (Breithaupt, 2007). Astaxanthin and canthaxanthin carotenoids are part of the diet of salmon, trout and shrimp grown in aquaculture. Lutein and zeaxanthin are mostly added to hens and poultry feed (Muntean *et al.*, 2007).

### 34.3.1 Terrestrial animal feed

Edible seaweed has been used as supplement or primary feed for livestock due to its low calorie and fat content, among other high quality biocomponents (Harun *et al.*, 2010). Currently, algal biomass of certain microalgae (*Spirulina*, *Chlorella* and *Scenedesmus* genera) is incorporated as an additive in pet and poultry feed (Pulz and Gross, 2004), partially replacing the fishmeal, groundnut meal and soybean meal used until recently (Habib *et al.*, 2008). The high nutritive value of such microalgae promotes weight control, shiny fur, healthy skin, better fertility and immune response in those animals that consume the algal biomass through their diet (Spolaore *et al.*, 2006). Apart from the use of the algae biomass for animal fodder, some algal pigments such as  $\beta$ -carotene are added to animal foods to enhance the fodder appearance and also to provide our pets (dogs, cats, birds, fishes) with a precursor to vitamin A (Dufossé *et al.*, 2005). The addition of lutein and zeaxanthin natural microalgae pigments to animal feed prevents macular degeneration, thus helping maintain optimal vision function

(EonSeon and Anastasios, 2003). Currently, DHA-enriched eggs and milk obtained after PUFA supplementation of the animal feed are available for human consumption (Sijtsma and Swaaf, 2004).

### 34.3.2 Poultry

As a result of the good deposition of xanthophylls (canthaxanthin, lutein, astaxanthin, etc.) in the flesh of poultry and egg yolk, these compounds are commercialized in fodder, thus contributing to their coloration. Pigmentation can be controlled through the animal's diet: astaxanthin-rich feeding can cause a pinkish egg yolk (Breithaupt, 2007); high lutein dosages stain poultry tissues and eggs with a greenish hue (Breithaupt, 2007; Sajilata *et al.*, 2008); canthaxanthin in excess causes an abnormal purple aspect, and  $\beta$ -carotene does not deposit well in the flesh (Sajilata *et al.*, 2008). Zeaxanthin natural dye is preferred to other pigments since it supplies a attractive pigmentation to the flesh and eggs (Sajilata *et al.*, 2008).

To obtain an appropriate coloring, a maximum of 80 mg/kg of xanthophylls is allowed in fodder, except in the case of canthaxanthin, for which concentration must not exceed 8 mg/kg, because a higher amount of this compound could generate crystals in the retina (Breithaupt, 2007).

It appears that the use of astaxanthin for poultry fodder is related to an increase in hen fertility, a decrease in chicken mortality and a reduction in salmonella infections (Higuera-Ciapara *et al.*, 2006; Lorenz and Cysewski, 2000). Chickens fed with freeze-dried algal biomass show a significant decrease in blood and egg cholesterol levels, while maintaining their body and egg weight as well as their egg production (Ginzberg *et al.*, 2000). Furthermore, a diet based on algal products enriched with DHA (docosahexaenoic acid) can be a natural source of  $\omega$ -3 fatty acid in yolk eggs, contributing to the reduction of coronary heart disease risk (Herber-McNeill and Van Elswyk, 1998).

### 34.3.3 Aquaculture

Another use of microalgae in feeding is the direct application in mariculture to stock up with food larval and juvenile bivalves, larvae of some crustaceans and fish, and also zooplankton. These microalgae must fulfill the following conditions: size at the nanoplankton level, high nutritional value, wide range of tolerance to salinity, temperature and light (Brown *et al.*, 1997), have a digestible cell wall and be non-noxious (Mata *et al.*, 2010).

The characteristic pinkish-red hue in the tissue of some organisms such as salmonids, trout, shrimp, lobster and crayfish is due to an astaxanthin-rich diet. These marine animals are not able to synthesize astaxanthin, thus they

acquire it from microalgae or microalgae-consumer zooplankton (EonSeon and Anastasios, 2003). Farmed fish and crustaceans acquire astaxanthin and canthaxanthin through their feed, as they do not have access to natural sources. Approximately 20–50 mg/kg astaxanthin is needed in aquaculture to obtain pigmentation in farmed fish similar to those that grow wild. However, 50–100 mg/kg canthaxanthin is added into the feed to avoid discoloration of the fish during cooking (Margalith, 1999). Currently, 25 mg/kg of canthaxanthin is allowed for fish feed in the European Union, and 80 mg/kg in the United States. Up to 100 mg/kg of astaxanthin is approved in the European Union (Breithaupt, 2007).

Although consumers demand natural products, the truth is that the synthetic forms of both pigments constitute the main source for feeding in aquaculture. Hoffman-LaRoche is the main distributor that commercializes synthetic astaxanthin and canthaxanthin under the name CaroPhyll Pink<sup>TM</sup> and CaroPhyll Red<sup>TM</sup>, respectively (Higuera-Ciapara *et al.*, 2006).

Even though canthaxanthin offers adequate fish pigmentation, astaxanthin is preferred because it has a better deposition in muscles, probably due to higher bioavailability in the digestive tract. A combination of both might be a good alternative. The absorption of these two pigments is species dependent (Higuera-Ciapara *et al.*, 2006).

Apart from color enhancement, astaxanthin is also related to some biological functions in fish, such as reproduction, egg survival and development, improving liver function, and influence on biodefense mechanisms (Baker and Günther, 2004; Higuera-Ciapara *et al.*, 2006). Carotenoids also might be related to an appropriate growth of the fish (Borowitzka, 1997). Canthaxanthin, in particular, seems to affect fertilization rate in trout (Baker and Günther, 2004).

## 34.4 Fertilizers

Approximately 1% of the seaweed harvested for industrial purposes is intended for use in agriculture as nutrient supplements and biostimulants or biofertilizers for plants (Craigie, 2010; Khan *et al.*, 2009), with an estimated production cost of U.S. \$5.10<sup>9</sup>/year (Pulz and Gross, 2004). People from coastal regions were the first to collect drift seaweed on the seashore and use them as fertilizers in nearby fields. Their high weight when wet probably prevented this practice from spreading inland (McHugh, 2003; Naylor, 1976).

Early writings mention the use of seaweed, such as *Ascophyllum*, *Ecklonia* and *Fucus*, either directly or mixed with soil, sand, or by making compost with straw, peat or other organic materials, producing more stabilized and aerated soils with a higher moisture retention (McHugh, 2003; Craigie 2010; Gualtieri and Barsanti, 2006), due to

the presence of insoluble carbohydrates that are contained in seaweed (McHugh, 2003).

The development of liquid seaweed biofertilizers made possible the use of these products by farmers in offshore areas. Unlike seaweed, these alkaline seaweed extracts or seaweed suspensions have a faster action on plants and are easier to transport. There are companies that commercialize liquid algal biofertilizers under various names: Maxicrop (United Kingdom), Kelpak 66 (South Africa), Seagrow (New Zealand), Algifert (Norway), Plantozyme, Shaktizyme (India), Goëmill (France), Seasol (Australia), Marinure (Scotland), etc. (McHugh, 2003; Craigie *et al.*, 1996).

Once seaweed or liquid seaweed extracts were added to land plants either directly or as foliar spray, there was an improvement on plant growth, health and crop yield. Although initially such an advance was attributed to the new soil texture, the moisture-holding capacity, and the addition of trace minerals or organic compounds from seaweed, researchers suggested that these conditions were not enough to produce those growth responses (McHugh, 2003). It is thought that seaweed extracts, applied in small dosages, elicit an increase in plant growth due to the presence of plant growth regulators/hormones such as cytokinins and auxins (Stirk and Staden, 1996) that together with betaines and sterols (Khan *et al.*, 2009) take part in this favorable plant development.

Commercial seaweed fertilizers have shown a wide range of benefits for plants, such as early seed germination, resistance to insects, bacterial or fungal pests, more resistance to frost, and prolonged fruit preservation (Khan *et al.*, 2009; Gualtieri *et al.*, 2006; Hong *et al.*, 2007). They are currently preferred over chemical fertilizers because they are eco-friendly, non-hazardous to animal life, non-toxic and biodegradable (Dhargalkar and Pereira, 2005).

Another way to introduce algae into soils is by inoculating blue green algae into those soils. Some blue green microalgae species used for that purpose are *Nostoc*, *Anabaena*, *Calothrix*, *Tolypothrix*, etc. These microorganisms are suitable for fixing nitrogen, providing soils with organic matter, and growth promoting substances. They are also able to permanently solubilize the insoluble phosphates of the soil. It has been proved that blue green algae can develop in rice paddies, where they have optimal conditions of temperature, humidity, light and nutrients (Metting, 1996; Kannaiyan, 2002; Henrikson, 2009).

## 34.5 Conclusion

Seaweeds are a great source of mineral salts, vitamins, proteins and trace metals necessary for human metabolism.

Although seaweeds have been consumed in the Orient for many centuries, the interest for introducing seaweed into human nutrition in the Western countries has increased in recent years. Seaweed can also be consumed indirectly by humans because they are a source of different compounds used in the food industry. Compounds such as algin, carrageenan, agar, alginate, furcellaran, and mannitol extracted from macroalgae are used in the food industry as gelling, stabilizing, and thickening agents. Microalgae are a good source of pigments, fatty acids, enzymes, fibers, proteins, polysaccharides, and vitamins used in the food industry to increase the nutritional value of their products, or to obtain functional ingredients used in functional foods consumed for their potential health benefits.

Seaweed or compounds extracted from seaweed are also used for animal feeding with the purpose of increasing the appearance of some animals or the nutritional properties of those animals to be used for human consumption. In recent years, the application of seaweed extracts as fertilizer in agriculture has also increased in importance due to the use of these extracts for increasing plant growth.

All these applications show the importance in the use of seaweed for human and animal food as well as the large number of applications from the industrial point of view.

## References

- Arterburn, L.M., Oken, H.A., Hoffman, J.P., *et al.* (2007) Bioequivalence of docosahexaenoic acid from different algal oils in capsules and in a DHA-fortified food. *Lipids*, **42**, 1011–1024.
- Baker, R. and Günther, C. (2004) The role of carotenoids in consumer choice and the likely benefits from their inclusion into products for human consumption. *Trends Food Sci. Technol.*, **15**, 484–488.
- Barrow, C.J., Nolan, C. and Jin, Y. (2007) Stabilization of highly unsaturated fatty acids and delivery into foods. *Lipid Technol.*, **19**, 108–111.
- Batista, A.P., Raymundo, A., Sousa, I., Empis, J. and Franco, J.M. (2006) Colored food emulsions – implications of pigment addition on the rheological behavior and microstructure. *Food Biophys.*, **1**, 216–227.
- Becker, E.W. (1994) *Microalgae: Biotechnology and Microbiology*. Cambridge University Press, Cambridge.
- Belay, A., Kato, T. and Ota, Y. (1996) Spirulina (Arthrospira): potential application as an animal feed supplement. *J. Appl. Phycol.*, **8**, 303–311.
- Belay, A., Ota, Y., Miyakawa, K. and Shimamatsu, H. (1993) Current knowledge on potential health benefits of *Spirulina*. *J. Appl. Phycol.*, **5**, 235–241.

- Bixler, H. and Porse, H. (2010) A decade of change in the seaweed hydrocolloids industry. *J. Appl. Phycol.*, doi 10.1007/s10811-010-9529-3.
- Borowitzka, M.A. (1997) Microalgae for aquaculture: Opportunities and constraints. *J. Appl. Phycol.*, **9**, 393–401.
- Breithaupt, D.E. (2007) Modern application of xanthophylls in animal feeding—a review. *Trends Food Sci. Technol.*, **18**, 501–506.
- Brown, M.R., Jeffrey, S.W., Volkman, J.K. and Dunstan, G.A. (1997) Nutritional properties of microalgae for mariculture. *Aquaculture*, **151**, 315–331.
- Campo, J.A., Garcia-Gonzalez, M. and Guerrero, M.G. (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Appl. Microbiol. Biotechnol.*, **74**, 1163–1174.
- Cardozo, K.H.M., Guaratini, T., Barros, M.P., et al. (2007) Metabolites from algae with economical impact. *Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol.*, **146**, 60–78.
- Ceron, M.C., Campos, I., Sanchez, J.F., Acien, F.G., Molina, E. and Fernandez-Sevilla, J.M. (2008) Recovery of lutein from microalgae biomass: development of a process for *Scenedesmus almeriensis* biomass. *J. Agric. Food Chem.*, **56**, 11761–11766.
- Chattopadhyay, P., Chatterjee, S. and Sen, S.K. (2008) Biotechnological potential of natural food grade biocolorants. *Afr. J. Biotechnol.*, **7**, 2972–2985.
- Chen, F. and Jiang, Y. (2001) *Algae and Their Biotechnological Potential*, Kluwer Academic Publishers, Dordrecht.
- Craigie, J.S. (2010) Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.*, doi: 10.1007/s10811-010-9560-4.
- Dhargalkar, V.K. and Pereira, N. (2005) Seaweed: Promising plant of the millennium. *Sci. Cult.*, **71**(3–4), 60.
- Dhargalkar, V.K. and Verlecar, X.N. (2009) Southern Ocean seaweeds: A resource for exploration in food and drugs. *Aquaculture*, **287**, 229–242.
- Dufossé, L., Galaup, P., Yaron, A., et al. (2005) Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? *Trends Food Sci. Technol.*, **16**, 389–406.
- EonSeon, J. and Anastasios, M. (2003) Microalgal biotechnology: Carotenoid production by the green algae *Dunaliella salina*. *Biotechnol. Bioproc. Eng.*, **8**, 331–337.
- Ferraro, V., Cruz, I.B., Jorge, R.F., Malcata, F.X., Pintado, M.E. and Castro, P.M.L. (2010) Valorisation of natural extracts from marine source focused on marine by-products: A review. *Food Res. Int.*, **43**, 2221–2233.
- Fredriksson, S., Elwinger, K. and Pickova, J. (2006) Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens. *Food Chem.*, **99**, 530–537.
- Ginzberg, A., Cohen, M., Sod-Moriah, U.A., Shany, S., Rosenshtrauch, A. and Arad, S. (2000) Chickens fed with biomass of the red microalga *Porphyridium* sp. have reduced blood cholesterol level and modified fatty acid composition in egg yolk. *J. Appl. Phycol.*, **12**, 325–330.
- González, C., García Álvarez, O. and Míguez Rodríguez, L. (1998) *Algas mariñas de Galicia: Bioloxía, gastronomía, industria*. Edicións Xerais de Galicia, S.A., Spain.
- Gouveia, L., Batista, A.P., Miranda, A., Empis, J. and Raymundo, A. (2007) *Chlorella vulgaris* biomass used as coloring source in traditional butter cookies. *Innovative Food Science & Emerging Technologies*, **8**, 433–436.
- Gualtieri, P. and Barsanti, L. (2006) *Algae: Anatomy, Biochemistry, and Biotechnology*. Taylor & Francis Group, Boca Raton.
- Guerin, M., Huntley, M.E. and Olaizola, M. (2003) Haematococcus astaxanthin: applications for human health and nutrition. *Trends Biotechnol.*, **21**, 210–216.
- Habib, M.A.B., Parvin, M., Huntington, T.C. and Hasan, M.R. (2008) A review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular No 1034. FAO, Rome.
- Hallmann, A. (2007) Algal transgenics and biotechnology. *Transg. Plant J.*, **1**, 81–98.
- Harun, R., Singh, M., Forde, G.M. and Danquah, M.K. (2010) Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable & Sustainable Energy Reviews*, **14**, 1037–1047.
- Harwood, J.L. and Guschina, I.A. (2009) The versatility of algae and their lipid metabolism. *Biochimie*, **91**, 679–684.
- Henrikson, R. (2009) *Earth Food Spirulina*. Ronore Enterprises, Hawaii.
- Herber-McNeill, S.M. and Van Elswyk, M.E. (1998) Dietary marine algae maintains egg consumer acceptability while enhancing yolk color. *Poultry Sci.*, **77**, 493.
- Herrero, M., Cifuentes, A. and Ibanez, E. (2006) Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae. *Food Chem.*, **98**, 136–148.
- Higuera-Ciapara, I., Felix-Valenzuela, L. and Goycoolea, F. (2006) Astaxanthin: a review of its chemistry and applications. *Crit. Rev. Food Sci. Nutr.*, **46**, 185–196.
- Hong, D., Hien, H. and Son, P. (2007) Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *J. Appl. Phycol.*, **19**, 817–826.
- Jin, E., Polle, J.E.W., Lee, H.K., Hyun, S.M. and Chang, M. (2003) Xanthophylls in microalgae: From biosynthesis to biotechnological mass production and application. *J. Microbiol. Biotechnol.*, **13**, 165–174.
- Kaliaperumal, N. (2003) *Products from Seaweeds*, SDMRI Research Publication N°3, India, 33.

- Kannaiyan, S. (2002) *Biotechnology of Biofertilizers*, Kluwer Academic Publishers, London.
- Khan, W., Rayirath, U.P., Subramanian, S., *et al.* (2009) Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.*, **28**, 386–399.
- Kumari, P., Kumar, M., Gupta, V., Reddy, C.R.K. and Jha, B. (2010) Tropical marine macroalgae as potential sources of nutritionally important PUFAs. *Food Chem.*, **120**, 749–757.
- Li, D. and Qi, Y. (1997) Spirulina industry in China: Present status and future prospects. *J. Appl. Phycol.*, **9**, 25–28.
- Liang, S., Liu, X., Chen, F. and Chen, Z. (2004) Current microalgal health food R & D activities in China. *Hydrobiologia*, **512**, 45–48.
- Lindsey Zemke-White, W. and Ohno, M. (1999) World seaweed utilisation: An end-of-century summary. *J. Appl. Phycol.*, **11**, 369–376.
- Lorenz, R.T. and Cysewski, G.R. (2000) Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. *Trends Biotechnol.*, **18**, 160–167.
- Margalith, P.Z. (1999) Production of ketocarotenoids by microalgae. *Appl. Microbiol. Biotechnol.*, **51**, 431–438.
- Mata, T.M., Martins, A.A. and Caetano, N.S. (2010) Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, **14**, 217–232.
- McHugh, D.J. (2003) A guide to the seaweed industry. FAO Fisheries Technical Paper. FAO, Rome.
- McLachlan, J. (1985) Macroalgae (seaweeds): industrial resources and their utilization. *Plant Soil*, **89**, 137–157.
- Metting, F.B., Jr (1996) Biodiversity and application of microalgae. *J. Industr. Microbiol. Biotechnol.*, **17**, 477–489.
- Muntean, E., Bercea, V., Dragos, N. and Muntean, N. (2007) Potential use of Mougeotia sp. algae in food production, based on its carotenoid content. *J. Agroaliment. Proc. Technol.*, **13**, 143–148.
- Naylor, J. (1976) Production, trade and utilization of seaweeds and seaweed products. *FAO Fisheries Technical Paper*, **159**, 1–71.
- Olaizola, M. (2003) Commercial development of microalgal biotechnology: from the test tube to the marketplace. *Biomolec. Eng.*, **20**, 459–466.
- Oren, A. (2005) A hundred years of Dunaliella research: 1905–2005. *Saline Systems*, **1**, 2.
- Osbourne, A.E. and Lanzotti, V. (2009) *Plant-derived Natural Products: Synthesis, Function, and Application*, Springer Verlag, Norwich.
- O'Sullivan, L., Murphy, B., McLoughlin, P., *et al.* (2010) Prebiotics from marine macroalgae for human and animal health applications. *Marine Drugs*, **8**, 2038–2064.
- Pereira, L., Critchley, A.T., Amado, A.M. and Ribeiro-Claro, P.J.A. (2009) A comparative analysis of phycocolloids produced by underutilized versus industrially utilized carrageenophytes (Gigartinales, Rhodophyta). *J. Appl. Phycol.*, **21**, 599–605.
- Perez-Garcia, O., Escalante, F.M.E., de-Bashan, L.E. and Bashan, Y. (2011) Heterotrophic cultures of microalgae: Metabolism and potential products. *Wat. Res.*, **45**, 11–36.
- Plaza, M., Herrero, M., Cifuentes, A. and Ibanez, E. (2009) Innovative natural functional ingredients from microalgae. *J. Agric. Food Chem.*, **57**, 7159–7170.
- Popescu, C., Iordan, M. and Cristian, B. (2007) Structure and properties of carragenan. *The annals of "Valahia" University of Targoviste*, **Fascicle VIII**, 27.
- Pulz, O. and Gross, W. (2004) Valuable products from biotechnology of microalgae. *Appl. Microbiol. Biotechnol.*, **65**, 635–648.
- Raja, R., Hemaiswarya, S. and Rengasamy, R. (2007) Exploitation of *Dunaliella* for  $\beta$ -carotene production. *Appl. Microbiol. Biotechnol.*, **74**, 517–523.
- Renn, D. (1997) Biotechnology and the red seaweed polysaccharide industry: status, needs and prospects. *Trends Biotechnol.*, **15**, 9–14.
- Sajilata, M.G., Singhal, R.S. and Kamat, M.Y. (2008) The carotenoid pigment zeaxanthin—a review. *Comp. Rev. Food Sci. Food Safety*, **7**, 29–49.
- Sijtsma, L. and Swaaf, M.E. (2004) Biotechnological production and applications of the  $\omega$ -3 polyunsaturated fatty acid docosahexaenoic acid. *Appl. Microbiol. Biotechnol.*, **64**, 146–153.
- Simopoulos, A.P. (1999) New products from the agri-food industry: the return of  $n$ -3 fatty acids into the food supply. *Lipids*, **34**, S297–S301.
- Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. (2006) Commercial applications of microalgae. *J. Biosci. Bioeng.*, **101**, 87–96.
- Stirk, W. and Staden, J. (1996) Comparison of cytokinin- and auxin-like activity in some commercially used seaweed extracts. *J. Appl. Phycol.*, **8**, 503–508.
- Trautwein, E. (2001)  $n$ -3 Fatty acids? physiological and technical aspects for their use in food. *Eur. J. Lipid Sci. Technol.*, **103**, 45–55.
- Wissgott, U. and Bortlik, K. (1996) Prospects for new natural food colorants. *Trends Food Sci. Technol.*, **7**, 298–302.
- Wood, C.G. (1974) Seaweed extracts. Unique ocean resource. *J. Chem. Educ.*, **51**, 449–452.
- Wroble, M., Mash, C., Williams, L. and McCall, R.B. (2002) Should long chain polyunsaturated fatty acids be added to infant formula to promote development? *J. Appl. Dev. Psychol.*, **23**, 99–112.

# 35

## A Dimensional Investigation on Seaweeds: Their Biomedical and Industrial Applications

Sudha Narayanan Parapurath<sup>1</sup>, Hebsibah Elsie Bernard<sup>2</sup>,  
Dhanarajan Malli Subramaniam<sup>3</sup> and Ramya Ramamurthy<sup>4</sup>

<sup>1</sup>Department of Chemistry, DKM College, Thiruvalluvar University, Vellore, Tamil Nadu, India

<sup>2</sup>Department of Biochemistry, DKM College, Thiruvalluvar University, Vellore, Tamil Nadu, India

<sup>3</sup>Jaya College of Arts and Science, Thirunindravur, University of Madras, Tamil Nadu, India

<sup>4</sup>Research Scholar, Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

### 35.1 Introduction

#### 35.1.1 Introduction to algae

Seaweeds (algae) are macroscopic marine subset of algae. There are about 10,000 species of seaweeds, of which 6000 are red algae (Rhodophytae), 2000 are brown algae and 2000 are green algae (Johnson, 2007). In Asia seaweeds have been consumed as a vegetable. Japanese eat 1.4 kg of seaweeds/person/day. In recent years, scientists have started looking towards marine organisms, especially seaweeds for the production of new drugs from natural products. These products are also increasingly being used in medical and biomedical research (Smit, 2004). For many years chemical preservatives have been used in food to act as antimicrobials or antioxidants or both. These chemical compounds are hazardous to health and cause asthma, cancer and suspected to be neurotoxic. Now seaweeds are used as a safer food preservative (Devi *et al.*, 2008).

#### 35.1.2 Types of seaweeds

The photosynthetic macrophytes are split into three major groups based on the pigment complement: green algae, brown algae, red algae.

##### *Green seaweeds*

The green color varies from brilliant grassy green to-dark greencolor. These seaweeds have a variety of morphologies, ranging from unicells and filaments to blades and fresh thalloid forms (Gabrielson *et al.*, 2000). In general they are found in intertidal zone and lower subtidal region. Some of the examples are *Ulva*, *Enteromorpha*, *Ascorsiphonia*, *Cladophora*, *Codium*, and *Prasiola*.

##### *Brown seaweeds*

They are typically brownish in color due to the presence of the pigments fucoxanthin,  $\beta$ -carotene and chlorophyll *a*

and *c.* They grow up to 50 m length and live up to 15 years. The brown seaweeds are economically and ecologically very important. The brown seaweeds are utilized as food products, in cosmetics, luxury spa items and as fertilizers. The cell wall is removed and utilized as emulsifiers, anticoagulants and in the production of textile and rubber. Examples of brown algae are *Sargassum* spp., *Fucus*, *Cystoseira* and *Pelvetiopsis* (Scagel, 1956).

### Red seaweeds

Most of the red seaweeds are found in fresh water. They have phycoerythrin and phycocyanin pigments, which give the spectrum of red color. They are utilized as food products. The cell wall of the component is used for agar and carrageenan production. Red algal byproducts are used in ice cream, toothpaste, etc., and can act as an antiviral agent (Richard *et al.*, 1978; Deig *et al.*, 1974; Luescher-Mattli, 2003). Common red seaweeds are *Mazzaella splendens*, *Porphyra* sp., *Chondracanthus exasperates*, *Microcladia coulteri*, *Mastocarpus* sp., *Prionitis lanceolata*, etc.

## 35.1.3 Components of algae

Seaweed has many secondary metabolites, which have antioxidant, antimicrobial, and antitumor properties. The secondary metabolites include flavonoids (which play a role in removal of toxins from skin), alkaloids and phenolic compounds (a powerful free radical scavenging agent) (Meenakshi *et al.*, 2009). It has minerals such as chromium, cobalt, copper, silicon, sodium, potassium, phosphorus, nickel, etc. It has vitamin A, vitamin B-complex, and vitamin C, which are powerful antioxidants. It has omega-3-fatty acids and omega-6-fatty acids, which prevent coronary heart diseases. It has different compositions of amino acids such as alanine, arginine, threonine, and serine, which are the building blocks of our body and act as an effective immune booster.

It has a high carbohydrate content (which boosts physical stamina) and nucleic acid that improves mental focus. 24-Hydroperoxy-24-vinyl-cholesterol was isolated from *Sargassum ringgoldianum* and *S. horneri*. It was the first isolation of this sterol from a plant source (Iwahori *et al.*, 2000). Methanol extracts of green algae belonging to the phyla Volvocophyta, Chlorophyta, and Charophyta, which live in freshwater habitats revealed the presence of D-norandrostane-16-carboxylic acid,  $\beta$ -sitosterol, and *trans*-phytol. The unsaturated fatty acids were found in larger proportion (54–94%) than the saturated fatty acids (6–40%). C15:0 and C16:0 were the most commonly occurring fatty acids, followed by C18:1, C19:1, C15:3, and C17:3.

These algae resembled green seaweeds in their fatty acid composition. They displayed a significant phytotoxic activity but non-significant cytotoxic, insecticidal, and antitumor activities (Ghazala and Shameel, 2005). Small amounts of D-sorbitol were extracted from 15 species of brown seaweeds. Sorbitol phosphate was also isolated (Miramand and Bentley, 1992).

### Carrageenans and gelatin

Carrageenans or carrageenins are a family of linear sulfated polysaccharides that are extracted from red seaweeds. *Chondrus crispus* is an industrial source of carrageenan. It is commonly used as a thickener and stabilizer in milk products such as ice cream and processed foods, including luncheon meat. In Europe it is indicated as E407 or E407b. It may also be used as a thickener in calico-printing and for fining beer or wine. Irish moss is frequently mixed with *Mastocarpus stellatus* (*Gigartina mammillosa*), *Chondracanthus acicularis* (*G. acicularis*), and other seaweeds with which it is associated in growth. Carrageenan and agar-agar are also used in Asia for gelatin-like desserts such as almond jelly. Presently, the major source of carrageenan is tropical seaweeds of the genera *Kappaphycus* and *Eucheuma*.

### Gelatin shots

A gelatin shot (usually called a Jell-O shot in North America and vodka jelly or jelly shot in the United Kingdom and Australia) is a shooter in which liquor, usually vodka, rum, tequila, or neutral grain spirit replaces some of the water or fruit juice that is used to congeal the gel (Regan, 2003).

### Agar

Agar, a product made from seaweed, is the traditional gelling agent in many Asian desserts. Agar is a popular gelatin substitute in quick jelly powder mix and prepared dessert gels that can be stored at room temperature. Compared to gelatin, agar preparations require a higher dissolving temperature, but the resulting gels congeal more quickly and remain solid at higher temperatures, 40°C (104°F).

There are three main commercial classes of carrageenan:

- Kappa forms strong, rigid gels in the presence of potassium ions; it reacts with dairy proteins. It is sourced mainly from *Eucheuma cottonii*.
- Iota forms soft gels in the presence of calcium ions. It is produced mainly from *Eucheuma spinosum*.
- Lambda does not gel and is used to thicken dairy products. The most common source is *Gigartina* from South America.

The primary differences that influence the properties of kappa, iota and lambda carrageenan are the number and position of the ester sulfate groups on the repeating galactose units. Higher levels of ester sulfate lower the solubility temperature of the carrageenan and produce lower strength gels, or contribute to gel inhibition (lambda carrageenan).

### 35.1.4 Nutritive value of seaweeds

Seaweeds are rich in polysaccharides (agar, carrageenans, ulvans, and fucoidans), which are not digested by humans and therefore can be regarded as dietary fiber (Lahaye and Thibault, 1990; Lahaye, 1991); water-soluble and water-insoluble fibers have been associated with different physiological effects. Many viscous polysaccharides (pectins, guar-gum, etc.) have been related with hypocholesterolemic and hypoglycemic effects. Water-insoluble polysaccharides (celluloses) are mainly associated with decreases in digestive tract transient time (Southgate, 1990). Among polysaccharides, fucoidans show particularly important biological activities and have potential and therapeutic applications (Charreau *et al.*, 1997; Nasu *et al.*, 1997; Angstwurm *et al.*, 1995). Laminarins are degraded by intestinal bacteria and lead to substantial production of short chain fatty acids. Seaweeds also contain micro and macro elements and contribute up to 30% of dry matter. *Fucus vesiculosus* is registered in the European pharmacopoeia for its high content of iodine. Fucals contain 500 to 1000 ppm dry weight of iodine. Seaweeds are utilized to reduce the high risk of calcium deficiency as the calcium content is as high as 7% of the dry weight in macroalgae and up to 25 to 34% in the chalky seaweed lithotamne.

Browns seaweeds have average of 5–15% dry weight of protein and green seaweeds have 10–30% dry weight of protein. In red seaweeds such as *Palmaria palmata* (dulse) and *porphyra tenera* (nori) protein content ranges from 35–47% of dry matter. *Ulva* species range from 15 to 20% dry weight and *Laminaria digitata*, *Ascophyllum nodosum*, *Fucus vesiculosus* and *Himanthalia elongata* have relatively low protein contents. *Spirulina* has high protein content – up to 70% of dry matter. Several papers have described *in vivo* and *in vitro* studies by proteolyzing enzymes such as pepsin, pancreatin, and pronase (Fujiwara-Arasaki, 1979; Ryu *et al.*, 1982). The seaweeds with high phenolic content have limited availability of protein. (Boussiba and Richmond, 1979). These have antioxidant properties and are used in prevention of treatment of neurodegenerative diseases caused by oxidative stress, gastric ulcers, and cancer (Gonzalez *et al.*, 1999; Padula and Boiteux, 1999; Ramirez *et al.*, 1999).

Seaweeds have high lipid and fatty acid (1–5% of dry matter). The red and brown algae are rich in fatty acids with

20 carbon atoms (eicosapentaenoic acid, EPA  $\omega$ -3C20:4 and arachidonic acid AA,  $\omega$ -6C20:4). *Spirulina* is a rich source of gamma linolenic acid (GLA), which is the precursor of prostoglandins, leukotrienes, and thromboxane, and are important for immunological and inflammatory and cardiovascular diseases (Renaud *et al.*, 1999; Fleurence, 1999).

## 35.2 Biomedical applications of seaweeds

### 35.2.1 Biomedical importance of seaweeds

The marine algae are essential in nature and directly valuable to humans since they have antimicrobial, antiviral, antitumor, anticoagulant, fibrinolytic, and antioxidant properties (Cannell, 1990; Guven *et al.*, 1991; Honya *et al.*, 1994; Fleurence, 1999). Seaweed fucoidans have all the essentials against the effects of stress, the relief of pain and in the treatment of diseases. The fluid extracts can be applied to broken skin, in the treatment of sprains and bruises. Dried seaweed powder can be mixed with Vaseline or wheatgerm oil for the treatment of swollen and stiff joints. Algin is used as a dressing to aid wounds that are having trouble healing. In the dental field it is used to make tooth impressions.

#### Treatment for various diseases

Halogenated furanones from *Delisea pulchra* are used as antifouling components (Smit, 2004). Ethanol extracts of brown seaweeds have antiallergic activities. They include *Sargassum tennerimum* (ST), *Sargassum cervicorne* (SC), *Sargassum graminifolium* turn (SG), *Sargassum thunbergii* (STH), and *Laminaria japonica* (LJ) (Samee *et al.*, 2009).

Seaweed strengthens circulation, balances blood pressure, lowers cholesterol, builds healthy blood, increases the veins' and heart's contractile force, restores and increases cardiac efficiency, nourishes and prolongs the life of the heart muscle, and encourages rhythmical working of the heart in all its aspects: physical, emotional, and inspirational. Daily use of seaweed provides optimum nourishment for the hormonal, lymphatic, urinary, and nervous systems. The hormonal system uses minerals and trace elements so richly available from seaweed to repair tissue, build new cells, and create hormones responsible for regulating blood pressure, metabolism, fertility, sexuality, and reaction to allergens.

The lymphatic and immune systems are avid users of seaweed compounds. The urinary system gets a special boost from seaweed's seeming excess of potassium and sodium: aiding in recovery from cystitis, kidney weakness, gout,

diabetes, kidney disease, and bladder weakness. Seaweed helps the digestive system by soothing, disinfecting, and nourishing irritated surfaces such as mucosa, helping out with the metabolism of lipids, and maintaining a healthy balance of digestive yeasts and bacteria in the intestines.

### Ulcer and allergy

Seaweed is an excellent medicine for gastric ulcers, duodenal ulcers, ulcerative colitis, colitis, constipation, watery stools, and other intestinal diseases and also for osteoporosis, breast cancer, mastitis, uterine cancer, irregular menstrual cycles, ovarian cancer, fibroids, ovarian cysts, infertility, fibrocystic breast conditions, and premenstrual/menopausal problems, such as water retention, emotional mood swings, chills and hot flashes, fatigue, lack of lubrication, loss of calcium, and general irritability. Seaweeds have antiradiation, anticancer, antioxidant, antitoxic, antirheumatic, antibiotic, antibacterial properties.

Studies on the health effects of fucoidan extracted from nine species of brown seaweed revealed that all fucoidans delivered anti-inflammatory effects. Bladderwrack-derived fucoidans appeared to prevent breast cancer cells from adhering to platelets, suggesting that these substances could help inhibit the spread of cancer. In *in vitro* research, scientists have found that fucoidan may possess anticoagulant (blood-thinning) properties. However, these findings also indicate that brown seaweed may help prevent blood clotting (Wong, 2010).

### 35.2.2 Antioxidant properties of seaweeds

During normal metabolism different types of oxygen derivatives such as superoxide anion, singlet state oxygen, and hydroxyl radicals, together with peroxides and transition metals are produced. These metabolites have a degenerative effect. Derivatives of molecular oxygen are the principal cause of cell damage. Antioxidants have an important preventative activity against oxidative damage and have excellent free radical scavenging effects.

The commercially available synthetic antioxidants have side effects, so the searches for antioxidants from natural sources has intensified in recent years (Duh *et al.*, 1992; Osawa and Namiki, 1985). Consuming seaweeds as a sea vegetable has been a long tradition all over the world (Jimenez-Escrig and Sanchez-Muniz, 2000; Nagai and Yukimoto, 2003). The potential antioxidant compounds in brown algae have been identified as fucoxanthin in *Hijikier fusidormis* and polyphosphorytin in *Eisemia bicyclis* (Kuda *et al.*, 2005).

*Sargassum boveanum* is a marine species present in coastal waters that has expressed high antioxidant activity (Jimenez-Escrig and Sanchez-Muniz, 2000; Sohrabi pour

and Rabii, 1999). *Sargassum marginatum*, *Padina tetrastomatica* and *Turbinaria conoides* are Indian brown seaweeds that show the presence of antioxidant properties. *Gracilaria chagii*, a marine alga from Malaysia has free radical scavenging effects (Sreenivasan *et al.*, 2007).

Preparations of *Kappaphycus alverzi*, *Gracilaria edulis*, and *Gelidium acerosa*, which were previously known for their nutritive value and are now identified with free radical scavenging effects. Antioxidants are very important in scavenging the free radicals that are responsible for cerebrovascular disease (Halliwell and Gutteridge, 1985), allergies and cardiovascular diseases (Wichi, 1988).

Antioxidants also prevent damage to DNA and RNA, which leads to cell injury and tissue damage and its associated diseases. Seaweeds with antioxidant properties also reduce the oxidant stress and reduce ischemic injury and inflammation (Athukorala *et al.*, 2003; Lim *et al.*, 2002). Antioxidant activity of *Hippophae rhamnoides* reduces the major toxic compounds of cigarette smokers that induces oxidative stress both *in vivo* and *in vitro* as well as other complications from smoking (Suleyman *et al.*, 2002). The antioxidant properties was investigated by DPPH-decolorization assay (Viturro *et al.*, 1999) and inhibition of lipid peroxidation  $Fe^{2+}$ /ascorbate assay.

### 35.2.3 Antibacterial effects of seaweeds

Seaweeds also have antimicrobial activity against human pathogens like viruses, fungi, and yeast. Extract of seaweeds belonging to Chlorophyceae (*Ulva lactuca*, *Halimeda gracilis*), Rhodophyceae (*Gracilaria edulis*, *Hypnea musiformis*) and Phaeophyceae (*Turbinaria conoides*, *Sargassum myricystum*) families are effective against human pathogen such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterococcus faecalis* (Lima-filo *et al.*, 2002).

Seaweeds like *Sargassum wightii*, and *Gracilaria edulis*, which have nutritional value also have antimicrobial properties (Vallinayagam *et al.*, 2009). Petroleum, ether, and methanol extracts of *Codium decorticatum* show the presence of secondary metabolites like saponins, photosterols, alkaloids, and glycosides and antibacterial activities against Gram-positive bacteria such as *Streptococcus pneumoniae* and *Staphylococcus aureus* and Gram-negative bacteria such as *E. coli*, *K. pneumoniae*, *Salmonella typhi*, and *Pseudomonas aeruginosa* (Anbu Jeba Sunilson *et al.*, 2009).

### 35.2.4 Antiviral properties of seaweeds

The naturally occurring sulfated carbohydrate molecule that is present in seaweeds has numerous physiological

activities (Franz *et al.*, 2000; Smit, 2004) including antiviral properties against some viruses like herpes simplex virus (HSV) type-1 and type-2, human cytomegalovirus, human immunodeficiency virus type-1, respiratory syncytial virus, and influenza virus (Mazumder *et al.*, 2002). Fucoidans from the brown seaweed *Adenocystis utricularis* have antiviral activity (Ponce *et al.*, 2002).

### 35.2.5 Heme-agglutinating properties of seaweeds

'Lectins', agglutinins or heme-agglutinins are proteins that have at least one non-catalytic domain that binds reversibly to a specific mono- or oligosaccharide (Peumans and Damme, 1998). The occurrence of agglutinin in extract of marine algae was first described by Boyd *et al.* (1966). The presence of lectin in extracts of marine algae has been reported in various studies (Benevides *et al.*, 1999). Twenty-eight brown seaweeds contain agglutinins that show activity against animal and human erythrocytes. Three brown seaweeds are found to have hemagglutinin activity: such as *Ectocarpus confervoides*, *Giffordia granulosa*, and *Cutleria multifida*. The highest agglutinin activity was found with rabbit erythrocytes, with maximum titers of *Fucus serratus*, *Laminaria saccharina*, and *Himanthalia elongata*. The species *Giffordia granulosa* showed certain specificity against human erythrocytes. The hemagglutinin activity of the brown seaweeds are polyphenolic in nature. Agglutinin activity of the seaweeds can be used as a taxonomic index (Fabregas *et al.*, 1986).

### 35.2.6 Hepatoprotective and anticancer properties

Seaweed extracts are found to be an effective hepatoprotective agents. Ethanolic extract of edible brown algae *Ecklonia stolonifera*, *Sargassum* species has hepatoprotective activity because of the presence of phlorotannins, which protects the Hep G2 cell against cytotoxic effects (Kim *et al.*, 2005). It also acts as an anticancer agent because of the presence of antioxidant properties of secondary metabolites (Halliwell and Gutteridge, 1985; Wichi, 1988). The secondary metabolites prevent the free radical formation and tissue damage thus preventing the spreading of cancer cells, especially melanoma and leukemia.

The natural product from marine macroalgae in Rhodophyta Phaeophyta and Chlorophyta are identified to have the biological and pharmacological activities. Kahalalide F from the *Bryopsis* is used as a possible treatment for lung cancer, tumors and acquired immune deficiency syndrome (Smit, 2004). *Laminaria japonica* has fucoxan-

thin, which is an antitumor promoter and inhibitor of the growth of neuroblastoma cells GOTO and of colon cancer (Liquin *et al.*, 2008). Polyphenolic components of *Halimeda macroloba* and *Halimeda opuntia* have a free radical scavenging effect and an inhibitory role of various stages of tumor development in an animal study (Yoshie *et al.*, 2002).

Sulfated fucans are the sulfated polysaccharides of *S. polycystum*, *S. oligocystum*, *S. mcclurei*, *S. swartzii*, and *Denticaprum* were extracted and fractionated on a DEAE-Sephadex A-25 column. On the basis of chemical and spectral analyses, the fucoidan fractions obtained were found to be sulfated fucogalactans containing sulfate ester groups and uronic acid, and composed essentially of fucose and galactose, as well as a minor amount of other sugars. The polysaccharide fractions were tested and found to have anticancer activity. The primarily obtained results showed that all fucoidan fractions isolated from *S. swartzii* demonstrated bioactivity effects against cancer cells, while fraction F5 with highest sulfate content exhibits the strongest anti-invasion activity.

This indicated that sulfate content plays an important role in the anticancer activity of the brown algal fucoidans (Ly *et al.*, 2005). Researchers found that dietary intake of bladderwrack (brown seaweed) produced antiestrogenic effects in three premenopausal women. According to the study's findings the authors suggest that bladderwrack may help reduce risk of estrogen-related cancers.

### 35.2.7 Seaweed consumption and weight loss

Even unprocessed dry seaweeds have healing benefits and therapeutic uses as well as the extract. The compound present in wakame (brown kelp, *Undaria pinnatifida*), which is used in the normal diet has fucoxanthin that promotes weight loss. These fucoxanthins are found mostly in edible brown seaweeds. The fucoxanthins induce expression of the fat burning protein UCP1 (uncoupling protein 1) that accumulates in fat tissue around internal organs and adipose tissue. UCP1 helps in oxidation of fatty acids (Indergaard, 1983). *Laminaria japonica* has fucoxanthin, which reduces white adipose tissue and enhances weight loss (Liquin *et al.*, 2008).

Fucoxanthin has strong antidiabetic effects by promoting docosahexaenoic acid (DHA) produced in the liver. DHA reduces the level of bad cholesterol associated with obesity and heart disease as demonstrated by Mi-Jin Yim *et al.* (2010). Brown seaweeds decrease the concentration of triglycerol in serum and liver without side effects. It has potential health benefits on cancer. Fucoxanthin helps in the death of human prostate cancer cells in culture

(Indergaard, 1983). Brown seaweeds are used for treatment of thyroid disorder since they contain di-iodo thyronine (DIT) (Suzuki *et al.*, 1965).

### 35.3 Industrial applications of seaweeds

#### 35.3.1 Seaweeds as a fertilizer

The brown seaweeds that are available in large quantities like *Ascophyllum* and *Ecklonia* are used as soil conditioners and fertilizers since they have a typical N:P:K ratio. These fertilizers are commercially available. Maerl is a fertilizer derived from red seaweeds (*Phymatolithon calcareum* and *Lithothamnion corallioides*), which gives positive results to many applications. They are resistant to pests and improve the yield of cultivation (Indergaard, 1983).

Seaweed fertilizers increase seed germination and assist plants to get more nutrients from the soil. They enhance resistance against frost and build the resistance to diseases and insect pests. They enhance root development and condition the soil. The seaweed suspension obtained from *Codium tomentosum* (green algae), *Gracilaria gracilis* (red algae) and *Cystoseira barbata* (brown algae) show the highest effects on seed germination of tomato, potato, and egg-plant (aubergine) at all temperatures (Demir, Dural and Yildirim, 2006). Seaweed fertilizers (about 12 000+ varieties in the ocean) have been identified to be valuable additions to the organic garden and can be abundantly available free for those living near the coast.

Seaweed, particularly bladderwrack, kelp or laminaria, can be either applied to the soil as a mulch (it tends to break down very quickly) or can be added to the compost heap where it is an excellent activator (Coleby-Williams, 2006). In terms of soil structure it does not add a great deal of bulk, but its jelly-like alginate content helps to bind soil crumbs together, and it contains all soil nutrients (0.3% N, 0.1% P, 1.0% K, plus a full range of trace elements) and amino acids. As well as fresh seaweed, dried “meal” forms or concentrated liquid extract, which is active in significantly smaller rates, are available commercially. Seaweed fertilizer is known as *vraic*. The activity of collecting *vraic* is being termed “vraicking”. In Scotland, it is used as fertilizer in lazybeds or *feannagan*.

#### 35.3.2 Seaweeds for cosmetics and agar production

Seaweeds, either in the form of liquid or powder, are added to cosmetics like face wash, body cream, lotion, etc. and found to be more effective than any other synthetic cos-

metics (Indergaard, 1983). *Gelidium* and *Gracilaria* species and other algae can be used as a raw material for agar productions (food-grade agar), which are commercially available. High-grade agar is used extensively as a bacteriological medium and in cell culture. Lower quality agar is used in the food and pharmaceutical industries and also used as gelling agent, preservatives, or stabilizer (Johnson, 2007).

#### 35.3.3 Seaweeds used for wastewater treatment

Seaweeds are used to remove phosphorus and nitrogen from waste water and to remove the toxic metals from industrial waste water and thus act as a potential purifier of waste water. Green seaweeds such as *Enteromorpha* and *Monostroma* are successfully used commercially in the removal of phosphorus and nitrogen on a large scale (Indergaard, 1983).

The purification process includes

##### 1 Removal of nitrogen

Nitrification (aerobic conditions)



Denitrification (anaerobic conditions)



##### 2 Removal of phosphorus.

This is an anaerobic process and the phosphorus-removing bacteria use dehydrolytic reactions (Neori *et al.*, 2000). Nitrogen is also removed by using macroalgae biofilters (Msuya and Neori, 2002). Seaweed utilizes phosphorus for its growth since the seaweeds cannot directly assimilate and utilize some organic matter, especially larger molecules; it offers an expanded adhesive base for microorganisms and the formation of a mutual subsystem. Brown algal cells are very porous and easily permeable to small ionic species. The bioadsorption process by seaweeds offers an advantage in low operating costs, minimization of the volume of chemical or biological sludge to be disposed of, and high efficiency in detoxifying very dilute effluent with no nutrient requirement. It increases the water quality by reducing the transparency, reducing chemical oxygen demand (COD), biochemical oxygen demand (BOD), and total suspended solids (TSS) (Belmont *et al.*, 2004; Redding *et al.*, 1997; Baruah *et al.*, 2006).

Brown seaweeds like *Ecklonia*, *Macrocystis*, and *Laminaria* are found to absorb copper, nickel, lead, zinc, and

cadmium ions from solution and to remove toxic metals from industrial waste water. Depending upon the concentration of metal in the waste water the process is done so economically. Seaweed filtration thus has the potential to improve the efficiency and productivity of recirculating aquaculture, via enhanced culture conditions and the production of economically valuable biomass (Cahill *et al.*, 2010). Aluminum removal has been investigated in synthetic and real waste waters from an aluminum surface treatment plant. Marine algae, obtained as beach cast seaweed (a refuse substance) were used as adsorption material. The influence of pH, metal concentration and time for aluminium elimination was studied by use of synthetic solutions by Lodeiro *et al.* (2010).

### 35.3.4 Seaweed as a fuel

Seaweeds like *Laminaria*, *Gracilaria*, and *Sargassum* are the biomass that is converted to methane by anaerobic fermentation and it was found that *Macrocystis* produced more gas (methane) than could be burnt to produce energy (Indergaard, 1983). Seaweeds are also used in the glass and soap industries. Brown seaweed *Fucus serratus* (Phaeophyta) is often employed in single species toxicity testing and to study the association between the pollutant and the biota in contaminated marine habitats. (Nielsen *et al.*, 2005).

## 35.4 Conclusion

The study of algae (phycology) in the exploitation of seaweed resources has attracted the attention of scientists all over the world because of the possible economic uses in various fields. This review described the various applications of seaweeds, and further detailed study can be extended toward isolation and purification of the unidentified compounds, and used in the medical and industrial fields.

## Acknowledgment

The Authors thank the authorities of DKM College, Thiruvalluvar University, Vellore, Tamil Nadu, India for their support.

## References

Anbu Jeba Sunilson, J., Suraj, R., Anandarajaopal, K. *et al.* (2009) Preliminary phytochemical analysis, elemental determination and antibacterial screening of *Codium decorticatum* – a marine green alga. *Int. J. Biol. Chem.*, **3**, 84–89.

Angstwurm, K., Weber, J.R., Segert, A. *et al.* (1995) Focoidin, a polysaccharide inhibiting leukocyte rolling, attenuates inflammatory responses in experimental pneumococcal meningitis in rats. *Neurosci. Lett.*, **191**, 1–4.

Athukorala, Y., Lee, K.-W., Song, C. *et al.* (2003) Potential antioxidant activity of marine red alga *Grateloupia filicina* extracts. *J. Food Lipids*, **10**, 251–265.

Baruah, K., Norouzitallab, P. and Sorgeloos, P. (2006) Seaweeds: an ideal component for wastewater treatment for use in aquaculture. *Aquaculture Europe*, **31**, 3–6.

Belmont, M.A., Cantellano, E., Thompson, S. *et al.* (2004) Treatment of domestic wastewater in a pilot-scale natural treatment system in central Mexico. *Ecol. Eng.*, **23**, 299–311.

Benevides, N.M.B., Oliveira, S.R.M., Holanda, M.L. *et al.* (1999) Seasonal variations in Hemagglutinating activity and chemical composition of two red marine algae *Gracilaria domigensis* and *Gelidium pusillum*. *Revista Brasileira de Fisiologia Vegetal*, **11**, 91–95.

Boussiba, S. and Richmond, A.E. (1979) Isolation and characterization of phycocyanins from the blue-green alga *Spirulina platensis*. *Arch. Microbiol.*, **120**, 155–159.

Boyd, W.C., Almodovar, L.R. and Boyd, L.G. (1966) Agglutinin in marine algae for human erythrocytes. *Transfusion*, **6**, 82–83.

Cahill, P.L., Hurd, C.L. and Lokman, M. (2010) Keeping the water clean — Seaweed biofiltration outperforms traditional bacterial biofilms in recirculating aquaculture. *Aquaculture*, **306**, 153–159.

Cannell, R.J.P. (1990) Algal biotechnology. *Appl. Biochem. Biotechnol.*, **26**, 85–105.

Charreau, B., Blondin, C., Boisson-Vidal, C. *et al.* (1997) Efficiency of fucans in protecting porcine endothelial cells against complement activation and lysis by human serum. *Transplant. Proc.*, **29**, 889–890.

Coleby-Williams J. (2006) Fact Sheet: Seaweed Fertiliser. *Gardening Australia*. ABC. <http://www.abc.net.au/gardening/stories/s1805258.htm> (accessed 20 April 2011).

Deig, E.F., Ehresmann, D.W., Hatch, M.T. *et al.* (1974) Inhibition of herpesvirus replication by marine algae extract. *Antimicrob. Agents Chemother.*, **6**, 524–525.

Demir, N., Dural, B. and Yildirim, K. (2006) Effect of seaweed suspensions on seed germination of tomato, pepper, and aubergine. *J. Biol. Sci.*, **6**, 1130–1133.

Devi, K.P., Suganthi, N., Kesika, P. and Pandian, S.K. (2008) Bioprotective properties of seaweeds: in vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. *BMC Complement. Altern. Med.*, **8**, 38.

- Duh, P., Yeh, D. and Yen, G. (1992) Extraction identification of an antioxidative component from peanut hulls. *J. Am. Oil Chem. Soc.*, **69**, 814–818.
- Fabregas, J., Muñoz, A. and Llovo, J. (1986) Hemagglutinins in brown seaweeds. *J. Exp. Mar. Biol. Ecol.*, **97**, 213–219.
- Fleurence, J. (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.*, **10**, 25–28.
- Franz, G., Pauper, D. and Alban, S. (2000) Pharmacological activities of sulfated carbohydrate polymer. In: *Bioactive Carbohydrates Polymers* (ed. B.S. Paulsen). Kluwer Academic Publishers, Dordrecht, pp. 47–58.
- Fujiwara-Arasaki, T. (1979) Proteins of two brown algae, heterochordaria abietina and laminaria japonica. *J. Jap. Soc. Food Nutr.*, **32**, 408–412.
- Gabrielson, P.W., Widdowson, T.B., Lindstron, S.C. *et al.* (2000) Keys to the benthic marine algae and seagrasses of British Columbia, southeast Alaska, Washington and Oregon. *Phycol. Contrib.*, **5**, 1–189.
- Ghazala, B. and Shameel, M. (2005) Phytochemistry and bioactivity of some fresh water green algae from Pakistan. *Pharm. Biol.*, **43**, 358–369.
- Gonzalez, R., Rodriguez, S., Romay, C. *et al.* (1999) Anti-inflammatory activity of Phycocyanin extract in acetic acid-induced colitis in rats. *Pharmacol. Res.*, **39**, 55–59.
- Guven, K.C., Ozsoy, Y. and Ulutin, O.N. (1991) Anticoagulant, fibrinolytic and antiaggregant activity of carageenans and alginic acid. *Bot. Mar.*, **34**, 429–432.
- Halliwell, B. and Gutteridge, J.M.C. (1985) *Free Radicals in Biology and Medicine*. Clarendon Press, Oxford.
- Honya, M., Kinoshita, T., Tashima, K. *et al.* (1994) Modification of the M/G ratio of alginic acid from *Laminaria japonica* areschong cultured in deep seawater. *Bot. Mar.*, **37**, 463–466.
- Indergaard, M. (1983) The aquatic resource. I. The wild marine plants: a global bio resource. In: *A Biomass Utilization* (ed. W.A. Cote). Plenum Publishing Corporation, New York, pp. 137–168.
- Iwahori, Y., Okada, Y., Tanaka, J. and Okuyama, T. (2000) Using chitin column chromatography, isolation of 24-hydroperoxy-24-vinyl-cholesterol from sargassaceae seaweeds. *Nat. Med.*, **54**, 265–267.
- Jimenez-Escrig, A. and Sanchez-Muniz, F.J. (2000) Dietary fiber from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res.*, **20**, 585–598.
- Johnson, C.R. (2007) Seaweed invasion: introduction and scope. *Bot. Mar.*, **50**, 321–325.
- Kim, Y.C., An, R.B., Yoon, N.Y. *et al.* (2005) Hepatoprotective constituents of the edible brown algae Ecklonia stolonifera on tacrine-induced cytotoxicity in Hep G2 cells. *Arch. Pharm. Res.*, **28**, 1376–1380.
- Kuda, T., Tsunekawa, M., Goto, H., and Araki, Y. (2005) Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *J. Food Comp. Anal.*, **18**, 625–633.
- Lahaye, M. (1991) Marine algae as sources of fibers: determination of soluble and insoluble dietary fibre contents in some 'sea vegetables'. *J. Sci. Food Agric.*, **54**, 587–594.
- Lahaye, M. and Thibault, J.F. (1990) Chemical and physicochemical properties of fibers from algal extracts by-products. In: *Dietary Fibers: Chemical and Biological Aspects* (eds D.A.T. Southgate, K. Worldron, I.T. Johnson and G.R. Fenwick). Royal Society of Chemistry, Cambridge, UK, pp. 68–72.
- Lim, S.N., Cheung, P.C.K., Ooi, V.E.C. and Ang, P.O. (2002) Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum*. *J. Agric. Food Chem.*, **50**, 3862–3866.
- Lima-filo, J.V.M., Carvalho, A.F.F.U. and Freitas, S.M. (2002) Antibacterial activity of extract of six macroalgae from the northeastern Brazilian coast. *Braz. J. Microbiol.*, **33**, 311–313.
- Liquin, Y., Pengcheng, L. and Shoujin, F. (2008) The extraction of pigments from fresh Laminaria japonica. *Chin. J. Oceanol. Limnol.*, **26**, 193–198.
- Lodeiro, P., Gudiña, Á., Luz Herrero *et al.*, (2010). Aluminium removal from wastewater by refused beach cast seaweed. Equilibrium and dynamic studies. *J. Hazard. Mater.*, **178**, 861–866.
- Luescher-Mattli, M. (2003) Algae, a possible source for new drugs in the treatment of HIV and other viral diseases. *Curr. Med. Chem. – Anti-Infective Agent*, **2**, 219–255.
- Ly, B.M., Buu, N.Q., Nhut, N.D. *et al.* (2005) Studies on Fucoidan and its production from Vietnamese brown seaweeds. *ASEAN J. Sci. Technol. Dev.*, **22**, 371–380.
- Mazumder, S., Ghosal, P.K., Pujol, C.A. *et al.* (2002) Isolation, chemical investigation and antiviral activity of polysaccharides from *Gracilaria corticate* (Gracilariaceae, Rhodophyta). *Int. J. Biol. Macromol.*, **31**, 87–95.
- Meenakshi, S., Manicka Gnanambigai, D., Tamilmozhi, S. *et al.* (2009) Total flavanoid and in vitro antioxidant activity of two seaweeds of Rameshwaram coast. *Global J. Pharmacol.*, **3**, 59–62.
- Miramand, P. and Bentley, D. (1992) Heavy metal concentrations in two biological indicators (*Patella vulgata* and *Fucus serratus*) collected near the French nuclear fuel reprocessing plant of La Hague. *Science of the Total Environment*, **111**, 135–149.
- Msuya, F.E. and Neori, A. (2002) Ulva reticulata and Gracilaria crassa: microalgae that can biofilter effluent from tidal fish ponds in Tanzania. *W. Ind. Ocean J. Mar. Sci.*, **1**, 117–126.

- Nagai, T. and Yukimoto, T. (2003) Preparation functional properties of beverages made from sea algae. *Food Chem.*, **81**, 327–332.
- Nasu, T., Fukuda, Y., Nagahira, K. *et al.* (1997) Fucoidin, a potent inhibitor of L-selectin function, reduces contact hypersensitivity reaction in mice. *Immunol. Lett.*, **59**, 47–51.
- Neori, A., Shpigiel, M. and Ben-Ezra, D. (2000) A sustainable integrated system for culture of fish, seaweed and abalone. *Aquaculture*, **186**, 279–291.
- Nielsen, H.D., Burrige, T.R., Brownlee, C. and Brown, M.T. (2005) Prior exposure to Cu contamination influences the outcome of toxicological testing of *Fucus serratus* embryos. *Mar. Poll. Bull.*, **50**, 1675–1680.
- Osawa, T. and Namiki, M. (1985) Natural antioxidants isolated from Eucalyptus leaf waxes. *J. Agric. Food Chem.*, **33**, 775–780.
- Padula, M. and Bioteux, S. (1999) Photodynamic DNA damage induced by phycocyanin and its repair in *Saccharomyces cerevisiae*. *Braz. J. Med. Biol. Res.*, **32**, 1063–1071.
- Peumans, W.J.A. and Damme, E.J.M. (1998) Plant lectins: specific tools for identification, isolation and characterization of O-linked glycans. *Crit. Rev. Biochem. Mol. Biol.*, **33**, 209–258.
- Ponce, N.M.A., Pujol, C.A., Damonte, E.B. *et al.* (2002) Fucoidans from the brown seaweed *Adenocystis utricularis*: extraction methods, antiviral activity and structural studies. *Carbohydr. Res.*, **338**, 153–165.
- Redding, T., Todd, S. and Midlen, A. (1997) The treatment of aquaculture wastewaters - a botanical approach. *J. Env. Manag.*, **50**, 283–299.
- Regan, G. (2003) *The Joy of Mixology*. Clarkson Potter, New York, pp. 15–16.
- Remirez, D., Gonzalez, A., Merino, N. *et al.* (1999) Effects of phycocyanin in zymosan-induced arthritis in mice—phycocyanin as an antiarthritic compound. *Drug Dev. Res.*, **48**, 70–75.
- Renaud, S.M., Thinh, L.-V. and Parry, D.L. (1999) The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. *Aquaculture*, **170**, 147–159.
- Richard, J.T., Kern, E.R., Glasgow, L.A. *et al.* (1978) Antiviral activity of extracts from marine algae. *Antimicrob. Agents Chemother.*, **14**, 24–30.
- Ryu, H.S., Satterlee, L.D. and Lee, H.H. (1982) Nitrogen conversion factors and in vitro protein digestibility of some seaweeds. *Bull. Korean Fish. Soc.*, **15**, 263–270.
- Samee, H., Li, Z.-X., Lin, H. *et al.* (2009) Anti-allergic effects of ethanol extracts from brown seaweeds. *Journal of Zhejiang University Science B*, **10**, 147–153.
- Scagel, R.F. (1956) Introduction of a Japanese alga, *Sargassum muticum*, into the northeast Pacific. *Fisheries Research Paper St. Washington*, **1**, 49–58.
- Smit, A.J. (2004) Medicinal and pharmaceutical uses of seaweed natural products: a review. *J. Appl. Phycol.*, **16**, 245–262.
- Sohrabi pour, J. and Rabii, R. (1999) A list of marine algae of seashores of Persian Gulf and Oman sea in the Hormozgan province. *Iran. J. Bot.*, **8**, 131–162.
- Southgate, D.A.T. (1990) Dietary fibre and health. In: *Dietary Fibre: Chemical and Biological Aspects* (eds D.A.T. Southgate, K. Waldron, I.T. Johnson and G.R. Fenwick). Royal Society of Chemistry, Cambridge, UK, pp. 282–284.
- Sreenivasan, S., Ibrahim, D. and Mohd. Kassim, M.J.N. (2007) Free radical scavenging activity and total phenolic compounds of *Gracilaria changii*. *Int. J. Nat. Eng. Sci.*, **1**, 115–117.
- Suleyman, H., Gumustekin, K., Taysi, S. *et al.* (2002) Beneficial effects of *Hippophae rhamnoides* L. on nicotine induced oxidative stress in rat blood compared with vitamin E. *Biol. Pharm. Bull.*, **25**, 1133–1136.
- Suzuki, H., Higuchi, T., Sawa, K. *et al.* (1965) Endemic coast goiter in Hokkaido, Japan. *Acta Endocrinol.*, **50**, 161–176.
- Vallinayagam, K., Arumugam, R., Raupathi Raja Kannan, R. *et al.* (2009) Antibacterial activity of some selected seaweeds from Pudumadam coastal region. *Global J. Pharmacol.*, **3**, 50–52.
- Vituro, C., Molina, A. and Schmede-Hirschmann, G. (1999) Free radical scavengers from *Mutsia friesiana* (Asteraceae) and *Sanicula graveoleans* (Apiaceae). *Phytother. Res.*, **13**, 422–426.
- Wichi, H.P. (1988) Enhanced tumor development by butylated hydroxyanisole (BHA) from the prospective of effect on fore stomach and oesophageal squamous epithelium. *Food Chem. Toxicol.*, **26**, 717–723.
- Wong, C. (2010) Health benefits of brown seaweed. *Alternative Medicine Guide*. About.com.
- Yim, M.-J., Hosokawa, M. and Miyashita, K. (2010) Regulation of adipocyte differentiation by marine allene carotenoids. In: *Proceedings of 101st AOCS Annual Meeting and Expo*. <http://www.aocs.org/archives/am2010/index.cfm?page=101st-AOCS-Annual-Meeting-Program-Tech-Prog&interest=Health%20and%20Nutrition>
- Yoshie, Y., Wang, W., Hsieh, Y.P. and Suzuki, T. (2002) Compositional difference of phenolic compounds between two seaweeds, *Halimeda* spp. *Journal of Tokyo University of Fisheries*, **88**, 21–24.

# 36

## Seaweed Polysaccharides – Food Applications

**Vazhiyil Venugopal Menon**

*Seafood Technology Section, Food Technology Division, Bhaba Atomic Research Center, Mumbai, India*

### 36.1 Introduction

Development of food products to satisfy consumer interests depends on appropriate uses of various ingredients at required levels to impart proper texture and flavor as well as storage stability to the processed products. Polysaccharides immensely contribute to food technology through their interesting functional properties, which can be exploited to impart attractive properties to foods. Besides, polysaccharides also enhance fiber contents and have potential to function as edible coatings to foods retaining their shelf lives. The wide sources of polysaccharides include those from terrestrial plants, seaweeds, crustacean shellfish, and microorganisms. This article will discuss food applications of major polysaccharides from seaweed. At the onset some basic aspects of polysaccharides as food additives will be briefly pointed out.

### 36.2 Major functions of polysaccharides in a food system

#### 36.2.1 Water-binding capacity

Polysaccharides are able to bind large amounts of water, because of the presence of various functional groups in their molecules (Mitchell, 1998). For example, alginates contain

carboxylic groups; carrageenans, sulfonic groups; and chitosan, amino groups, which help these polysaccharides bind water several times their weights, making them referred to as 'hydrocolloids'. Through this property these compounds are able to modify texture, control of syneresis (a phenomenon of separation of water during freeze–thawing of foods), and to stabilize the food matrix. Furthermore, the ability to bind water also helps polysaccharides function as a cellular scaffold, biodegradable packaging material, and carriers of drugs and nutraceuticals (Tharanathan, 2002; Kavanagh and Ross-Murphy, 1998)

#### 36.2.2 Gelation

The ability to undergo gelation is probably the most important functional property of polysaccharides. A gel is composed of at least two components, where a polymer forms a three-dimensional network in a liquid medium such as water. A minimum amount of water is required for ensuring flexible and elastic properties of the gel. The process is reversible; melting of the gel is therefore possible by heating. Such gels may be covalently cross-linked networks or physical gels that involve non-covalent interactions, such as hydrogen bonding, hydrophobic, and ionic interactions among the constituents. Food gels are mostly physical gels resulting from cooling of heated solutions of polymers; their minimum concentration required for gelation varies from 0.1 to 15% (w/w). The gels can be rigid, flowing,

brittle, sparingly, firm, soft, spreadable, sliceable, rubbery, or grainy, depending upon the degree of interactions of polymers. (Nishinari and Zhang, 2004; Barbucci *et al.*, 2002; Morris, 1998).

### 36.2.3 Emulsions and foams

Emulsions and foams represent fine dispersions of oil, water, or air (droplets and air bubbles) in an immiscible liquid. Food emulsions can be mainly of three types:

- 1 *Oil-in-water* or *water-in-oil* emulsions. A system that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water (O/W) emulsion, whereas a system that consists of water droplets dispersed in an oil phase is called water-in-oil (W/O) emulsion).
- 2 *Foam*, in which air (gas) bubbles are dispersed in an aqueous medium.
- 3 *Sol*, which is small solid particles dispersed in liquid medium.

Multilayer emulsions of oil and water such as W/O/W and O/W/W are also possible. Most food emulsions are O/W emulsions, which include milk, cream, mayonnaise, sauces, salad dressing; custard, cake batter, mayonnaise, and sauces; butter; margarine, and spreads are examples of W/O emulsions. Since emulsion results in a large interfacial area between two immiscible phases (usually oil and water), they are thermodynamically unstable; tending to undergo phase separation over time due to gravitational separation, flocculation, coalescence and other reasons, gravitational separation being most common in food emulsions. Emulsifiers are molecules that facilitate the formation and stabilization of emulsions. Polysaccharides are able to function as emulsifiers. During emulsification, large deformable drops must be broken down, which can be accomplished using homogenizers such as high shear mixers, high-pressure homogenizers, colloid mills, ultrasonic homogenizers, and membrane homogenizers. The science and technology of emulsions have been described (Friberg *et al.*, 2007; McClements, 2006; Norton and Foster, 2002)

## 36.3 Interactions of polysaccharides with food components

Interactions of polysaccharides with food components have profound influence on the quality and stability of processed

foods. Apart from their structural features, physicochemical factors such as pH, ionic strength, temperature, pressure, shearing rate, mixing time, ratio of polysaccharide to other food components such as proteins, their charges and molecular weights dictate these interactions. Food polysaccharides may also interact among themselves to give mixed polymer gels that impart novel texture to food products. The minimum concentration of polysaccharide for gelation usually decreases when another incompatible biopolymer is added, presumably due to an exclusion effect. Careful selection of hydrocolloid types and their concentrations can lead to the formation of a broad range of gel textures. Methodologies to understand polysaccharide–polysaccharide and polysaccharide–protein interactions include differential scanning calorimetry (DSC), rheometry, UV absorption and circular dichroism (CD) measurements (de Kruif and Tuinier, 2001; Narchi and Djelvehl, 2009; Dickinson, 2008).

## 36.4 Major food applications of polysaccharides

Gelation and other functional properties of polysaccharides, briefly discussed above, make them valuable additives as thickening and texture modifiers, stabilizers, water retention compounds, emulsifiers, foam stabilizers, binders of ingredients, and modifiers of viscosity. They also control syneresis and starch retrogradation, enhance flavor, retard crystal growth, replace fat and improve satiety and fiber contents of foods. Hydration (solubility, viscosity), structure (aggregation, gelation) and surface (foaming, emulsifying) properties of polysaccharides and their complexes can be exploited to optimize the sensory properties of foods. Food texture is perceived when the food materials are stirred, poured, pumped, stretched and finally, eaten. Mixed dispersions of polysaccharides may evoke entirely new oral sensations as compared with those containing individual biopolymers (Williams and Phillips, 2003). These changes may be evaluated by sensory analysis, which use trained panelists to evaluate specific textural attributes such as ‘hardness’ and ‘stickiness’ and/or by ‘texture profile analysis’ using texturometers, which gives a force–displacement curve obtained from a double compression test providing interpretation to a number of texture features such as hardness, cohesiveness, viscosity, elasticity, adhesiveness, brittleness, chewiness and gumminess (Walkenstrom, 2003). Polysaccharides, at concentrations varying from 0.1 to 1.0% contribute significantly to development of food emulsions and foams to aid in keeping solids dispersed in medium such as chocolate in milk, air in whipping creams and carbonated soft drinks, fat in salad dressings, canned meats

or fish, marshmallows and jelled candies, ice cream, sauces and dressings. In bakery products, they are used to enhance dough strength and stability, preserve freshness, viscoelastic properties and other quality criteria such as increased water absorption, specific and loaf volume. They can also replace the wheat protein, gluten, without adversely affecting the texture and also control retrogradation of starch. Polysaccharides retain volatile flavor compounds in many food systems, ranging from wine to salad dressing and dessert gels (Koliandris, 2008). Besides, polysaccharides can also function as dietary fiber and have potential for use as biodegradable edible films and encapsulation materials possessing excellent barrier properties against moisture and gases. Many of them also exhibit antimicrobial and/or antioxidant properties (Venugopal, 2011; Walkenstrom, 2003). Being of natural origin, they are quite safe, unlike many synthetic food additives.

### 36.4.1 Seaweed polysaccharides

Seaweeds are widespread throughout the world's oceans. They have traditionally been used as food in several parts of the world, especially in East and South-east Asia. They enjoy wide popularity as food due to their low calorie contents, high amounts of fiber and minerals such as potassium, magnesium, iron, and also iodine. Some of the popular edible seaweed species include nori (laver) (*Porphyra* spp.), Irish moss (*Chondrus* spp.), kombu (*Laminaria* spp.), wakame (*Undaria* spp.), and dulse (*Euchema* spp.). Nori (laver and also called purple laver, or sea tangle) is commonly eaten, especially by the Japanese. Blanched and salted seaweed prepared from wakame (*U. pinnatifida*) is another popular product that has high dietary fiber content and has health benefits, including protection against diabetes and fat-burning properties. The brown seaweed, *Sargassum* (also called gulfweed and sea holly) is used in soups. Because of their commercial importance, some seaweed species such as nori (*Porphyra* spp.), kombu (*Laminaria* spp.) and wakame (*Undaria* spp.) are also grown by aquaculture. Processed *Euchema* seaweed (PES) also known as Philippines Natural Grade (PNG), semirefined carrageenan (SRC), alternatively refined carrageenan (ARC) or alkali modified flour (AMF) prepared from *E. cottonii* and *E. spinosum* contains polysaccharides, particularly, carrageenan, imparting significant water- and fat-holding capacities and hence making it an interesting food additive. Other products include 'Modifilan', a patented commercial extract of *Laminaria* spp. (contains significant amounts of organic iodine, fucocanthine, alginate, fucoidan and laminarin). Microparticles of red seaweed *Gracilaria rhodophyta*, developed by

high speed shearing techniques, have potentials as low-cost fat replacers for food and texturizers for beverages. The composition of seaweed and their nutritional values have been discussed recently in detail (Braune and Guiry, 2011; Venugopal, 2009a; Kumar *et al.*, 2008; McHugh, 2003; Rudolph, 2000; Ito and Hori, 1989). Seaweed species are rich sources of polysaccharides, which include agar, alginate and carrageenans, which are being isolated for their food and other applications (Kohajdová *et al.*, 2007; Sen, 2005; Roller and Dea, 2002). Incorporation of these polysaccharides have shown numerous functional benefits, as indicated in Table 36.1.

The major seaweed polysaccharides and their food applications are described below:

#### Agar

The term 'agar' (synonymous with agar-agar, the Japanese gelatin, Japanese isinglass, vegetable gelatin and angel's hair) accumulates in the cell walls of agarophyte red seaweed; its content varying with seasons. Agar is mostly extracted from seaweed such as *Gracilaria* spp. and *Gelidium* spp. belonging to the Rhodophyceae. For extraction, the algae are boiled (4–10 h) in acidified water (pH 5–6) containing bleaching agents. After extraction, the sediments are allowed to settle and the crude extract is filtered out usually under pressure. The extract is dried outdoors and the crude agar is subjected to repeat freezing and thawing, draining out the liquid formed that contain salts and other impurities followed by further drying in the sun for 15 to 30 days. The dried agar is packed as strips, threads or shredded or powdered form and graded according to color, luster, gel strength, etc. Agar from the crude extract may also be precipitated by alcohol. The yield varies with species and ranges between 30–35% (Venugopal, 2009b, 2011; Li *et al.*, 2008; Chattopadhyay, 2007; Naidu, 2000; Wheaton and Lawson, 1985). Commercial agar is shiny, semitransparent, tasteless and odorless, having less than 20% moisture and about 7% ash. It is a hydrophilic colloid, composed of two polysaccharides, agarose and agarpectin. Agarose consists of alternating 1,4-linked 3,6 anhydro- $\alpha$ -L-galactopyranose and 1,3-linked  $\beta$ -D-galactopyranose. Agarpectin is more complicated in structure and contains sulfonic, pyruvic, and uronic acids. Agar contains 3–5% sulfate groups. Agar forms a strong gel, due to coil-helix transition followed by aggregation of helices, holding water molecules within the interstices, when a hot aqueous solution of agar is cooled. Difference in gelling (32–40 °C) and melting (85 °C) temperatures of agar, known as "hysteresis", makes it useful in food, microbiological and pharmaceutical applications (Rodriguez *et al.*, 2009; Prasad *et al.*, 2007; Lahaye and Rochas, 1991).

**Table 36.1** Functional claims made by seaweed polysaccharides in diverse food products

Product categories	Functional claims by major seaweed polysaccharides	
Baked goods	Alginate	Forms gel
Beverages		Emulsifier
Confectionery		Fat replacer
Dairy		Water binding agents
Desserts		Controls syneresis
Dressings and dips		Provides smooth texture
Fried foods		Creates creamy mouth feel
Frozen foods		Enhances fiber content
Meat analogs		Antioxidant activity
Meat products		Antimicrobial activity
Pasta		Increase yield
Restructured products		Reduce production costs
Sauces and gravies	Carrageenan	Forms heat stable gel
Snack foods		Controls syneresis
Soups		Emulsifier
		Adds to mouth feel
		Adds viscosity
		Antioxidant activity
		Antimicrobial activity
		Anti-browning activity
		Moisture retention
		Enhances texture
	Agar	Forms creamy and smooth gel
		Enhances fiber content
		Increase yield
		Reduce production costs
		Forms gel
		Syneresis control
		Emulsifier
		Adds texture
		Reduces sugar bloom
		Enhances fiber content
		Increase yield
		Reduce production costs

### Food uses

Agar is a popular thickener, gelling agent, stabilizer, lubricant, emulsifier, and absorbent. Unlike starch, agar is not readily digested and therefore has little calorific value. Al-

though agar costs more than some synthetic and natural gelling agents, it is usually superior to such products because of its greater transparency, strength and stability over a range of acidities and alkalinities. Food grade agar is used as a stabilizer in canned meat, confectionery, and glazing and as icing in the baking industry. It is also used to make jellies, puddings, and custards. Interaction of agar with sugar increases the strength of the gel, by a phenomenon called “sugar reactivity”. Because of its bland taste, agar does not interfere with the flavors of foodstuffs. The popular Japanese sweet dish, *mitsumame* consists of cubes of agar gel containing fruit and added colors. It can be canned and sterilized without the cubes melting. In Indian cuisine, agar is known as “China grass” and is used for making desserts. It has been used to clarify wines, especially plum wine, which is difficult to clarify by traditional methods. It improves the texture of dairy products like cream cheese and yoghurt (Meena *et al.*, 2006; Armisen, 1999).

In bakery products, agar modifies starch properties resulting in reduction in starch pasting temperatures (which could be measured by the amylograph parameters). This property is important since it indicates an early start of starch gelatinization and, in turn, an increase in the susceptibility of starch to the enzyme amylase. Due to its high water-binding properties agar also helps improves crust characteristics such as texture, color and moisture, and viscoelastic characteristics of baked products. In addition, it improves dough stability during fermentation and hence causes increase in the specific volume. The effects of the hydrocolloid in this respect, however, were highly dependent on flour types (white and whole flours) and the bread making process. Agar is usually added at 0.8% (w/w) level in baked goods and baking mixes; 2.0% in confections and frostings; and 1.2% in soft candies. As an emulsifier, it exhibits both synergistic and antagonistic interactions among antistaling additives. It is well known that the characteristic textures of bread and other baked products are due to the interactions between the gluten protein of wheat and its polysaccharides (starch, pentosans). However, certain population is intolerant to gluten, reflected as celiac disease. Agar can replace gluten to develop gluten-free breads for these individuals. Besides, it can also function as fat replacers in such breads (Kohajdova and Karovcova, 2009; Guarda *et al.*, 2004).

With the incidences of bovine spongiform encephalopathy (BSE, or mad cow disease) and foot-and-mouth disease, agar, is finding a role as substitutes for bovine gelatin in jelly candies, marshmallows, puddings, fruit batters, and jams (Karim and Rajiv, 2009). Because of its higher melting temperature and gel strength, the polysaccharide is added to frozen desserts of fruit juice, soy, water or milk, and ice cream at about 0.1% (w/v), often in combination with

gum tragacanth and locust bean gum (LBG). An amount of 0.1–1% (w/v) agar stabilizes yoghurt, some cheeses, and candy and pastry fillings. It is also added to desserts and pre-treated instant cereal products. In confectionery, jelly-type candies are made with agar, at concentrations ranging from 0.3 to 1.8% by weight. An agar-containing diet has been developed for obesity and for patients with impaired glucose tolerance and type 2 diabetes. The diet resulted in marked weight loss due to the maintenance of reduced calorie intake (Maeda *et al.*, 2005). The various food uses of agar have been discussed recently (Venugopal, 2011). There is recent interest to modify the gelling properties and solubility of agar for novel food uses. Microparticles of agar have been prepared by high-speed shearing of gels dispersed in cold water. The product can confer a range of textures to fluid gels including beverages (Ellis and Jacquier, 2009). Table 36.2 shows the major applications of agar in food processing.

### Alginic acid and alginates

The term ‘algin’ or ‘alginate’ is used as a generic name for the salts of the alginic acid such as sodium, potassium, ammonium, calcium, and propylene glycol alginates. The important algal sources of alginate are the brown seaweed species, *Macrocystis pyrifera*, *Ascophyllum nodosum* and *Laminaria* spp., *Ecklonia maxima*, *E. cava*, *E. bicyclis*, *Lessonia nigrescans*, and *Sargassum* spp (Draget *et al.*, 2005). The two popular methods for production of alginate are the Green and

Le Gloahec-Herter processes (Sen, 2005; Owusu-Apenten, 2004). In the Green’s process, fresh alga is demineralized with 0.3% aqueous HCl, pulverized and treated with an aqueous 8% soda ash (pH 10–11). The treated material is ground well, diluted with water and allowed to settle. The supernatant is heated to 50 °C, passed through a filter press and then mixed with 10–12% aqueous CaCl<sub>2</sub>, when the insoluble calcium alginate floats to the surface. It is separated, bleached with 10% aqueous sodium hypochlorite, drained and mixed with 5% HCl. The precipitated alginic acid is thoroughly washed and is converted to desired salt by treatment with appropriate carbonate, oxide or hydroxide, which is dried, grounded and packed. (McHugh, 2003; Zvyagintseva, 1999; Wheaton and Lawson, 1985)

Alginates are linear unbranched polymers containing  $\beta$ -(1→4)-linked D-mannuronic acid (M) and  $\alpha$ -(1→4)-linked L-guluronic acid (G) residues. The ratio of D-mannuronic to L-glucuronic acids in alginic acids vary with type of seaweed, its age, portions of the seaweed used, and its location. Alginic acid is essentially insoluble in water; monovalent ions such as sodium and ammonium interact with the carboxyl groups of alginic acid to form water-soluble salts. G blocks are believed to be important since they determine binding capacity of alginate with Ca<sup>2+</sup>. Molecular weights of alginic acid range between 32 and 200 kDa. Biochemical and biophysical properties of alginate are dependent on the molecular weights and G:M ratios, which are usually in the range of 1.45 to 1.85 (Draget *et al.*, 2005; Clementi, 1999). Addition of divalent alkali metal ions (Ba<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Sr<sup>2+</sup>, etc.) induces gelation of alginate, which occurs without any heating or cooling. During gelation junction zones are formed due to formation of metal ions cross-links with guluronic acid residues from adjacent poly-G chains. The structure of the alginate gels has been described by the so-called “egg-box model”, in which each divalent cation (e.g., Ca<sup>2+</sup>) is coordinated to the carboxyl and hydroxyl groups of four guluronate monomers from two adjacent chains of the polymer (Rastall, 2001).

### Food uses

Alginic acid and alginates are used as thickening and stabilizing agents in a variety of food products, which include salad dressings, sauces, syrups, milk shakes, ice cream toppings, pie fillings, cake mixes, canned meat, and vegetables. Alginate is used in a variety of other gel products such as cold instant puddings, fruit gels, dessert gels, onion rings, among others. The polysaccharide prevents formation of large crystals in ice cream during storage and improves stability of salad dressings, milk chocolates, and fresh fruit juice, among others. Sodium alginate had better stabilizing effect improving textural quality and acceptance of

**Table 36.2** Applications of agar in food products

Food products	Applications
Bakery items	Texture improvement, stabilization of dough, reduction of pasting temperatures of starch, replacement of gluten, prevention of adhering of product to packages. Micro-particles of agar are particularly useful for texture modification
Dairy and related products	Stabilizes yoghurt, cheeses, and candy and pastry fillings. In confectionery, jelly type candies are made with agar
Vegetable products	Improves texture Replacement of gelatin. Reduction of torque in extrusion cooked products
Miscellaneous	Hydrogels of agar (and also alginates and carrageenans) can enhance texture. Enhances satiety and hence useful as dietetic foods

ice creams during prolonged storage. Furthermore, alginate provides uniform viscosity during aging, lighter color, smoother and cleaner melt down and better flavor. One of the major uses of alginate is as suspending agent for cocoa in chocolate milk. It is used as a foam stabilizer in beer and cider and emulsifier in high oil salad dressings. The whipping ability of mixes containing alginate is significantly more than that of similar mixes containing gelatin. It is also used in making soft cheese spreads at 0.1–0.2%. The colloid should be dissolved in hot water and added to the cream before pasteurization. The hydrocolloid is also used in several bakery products such as icing, filling, marshmallow toppings, jellies, glazes, syrup, and bread. It is also added to puddings, cheese spreads, and confectioneries. In most of these products it helps to retain moisture, while in some others it thickens the batter, besides aiding moisture retention. The disadvantage of alginate, namely its insolubility when added to cold mixes, could be overcome by warming the mixture to 68–70 °C before its addition. The remarkable gelling properties of alginic acid have also found unique application in restructured foods due to its interactions with proteins. Wherever alginate is required at high levels for specific functionality, such as stabilization of emulsion, less expensive hydrocolloids such as xanthan could be used to partially replace alginate to reduce the costs. Propylene glycol alginate (PGA) is the only commercial chemically modified alginate (Onsoyen, 1997; Lee *et al.*, 2009).

Alginate and also other hydrocolloids (xanthan and  $\kappa$ -carrageenan) at concentrations as low as 0.1% (w/w, flour basis) improve properties of bread in terms of specific volume index, width/height ratio, crumb hardness, sensory characteristics (visual appearance, aroma, flavor, crunchiness) and overall acceptability. The hydrocolloids also prevent staling in bread stored for 24 h, retain moisture content and reduce the dehydration of crumb during storage. Further, the syneresis of the wheat flour gel was significantly reduced during freeze-thaw treatments when alginate was incorporated. (Kohajdova and Karovcova, 2009). The alginate-containing wheat noodles exhibit an increase in the cooked weight and a decrease in the cooking loss, besides significant increase in the cutting and tensile forces (Lee *et al.*, 2009). Coating with alginate prevents loss of quality of onion rings during storage enabling providing extended shelf-life (Hershko and Nussinovitch, 1998).

Restructured meat products are made by binding meat pieces together and shaping them to resemble usual cuts of meat, such as nuggets, roasts, meat loaves, and even steaks. A mixture of sodium alginate along with calcium carbonate, lactic acid and calcium lactate can bind the meat pieces together (Chidanandaiah *et al.*, 2009). Alginate, along with carrageenan (see below), could be used to develop low-fat, precooked, beef patties. These products are comparable

with respect to yields and textural properties to regular beef patties having 20% fat (Weilin and Keeton, 1998). If beef cuts are coated with calcium alginate films before freezing, the meat juices released during thawing are reabsorbed into the meat. The coating also prevents bacterial contamination of the product (McHugh, 2003). Similarly, alginate can also be used to modify the texture of restructured shrimp, crab or fish meat products, which contain proteins such as soy protein concentrate and seafood flavors. The ingredient mixture is extruded into a calcium chloride bath to form edible fibers which are chopped, coated with sodium alginate and shaped in a mold. Alginate at 0.5 % (w/w) helped retain water-holding capacity of raw whiting muscle and also prevented increase in toughness of the minced fillets stored at –18 °C for 2 months. Calcium alginate, besides modifying texture also functioned as a cryoprotectant in frozen fish products to control denaturation of proteins (Perez-Mateos *et al.*, 2002; Lian *et al.*, 2000). Freezing fatty fish such as herring and mackerel in calcium alginate jelly controls rancidity development during storage (McHugh, 2003).

Alginate gels in the form of sponges can be useful as a carrier of vitamin A. Administration of the sponges to children having endemic vitamin A deficiency enhanced their vitamin A level (Reifen *et al.*, 1998). A low viscosity soybean beverage was prepared through a lactic acid fermentation of soy milk with *Lactobacillus casei*. Incorporation of PGA along with calcium lactate suppressed fermentation-related undesirable powdery-gritty sensation and provided emulsion stability to the product (Sugimoto *et al.*, 1982). Alginate in foods also functions as dietary fiber, since it is non-digestible. It reduces intestinal absorption, increases satiety, reduces glycemic index value, modulates colonic microflora, elevates colonic barrier function, and stimulates the immune system. Consumption of alginate at a rate of 10 g once a day for two weeks has beneficial effect on the levels of bifidobacteria, which increase significantly, while the levels of Enterobacteriaceae decrease. Because of these benefits, alginates are components of slimming diet foods, particularly biscuits (Brownlee *et al.*, 2005). Table 36.3 depicts common uses of alginates in food products.

### Carrageenan

The anionic polysaccharide carrageenan is classified into three industrially relevant types, kappa ( $\kappa$ ), iota ( $\iota$ ) and lambda ( $\lambda$ ). A hybrid form consisting of  $\kappa$ - and  $\iota$ -carrageenans is also found. Main sources of carrageenans are red seaweed such as *Gigartina*, *Chondrus*, *Euchema*, and *Furcellaria* spp. belonging to the Rhodophyceae, the principal species being *E. cottoni*, *E. spinosum*, *C. crispus* (known as Irish moss), and *G. stellata*. *Chondrus* spp. are abundant

**Table 36.3** Common uses of alginates in food products

Applications	Remark
Foam stabilizer in beer	Propylene glycol alginate (PGA) provides better head retention and prevents foam-negative contaminants
Texturized foods	Endows food products with thermostability and desired consistency
Other uses	PGA is acid stable and resists loss of viscosity. Has unique suspension and foaming properties. Hence used in soft drinks, milk drinks, sorbet, ice cream, noodles, pasta, etc.
Bakery products	Provides freeze-thaw stability and reduced syneresis to some products
Fruit preserves	Commonly used as thickening, gelling, stabilizing agents in jams, marmalades and fruit sauces. Alginate-pectin gels are heat reversible and gives better gel strength than individual components
Ice cream	Gives ideal viscosity, prevents crystallization and shrinkage, help homogenous melting without whey separation. Used in combination with other gums
Others	Desserts, emulsions (e.g., low fat mayonnaise) and sauces, extruded foods (noodles and pasta)

in the Atlantic coast of North America, particularly Canada, while *Eucheuma* spp. occurs in the Philippines, Indonesia and East Africa (McHugh, 2003). The relative content of carrageenan in seaweed depends on the algal source, season of its harvest as well as on the extraction procedure used. For isolation of the polysaccharide, the thoroughly washed seaweed is subjected to extraction with dilute hot calcium or sodium hydroxide. Carrageenan is precipitated from the supernatant using isopropyl alcohol, dried under vacuum, ground and packed (Hilou *et al.*, 2006; McHugh, 2003; Sen, 2005). Alkali treatment, however, could influence the sulfate and 3,6-anhydro-D-galactose contents of the sample (McHugh, 2003).

Commercial carrageenans, which are usually mixtures of  $\kappa$ -,  $\iota$ - and  $\lambda$ -carrageenans, have molecular weights in the range of  $10^5$  to  $10^6$  Da. The three carrageenans differ prominently in their sulfate group contents.  $\kappa$ -Carrageenan has only one sulfate ester group, making it less hydrophilic and less soluble in water. The

polysaccharide is composed of D-galactose, 3,6-anhydro-D-galactose and ester bound sulfate in a molar ratio of 6:5:7.  $\lambda$ -Carrageenan has no 3,6-anhydrogalactose, but has three sulfate groups, and hence readily soluble in water due to good hydrophilic character.  $\iota$ -Carrageenan is intermediate with a 3,6-anhydrogalactose and two sulfate ester groups. The structure of  $\lambda$ -carrageenan consists of an alternating disaccharide repeating unit of (1-3)-linked  $\beta$ -D-galactopyranosyl 1-4-sulfate and (1-4)-linked-3,6-anhydro- $\alpha$ -D-galactopyranosyl 1,2-sulfate residues.  $\kappa$ - and  $\iota$ -carrageenans exist as right-handed, threefold helices that form double helices reversibly. The double helical segments can then interact to form a three-dimensional network. (McHugh, 2003; Belitz *et al.*, 2004; MacArtain *et al.*, 2003; Angelin *et al.*, 2004) Upon heating and subsequent cooling,  $\iota$ - and  $\kappa$ -carrageenan form thermoreversible gels in the presence of gel-promoting cations. Salts enhance the interaction effect in the following order:  $\text{Na}_2\text{SO}_4$ , NaCl, KCl, and  $\text{NH}_4\text{Cl}$ .  $\text{K}^+$  salts must be added to the system before cooling below the gelling temperature (García-García and Totosaus, 2008). Carrageenans are strongly negatively charged over the entire pH range usually encountered in food. As pH value decreases below 6.0, carrageenan solutions become increasingly unstable when heated, resulting in loss of viscosity due to irreversible cleavage of polymer chains (MacArtain *et al.*, 2003; Angelin *et al.*, 2004). These aspects need to be considered in developing foods using the polysaccharide as food additive. Measurement of carrageenans in food: challenges, progress, and trends in analysis have been discussed (Roberts, and Quemener, 1999).

### Food uses

Carrageenans are ideal food additives. They have a range of gelling and emulsifying properties. The important applications of the hydrocolloid as additives in foods include modification of texture, reduction of fat and salt, enhancement of storage stability and flavor, fiber contents, antioxidant and antimicrobial activities, among others. The concentrations of the additive required for these applications range from 0.005–3% (w/w) (Venugopal, 2011). They have a high reactivity with a range of materials including, most importantly, milk proteins, being widely used at low concentrations in dairy products to prevent fractionation of milk constituents. Formation of a stable complex between carrageenan and protein is through the sulfate groups of carrageenan with anions groups in proteins (Verbeke *et al.*, 2004). Carrageenans allow textural modification of diverse food products through changes in water binding, emulsifying and foaming properties. Gelation properties and interactions of the polysaccharide with other food components including cations significantly influence these

**Table 36.4** Comparison of properties of carrageenans

Medium	$\kappa$ -carrageenan	$\iota$ -carrageenan	$\lambda$ -carrageenan
Hot water	Soluble at $> 60^{\circ}\text{C}$	Soluble at $> 60^{\circ}\text{C}$	Soluble
Cold water	Sodium salt soluble K and Ca salts insoluble	Sodium salt soluble K and Ca salts give thixotropic dispersion	Sodium salt soluble
Hot ( $80^{\circ}\text{C}$ ) milk	Soluble	Soluble	Soluble
Cold ( $20^{\circ}\text{C}$ ) milk	Na, Ca, Ki salts insoluble, but swells	Insoluble	Soluble, thickens
Gelation	Gels, strongest with $\text{K}^{+}$	Gels strongest with $\text{Ca}^{2+}$	No gelation
Concentrated sugar solution	Soluble, when hot	Soluble with difficulty	Soluble, when hot
Concentrated salt solution	Insoluble	Soluble, when hot	Soluble, when hot
Stability			
Freeze–thaw	No	Yes	Yes
pH $> 5$	Stable	Stable	Stable
Syneresis	Yes	No	No
Salt tolerance	Poor	Good	Good

Source: Adapted from Rudolph (2000).

modifications. The net effects could be additive or sometimes, synergistic. Addition of limited amounts of carrageenan has been found to be useful to reduce fat-free palatable, healthier, and convenient third-generation foods. Functional properties of carrageenans in food products, however, depend on types of carrageenan. These properties are also influenced by process variables such as temperature, pH, ionic strength, cations, etc. The  $\iota$ -carrageenan is particularly thixotropic, that is, a gel that has been broken will reform if left for sometimes without disturbance.  $\iota$ -Carrageenan is often used in cold-filled, ready to consume desserts. The carrageenan is also freeze–thaw stable. To avoid agglomeration, the carrageenans are often premixed with high concentrations of other ingredients such as sugar, usually in a ratio of 1:10. If premixing is not possible stirring with a high-speed mixer together with slow addition of carrageenan can prevent agglomeration. In instant preparations, carrageenan must be used as a powder to be mixed with cold water, when a thickening effect is caused by the swelling of the hydrocolloid. In solution, with a high content of soluble solids ( $>50\%$ ), the temperature is increased to a level favoring gelation of the polysaccharide. Mixed gels of LBG and carrageenan are brittle, slightly elastic gels, whereas xanthan gum and  $\kappa$ -carrageenan form soft cohesive gels. Combinations of carrageenan with LBG and starch could be used in sausages for improved texture (Soumya and Ryan, 2003). Carrageenan at 0.25 to 0.75% helped develop low-fat ground pork patties that had better cooking yield and higher moisture contents. The polysaccharide can also significantly reduce NaCl without affect-

ing texture and sensory properties of the products. (García-García and Totosa, 2008; Manish Kumar and Sharma, 2004) Table 36.4 compares properties of carrageenans with respect to their food uses.

Carrageenan can also function as a fat substitute in food products including emulsified cheese spreads, meat balls and beef frankfurters, among others (Mahungu *et al.*, 2002). Lambda carrageenan at 0.1 to 0.5% (w/w) levels suppressed release of aroma compounds including aldehydes, esters, ketones and alcohols in thickened viscous solutions containing 10% of sucrose (Bylaite *et al.*, 2004). Similarly,  $\kappa$ -carrageenan enhanced flavor in a formulation containing a mixture of spices, hydrolyzed vegetable protein, and salt (Mahungu *et al.*, 2002). Carrageenan at a concentration of 0.5 to 0.7% can be used to increase fiber content of low-fiber foods such as fishery products (e.g., salmon burger), besides improving their texture (Borderías *et al.*, 2006).  $\kappa$ -Carrageenan and its oligosaccharides can also provide significant antioxidant activities to foods (Yuaw *et al.*, 2005; Venugopal, 2011). Carrageenans ( $\iota$ -,  $\kappa$ -, or  $\lambda$ ) at 0.1% level with 0.5% citric acid synergistically prevent browning in apple juice and dried apples containing 0.1% sodium benzoate for up to 3 months at  $3^{\circ}\text{C}$  (Tong and Hicks, 1991). Carrageenans, particularly  $\iota$ -carrageenan, can possess antimicrobial (bacteriostatic) activities against food-borne pathogens such as *Salmonella enteritidis*, *S. typhimurium*, *Vibrio mimicus*, *Aeromonas hydrophila*, enterotoxigenic *Escherichia coli* O157:H7 and *Staphylococcus aureus*. The anionic properties of carrageenan, due to its sulfate content, play an essential role in this effect. This property can be

**Table 36.5** Typical dairy applications of carrageenans

Product	Function	Product	Use level (%)
<i>Milk gels</i>			
Cooked flans or custards	Gelation	K, K + I	0.20–0.30
Cooked prepared custards (with TSPP)	Thickening Gelation	K, I, L	0.20–0.30
<i>Pudding and pie fillings</i>			
Dry mix cooked with milk	Level starch gelatinization	K	0.10–0.20
Ready-to-eat	Syneresis control, bodying	I	0.10–0.20
Whipped products	Stabilize overrun	L	0.05–0.15
Aerosol whipped cream	Stabilize overrun and emulsification	K	0.02–0.05
<i>cold prepared milks</i>			
Instant breakfast	Suspension, bodying agent	L	0.10–0.20
Shakes	Suspension, bodying, overrun	L	0.10–0.20

'K', k-carrageenan; 'I', i-carrageenan; and, 'L', λ-carrageenan.

Adapted from Rudolph (2000).

used to control pathogens in poultry and meat products (Yamashita *et al.*, 2001).

In dairy products carrageenan is preferred over other gums to impart texture for functional and economic reasons. The polysaccharide stabilizes cocoa particles and fat suspensions, preventing separation of fat in chocolate milks. It also prevents separation of whey in ice cream while thawing. Such stabilizing interactions are also useful in producing condensed milk, infant formula and whipped creams. Carrageenan of the λ type, which is insensitive to cations, gives evaporated skim milk consistency like that of cream. Carrageenans can be used to improve sensory properties of dairy products such as water dessert gels, whipped toppings, instant whipped desserts, and egg-less custards and flavors; they also function as emulsifiers and stabilizers. At a concentration as low as 0.025% a weak thixotropic gel is formed in milk via interaction of κ-carrageenan with casein micelles, by a phenomenon, known as “milk reactivity”. Because of this, the thickening effect of κ-carrageenan in milk is 5 to 10 times greater than it is in water λ-Carrageenan has the ability to disperse in milk at 5–10 °C and thicken it without any salts (Verbeken *et al.*, 2004). Carrageenans offer smoothness and a sensation of richness to cheese, ice creams and egg-less milk puddings, preventing separation of fat and syneresis. Carrageenan gels do not require refrigeration because they do not melt at room temperature. ι-Carrageenan at concentrations of 0.15–0.25% (w/w) was effective in preventing syneresis and increasing the rigidity of processed cheeses in comparison with κ-carrageenan. The effect of carrageenan was also influenced by the fat

content of the cheese (Cernikova *et al.*, 2008). Multilayer emulsions containing carrageenan or other biopolymer provide better stability against droplet aggregation than single-layer emulsions under same environmental conditions of pH, ionic concentrations, temperature, etc., providing enhanced stability to dairy products (Hansen, 1994). Table 36.5 indicates the typical dairy applications of carrageenans.

In bakery products, carrageenan improves batter quality and properties of dough and pastes associated with higher water absorption and loaf volume (Techawipharat *et al.*, 2008). Coated, deep fat fried and frozen products constitute an important sector in the ready-meals market and include chicken nuggets, “fish fingers” (prepared from fish such as cod, haddock, pollock, perch, catfish, etc.) and crustacean products (shrimp, crab cakes, and crawfish). For coating, the raw materials are exposed to a batter that contains starch, proteins, fat/hydrogenated oils and seasonings suspended in water. Incorporation of carrageenan in the batter improves viscosity, suspension-characteristics, emulsifying capacity to the raw material, in addition to giving enhanced refrigerated shelf life for the coated products. (Other ingredients useful in this respect are methylcellulose and alginates, among others). Besides, presence of carrageenan is also beneficial to reduce oil uptake during deep fat frying of the products, prior to freezing (Annapure *et al.*, 1999).

In muscle products, injection of carrageenan improves firmness and color and decreased cooking loss. The hydrocolloid also finds application as binder in making restructured products such as turkey rolls, beef rolls, chicken

**Table 36.6** Some applications of carrageenan in food product development

Products	Polysaccharide and action	References
Bakery products	Carrageenan enhanced loaf volume, water absorption and improved crumb grain score	Kohajdova and Karovcova (2009); Guarda <i>et al.</i> (2004)
Fishery products such as surimi and other gel and novel fish products, e.g., fish burgers, sausages	Carrageenan and alginate enhance cooking yield, hardness and bind strength, texture and fiber content	García-García, and Totosa (2008); Lian <i>et al.</i> (2000)
Red meat products such as turkey, restructured beef products, low-fat meat balls, beef burgers, etc.	Carrageenan increases yield, improved visual appearance, sliceability and rigidity and decreased expressible juice. Enhances storage stability	Trius. and Sebranek (1996); García-García, and Totosa (2008). Cierach <i>et al.</i> (2009)
Vegetable products	Reduces or replaces pectin in jams and jellies and helps low sugar products, improves texture, controls browning	Hamza-Chaftai (1990); Mahungu <i>et al.</i> (2002); Tong and Hicks (1991)
Flavored soy milk	$\iota$ -carrageenan increases viscosity and sensory values	Wang <i>et al.</i> (2001)
Fruit juices	$\iota$ -, $\kappa$ -, or $\lambda$ -carrageenan, alone or with citric acid inhibit browning	Hamza-Chaftai (1990)
Clarification of wine, Colloidal stabilization of beer	Carrageenan, alginic acid	Cabello-Pasini <i>et al.</i> (2005)
Goat milk	Improves viscosity to goat milk	Hansen (1994) See also Table 36.5 for other dairy products

rolls, sausages and cutlets from meat trimmings (Cierach *et al.*, 2009; Trius and Sebranek, 1996). All the three types ( $\kappa$ ,  $\iota$  and  $\lambda$ ) improved water-holding ability of cooked surimi (concentrate of washed fish myofibrillar proteins) prepared from fish such as Atlantic pollock and blue whiting (Perez-Mateos *et al.*, 2002). Carrageenan and also alginic acid have good wine stabilization capacity. Protein flocculation and precipitation capacities of carrageenan and alginic acid were two times greater than that of agar (Cabello-Pasini *et al.*, 2005). In general, agar, alginate and carrageenan have wide applications in the food processing sector, showing wide potential for further enhancement. It has been concluded that in general, the three polysaccharides modify food texture from firm, brittle to soft in the order agar >  $\kappa$ -carrageenan > high 'G' alginate > high 'M' alginate >  $\iota$ -carrageenan (Rudolph, 2000). Table 36.6 gives some applications of carrageenan in food product development.

#### Other seaweed polysaccharides

Some of the other seaweed polysaccharides that have shown food applications are furcellaran, fucoidans, ulvan, and

floridean starch. Furcellaran forms thermally reversible aqueous gels, similar to  $\kappa$ -carrageenan; gelation being influenced by the cations such as  $K^+$ ,  $NH_4^+$  and  $Cs^+$ , while  $Na^+$  prevents gel formation. Presence of sugar influences the gel texture changing it from brittle to elastic. The gel is stable at low pH. Furcellaran gel is used in puddings, cake fillings and icings and in marmalades has the advantage over pectin since it allows stable gel at sugar concentrations below 50–60%.

Furcellaran is also used in processed meat products, such as meat spreads, pastes and pastry fillings (Belitz, 2004). It can be mentioned that fucoidans and laminarins are more important for their health applications, rather than food applications. The anti-inflammatory, antiangiogenic, anticoagulant and antiadhesive properties of fucoidans have been well recognized. Furthermore, they can have antitumor, antimutagenic, anticomplementary, immunomodulating, hypoglycemic, antiviral, hypolipidemic, and anti-inflammatory activities (Venugopal, 2011). Ulvan is a polysaccharide from *Ulva* spp., commonly referred to as sea lettuce, one of the commonly consumed seaweeds. It is not fermented by colonic bacteria, because of its particular chemical structure. Dietary fibers from sea lettuce

**Table 36.7** Seaweed polysaccharides in the Food Additives Status List of the US FDA

Polysaccharide	Description	Applications
Agar-agar	MISC, GRAS/FS, GRAS	In baked goods and baking mixes 2%); in confectionery/frosting, 1–2%, soft candies (0.25%)
Ammonium alginate	MISC, REG	Boiler water additive
Potassium-alginate	GRAS	Stabilizer & thickener, <0.1% in confections & frostings, <0.7% in gelatins & puddings, <0.25% in processed fruits & fruit juices, <0.01% in all other food categories
Alginic acid/algin	GRAS/FS	Cheeses, frozen desserts, jellies, preserves
Sodium alginate	STAB, GRAS/FS	Cheeses, frozen desserts <0.5%, finished products
Calcium or potassium alginate	GRAS	Confectioneries, gelatin, pudding, processed foods
Carrageenan and its NH <sub>4</sub> /K/Na/Ca salts/ <i>Gigartina</i> extracts	STAB, REG, GMP, REG/FS	<0.8% in finished cheese. Also added with polysorbate80 at a maximum of 500 ppm level
Furcellaran and its K/Na/Ca salts	MISC, EMUL, STAB, REG/FS, GMP	Ice cream

EMUL, emulsifier; STAB, stabilizer; FS, substances permitted as optional ingredient in a standardized food, GRAS, Generally Recognized As Safe; GRAS/FS, Substances generally recognized as safe in foods but limited in standardized foods where the standard provides for its use, REG, Food additives for which a petition has been filed and a regulation issued, REG/MS, Food additives regulated and included in a specific food standard, MISC, Miscellaneous. Source: US FDA (2009).

can function as bulking agents with little effect on nutrient metabolism. Floridean starch, isolated from red seaweed exhibits low gelatinization temperature, low viscosity, high clarity and little or no retrogradation upon repetitive freeze-thaw cycles. These properties make floridean starch suitable for applications such as in instant noodles and deep-frozen food (Venugopal, 2011; Yu, *et al.*, 2002).

## 36.5 Regulatory and commercial aspects

Agar, alginate and carrageenan have received regulatory approvals from the United States Food and Drug Administration (US FDA), the European Council (EC) and the Codex Alimentarius Commission (CAC) of the Food and Drug Administration (FAO), Rome. Agar enjoys Generally Regarded As Safe (GRAS) status of the US FDA. Alginic acid, its sodium, potassium, ammonium and calcium salts and PGA and agar have been approved by the EC with approval numbers from E400 to E406 (International Numbering System numbers: INS 400 to INS 406). In North America, since 1990, the PES has been approved and labeled as carrageenan. Whereas carrageenan *per se* is safe, its degradation products (poligeenans) having molecular weights of 20 to 30 kDa could exhibit toxicological properties. The US FDA considers natural carrageenans (E407) to be safe as food

additive but advises molecular weight determination to be made on samples prior to their use in foods, to ensure degraded products are not used (FDA, 2006). Table 36.7 shows marine polysaccharides in the Food Additives Status List of the US FDA and Table 36.8 gives seaweed polysaccharides permitted by the European Council.

Seaweed species as sources of commercial hydrocolloids approved by the Codex Food Standards of the FAO, Rome and the World Health Organization (WHO), Geneva, include Danish agar (*Furcellaria fastigiata*), Eucheuman (*Eucheuma* spp.), Furcellaran agar (*Furcellaria fastigiata*), Hypnean (*Hypnea* spp.), Iridophycan (*Iridaea* spp.) and Irish moss (*Chondrus* spp.). Alginate, carrageenan and agar have shown significant growth in the last decade the value which has increased from US \$644 million in 1999 to US \$1020 million in 2009. Reported production and value of these polysaccharides during the year 2009 were as follows: alginate, 26 500 t valued at US \$318 million; agar, 9600 t, US \$173 million; and carrageenan, 50 000 t, US \$527 million (Bixler, 2010). Agar is currently facing increasing competition from other gelling agents. About 30% of the total production of alginate is used by the food industry, chiefly as sodium and calcium alginates, the only commercially important derivative being PGA. Almost all the carrageenan produced is used by the food industry, (often labeled as “Natural Food Stabilizer” by some food companies). However, pure carrageenan is facing competition from Processed

**Table 36.8** Selected seaweed polysaccharides permitted by the European Council

Name of polysaccharide and E Number	Source	Functions, food products and permitted levels
Alginic acid (E400), Sodium alginate (E401), Potassium alginate (E402) Ammonium alginate (E403) Calcium alginate (E404) Propylene glycol alginate(E405)	Large brown seaweeds such as <i>Laminaria hyperborea</i> , <i>Ascophyllum nodosum</i> and <i>Macrocystis</i> species	<p><i>Functions</i> Emulsifier, suspending, stabilizer, gelling agent, thickener</p> <p><i>Products</i> Jam, jellies and marmalades Sterilized, pasteurized and UHT cream, low calorie cream, pasteurized low fat cream, weaning foods for infants and young children in good health</p> <p><i>Permitted levels</i> 10 g/kg (individual or in combination) 0.5 g/kg in weaning foods (individual or in combination)</p>
Agar (E406)	Mainly species of <i>Gelidium</i> , <i>Pterocladia</i> , and <i>Gracilaria</i>	<p><i>Functions:</i> Emulsifier, stabilizer, gelling agent, thickener.</p> <p><i>Products:</i> Ice-creams, tinned goods, glaze for meats, etc Ice-creams, milk shakes, instant desserts, custard tarts. Suspending agent in soft drinks. Spreads and many others.</p>
Carrageenan (E407)	Mainly <i>Eucheuma</i> , <i>Betaphycus</i> , <i>Kappaphycus</i> , and <i>Chondrus crispus</i>	Partially dehydrated and dehydrated milk
Carrageenan (E407)		<p>Permitted level <i>Quantum satis</i></p> <p><i>Functions</i> Emulsifier, stabilizer, gelling agent, thickener</p> <p><i>Products</i> Ice-creams, milk shakes, instant desserts, custard tarts. Suspending agent in soft drinks. Spreads and many others</p> <p><i>Permitted level</i> 0.3g per l in infant formulae</p>

Source: Directive 95.2 EC 20 February 1995 European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colors and sweeteners.

Eucheuma seaweed (PES). (Seisun, 2009). A recent survey has shown world wide availability of about 200 carrageenan-containing food products (Shah and Huffman, 2003). In summary, seaweed polysaccharides have established themselves as food additives having interesting functional properties.

## References

- Angelin, T.S. *et al.* (2004) Physicochemical properties of carrageenans extracted from *Sarconema filiforme* and *Hypnea valentiae*. *Seaweed Research and Utilization*, **26**, 197.

- Annapure, U.S., Singhal, R.S., Kulkarni, P.R. (1999) Screening of hydrocolloids for reduction in oil uptake of a model deep fat fried product, *Lipids*, **101**, 217.
- Armisen, R (1999) Agar. In: *Thickening and Gelling agents for food*, 2nd edn. (ed. A. Imesen). Blackie Academic & Professional, London, p. 1.
- Barbucci, R., et al. (2003) Polysaccharides based hydrogels for biological applications, *Macromolecular Symposia*, **204**, 37–58.
- Belitz, H.D., Grosch, W. and Schieberle, P. (2004) Carbohydrates. In: *Food Chemistry*, 3rd edn. Springer-Verlag, Berlin, Heidelberg, p. 245.
- Bixler, H. (2010) [http://www.seaweed.ie/uses\\_general/industrialgums.html](http://www.seaweed.ie/uses_general/industrialgums.html) (accessed January, 2011).
- Borderías, A.J., Sánchez-Alonso, L. and Pérez-Mateos, M., (2006) New applications of fibers in foods: Addition to fishery products, *Trends Food Sci. Technol.*, **16**, 458–462.
- Braune, W and Guiry, M. (2011) *Seaweeds: A Colorful Identification Guide to Seaweeds of the World*. Koeltz Scientific Books, Koenigstein, Germany.
- Brownlee, A., Allen, J.P., Datamar, P.W. et al. (2005) Alginates as a source of dietary fiber. *Crit. Rev. Food Sci. Nutr.*, **45**, 497–510.
- Bylaite, E., Igūnaitė, Z., Anne, S., Meyer, A.S. and Adler-Nissen, J. (2004) Influence of  $\lambda$ -carrageenan on the release of systematic series of volatile flavor compounds from viscous food model systems. *J. Agric. Food Chem.*, **52**, 3542–3546.
- Cabello-Pacini, A., Victoria-Cota, N., Macias-Carranza, V., Hernandez-Garibay, E. and Muñoz-Salazar, R. (2005) Clarification of wines using polysaccharides extracted from seaweed. *Am. J. Enol. Viticuli.*, **56**, 52–56.
- Cernikova, M. et al. (2008) Effect of carrageenan type on viscoelastic properties of processed cheese. *Food Hydrocolloids*, **22**, 1054–1061.
- Chattopadhyay, K. (2007) Sulphated polysaccharides from Indian samples of *E. compressa* (Ulvaes, Chlorophyta): Isolation and structural features. *Food Chem.*, **104**, 928–935.
- Chidanandaiah, K., Leshri, R.C. and Sanyal, M.K. (2009), Effect of sodium alginate coating with preservatives on the quality of meat patties during refrigerated storage. *J. Muscle Foods*, **20**, 275–292.
- Cierach, C., Modzelewska-Kapituła, M. and Szaciło, K. (2009) The influence of carrageenan on the properties of low-fat frankfurters, *Meat Sci.*, **82**, 295–299.
- Clementi, F., Crudele, M.A. and Parente, E., et al. (1999) Production and characterisation of alginate from *Azotobacter vinelandii*. *J. Sci. Food Agric.*, **79**, 602–610.
- de Kruijff and Tuinier, R. (2001) Protein-polysaccharide interactions. *Food Hydrocolloids*, **15**, 555–563.
- Dickinson, E. (2008) Interfacial structure and stability of food emulsions as affected by protein–polysaccharide interactions. *Soft Matter*, **4**, 932–941.
- Dragnet, K. I., Smidsrod, O. and Skjak-Broek, G. (2005) Alginates from algae. In: *Polysaccharides and Polyamides in the Food Industry*. (eds A. Steinbucke and S.K. Rhee). Wiley-VCH, Weinheim, p. 1.
- Ellis, A. and Jacquier, J. C. (2009) Manufacture and characterisation of agarose microparticles. *J. Food Eng.*, **90**, 141–147.
- Friberg, S. Larsson; K. and Sjoblom, J. (eds) (2007) *Food Emulsions*, Vol. 1. Taylor & Francis, Boca Raton, FL, p. 86.
- García-García, E. and Totosaús, A. (2008) Low-fat sodium-reduced sausages: Effect of the interaction between locust bean gum, potato starch and  $\kappa$ -carrageenan by a mixture design approach. *Meat Sci.*, **78**, 406–411.
- Guarda, A., Rosell, C.M., Benedito, C. and Galotto, M.J. (2004), Different hydrocolloids as bread improvers and anti-staling agents. *Food Hydrocolloids*, **18**, 241–249.
- Hamza-Chaftai, A. (1990) Effect of manufacturing conditions on rheology of banana gelified milk: optimization of the technology. *J. Food Sci.*, **55**, 1630–1635.
- Hansen, P.M.T. (1994) Food hydrocolloids in the dairy industry. In: *Food Hydrocolloids*. (eds E. Nishinari, and K. Doi) Plenum Press, New York, p. 211.
- Hershko, V. and Nussinovitch, A. (1998) Physical properties of alginate-coated onion (*Allium cepa*) skin. *Food Hydrocolloids*, **12**, 115–119.
- Hilou, L. et al. (2006) Effect of extraction parameters on the chemical structure and gel properties of k/i-hybrid carrageenans obtained from *Mastocarpus stellatus*. *Biomolec. Eng.*, **23**, 201–208.
- Ito, K. and Hori, K. (1989) Seaweed, chemical composition and potential food uses. *Food Rev. Int.*, **5**, 101–107.
- Karim, A.A. and Rajiv, B. (2009) Gelatin alternatives for the food industry: recent developments, challenges and prospects. *Trends Food Sci. Technol.*, **19**, 644–639.
- Kavanagh, G.M. and Ross-Murphy, S.B. (1998) Rheological characterization of polymer gels. *Progr. Polym. Sci.*, **23**, 533–562.
- Kohajdova, Z. and Karovcova, J., (2009) Application of hydrocolloids as baking improvers. *Chemical Papers*, **63**, 26–38.
- Kohajdová, Z. et al. (2007), Marine biotechnology for production of food ingredients. *Adv. Food Nutr. Res.*, **52**, 237–251.
- Koliandris, A. Lee, A., Ferry, A.-L., Hill, S., and Mitchell, J. (2008) Relationship between structure of hydrocolloid gels and solutions and flavor release, *Food Hydrocolloids*, **22**, 623–630.

- Kumar, C.S., Ganesan, P., Suresh P.V., and Bhaskar, N. (2008) Seaweed as a source of nutritionally beneficial compounds – A review. *J. Food Sci. Technol.*, **45**, 1–9.
- Lahaye, M. and Rochas, C. (1991) Chemical structure and physicochemical properties of agar. *Hydrobiologia*, **221**, 137–142.
- Lee, S. *et al.* (2009) Physicochemical, textural and noodle-making properties of wheat dough containing alginate. *J. Text. Stud.*, **39**, 393–404.
- Li, H., Yu, X., Jim, Y., Zhan, W., and Liu, Y. (2008) Development of an eco-friendly agar extraction technique from the red seaweed *Gracilaria lemaneiformis*. *Bioresour. Technol.*, **99**, 3301–3305.
- Lian, P.Z., Lee, C.M. and Hufnagel -L. (2000) Physicochemical properties of frozen red hake (*Urophycis chuss*) mince as affected by cryoprotective ingredients. *J. Food Sci.*, **65**, 1117–1121.
- MacArtain, P. Jacquier, J.-C. and Dawson, K-A (2003) Physical characteristics of calcium induced  $\kappa$ -carrageenan networks. *Carbohydr. Polym.*, **53**, 395.
- Maeda J. *et al.* (2005) Effects of agar (kanten) diet on obese patients with impaired glucose tolerance and type 2 diabetes. *Diabet. Obesity Metab.*, **7**, 40–45.
- Mahungu, S.M., Hansen, S.L. and Artz, W.E. (2002) Fat substitutes. In: *Food Additives* (eds A.L. Brown, P.M. Davidson, S. Salminen and J.H. Thorngate III). Marcel Dekker, New York, p. 311.
- Manish Kumar and Sharma, B. D. (2004) The storage stability and textural, physico-chemical and sensory quality of low-fat ground pork patties with carrageenan as fat replacer. *Int. J. Food Sci. Technol.*, **39**, 31–39.
- McClements, D.J. (2006) Formation, stability and properties of multilayer emulsions for application in the food industry. *Adv. Colloid Interf. Sci.*, **128**, 227–248.
- McHugh, D.J. (2003) A guide to the seaweed industry. *FAO Fisheries Technical Paper No. 441*, Food and Agriculture Organization of the United Nations, Rome, p. 105.
- Meena, R., Prasad, K. and Siddhanta, A. K (2006) Studies on “sugar-reactivity” of agars extracted from some Indian agarophytes. *Food Hydrocolloids*, **20**, 1206–1211.
- Mitchell, J. R (1998) Water and food macromolecules. In: *Functional Properties of Food Macromolecules* (eds S.E. Hill, D.A. Ledward and J.R. Mitchell). Aspen Publ., New York, p. 50.
- Morris, V.J (1998) Gelation of polysaccharides. In: *Functional Properties of Food Macromolecules* (eds S.E. Hill, D.A. Ledward and J.R. Mitchell). Aspen Publ., New York, p. 143.
- Naidu, A.S. (2000) Agar. In: *Natural Food Antimicrobial Systems* (ed. A.S. Naidu). CRC Press, Boca Raton, FL, p. 417.
- Narchi, Ch. and DjelvehI, V. G. (2009) Effect of protein–polysaccharide mixtures on the continuous manufacturing of foamed food products. *Food Hydrocolloids*, **23**, 188–201.
- Nishinari, K. and Zhang, H. (2004) Recent advances in the understanding of heat set gelling polysaccharides. *Trends Food Sci. Technol.*, **15**, 305–312.
- Norton, I. T. and Foster, T. J. (2002) Hydrocolloids in real food systems. In: *Gums and Stabilizers for the Food Industry*, Vol. 11 (eds P.A. Williams and G.O. Philips). The Royal Society of Chemistry, Cambridge, p. 187.
- Onsoyen, E. (1997) Alginates. In: *Thickening and Gelling Agents for Food* (ed. A. Imeson). Blackie Academic and Professional, London, p. 22.
- Owusu-Apenten, R.K. (2004) *Introduction to Food Chemistry*. CRC Press, Boca Raton, FL, p. 55.
- Perez-Mateos, M., Solas, M. and Montero, P. (2002) Carrageenans and alginate effects on properties of combined pressure and temperature in fish mince gels. *Foods Hydrocolloids*, **16**, 225–233.
- Prasad, K., Sidhartha, M., Ganesan, B.K., Ranewal, B.K., Jha, B., and Ghosh, P.K. (2007) Agars of *Gelidiella acerosa* of west and southeast coasts of India. *Biores. Technol.*, **98**, 1907–1915.
- Rastall, R. (2001) Tailor-made food ingredients: enzymatic modulation of nutritional and functional properties. IFIS Publ. <http://www.foodsciencecentral.com/fsc/ixid3729> (accessed 20 April 2011).
- Reifen, R., Edris, M and Nussinovitch A. (1998) A novel, vitamin A-fortified, edible hydrocolloid sponge for children. *Food Hydrocolloids*, **12**, 111–114.
- Roberts, M.A. and Quemener, B. (1999) Measurement of carrageenans in food: challenges, progress, and trends in analysis. *Trends Food Sci. Technol.*, **10**, 169.
- Rodriguez, M.C., Matulewicz, M.C., Nosedá, M.D., Ducatti, D.R.B. and Leonardi, P.I. (2009) Agar from *Gracilaria gracilis* of the Patagonic coast of Argentina – content, structure and physical properties. *Biores. Technol.*, **100**, 1435–1441.
- Roller, S. and Dea, I.C.M. (2002) Biotechnology in the production and modification of biopolymers for foods. *Crit. Rev. Biotechnol.*, **12**, 261–275.
- Rudolph, B. (2000) Seaweed products: Red algae of economic importance. In: *Marine & Freshwater Products Handbook*. VCH Publ., Lancaster, p. 315.
- Seisun, D. (2009) *Hydrocolloid News*. IMR International, San Diego, CA, 92127, USA.
- Sen, D.P., (2005) Selected byproducts from the sea. In: *Advances in Fish Processing*, Allied Publishers, New Delhi, p. 616.
- Shah, Z.C. and Huffman, F. G. (2003) Current availability and consumption of carrageenan-containing foods. *Ecol. Food Nutr.*, **42**, 357–362.
- Soumya, R. and Ryan, A.L (2003) Method of preparing food products with carrageenan, US Patent 6663910B2.

- Sugimoto, S. *et al.* (1982). Improvement of organoleptic quality of fermented soybean beverage by additions of propylene glycol alginate and calcium lactate. *J. Food Process. Preserv.*, **5**, 83–88.
- Techawipharat, J., Supphantharika, M. and BeMiller, J.N. (2008) Effects of food gums on viscosities of starch suspensions during pasting. *Carbohydr. Polym.*, **73**, 417–426.
- Tharanathan, R. N. (2002). Food-derived carbohydrates – structural complexity and functional diversity. *Crit. Rev. Biotechnol.*, **22**, 65–84.
- Tong, C.B.S. and Hicks, K.B. (1991) Sulfated polysaccharides inhibit browning of apple juice and diced apples. *J. Agric. Food Chem.*, **39**, 1719–1722.
- Trius, A. and Sebranek, J. G. (1966) Carrageenans and their use in meat product. *Crit. Rev. Food Sci. Nutr.*, **36**, 69.
- US FDA, US Food and Drug Administration (2006) Food additives permitted for direct addition to food for human consumption 21CFR172, subpart C. US FDA, Silver Spring, MD.
- US FDA (2009) Listing of Food Additive Status Part I. <http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/FoodAdditiveListings/ucm091048.htm> (accessed 20 April 2011).
- Venugopal, V (2011) Seaweed, microalgae, and their polysaccharides: food applications. In: *Marine Polysaccharides: Food Applications*. CRC Press, Boca Raton, Florida, Ch. 7.
- Venugopal, V. (2009a) Seaweed: nutritional value, bioactive properties and uses. In: *Marine Products for Healthcare*. CRC Press, Boca Raton, FL, Ch.9
- Venugopal, V. (2009b) Seaweed hydrocolloids. In: *Marine Products for Healthcare*. CRC Press, Boca Raton, FL, Ch. 10.
- Verbeken, D. Thas, O. and Dewittinck, K. (2004) Textural properties of gelled dairy desserts containing  $\kappa$ -carrageenan and starch. *Food Hydrocolloids*, **18**, 817–823.
- Walkenstrom, P. (2003) The creation of new food structures and textures by processing. In: *Texture in Food*, Vol.1 (ed. B.M. McKenna). Woodhead Publishers, Cambridge, UK, p. 201.
- Wang, B., Xiong, Y.L. and Wang, C. (2001) Physico-chemical and sensory characteristics of flavored soymilk during refrigeration storage. *J. Food Quality*, **24**, 513–519.
- Weilin, K. and Keeton, J.T. (1998) Textural and physico-chemical properties of low-fat, precooked ground beef patties containing carrageenan and sodium alginate. *J. Food Sci.*, **63**, 571–574.
- Wheaton, F.W. and Lawson, T. B. (1985) *Processing of Aquatic Food Products*. John Wiley & Sons, New York, p. 42.
- Williams, P. A. and Phillips, G.O. (2003) The use of hydrocolloids to improve food texture. In: *Texture in Food*, Vol. 1 (ed. B.M. McKenna). Woodhead Publishers, Cambridge, UK, p 251.
- Yamashita, S., Sugita, K.Y and Shimizu, -M. (2001) In vitro bacteriostatic effects of dietary polysaccharides. *Food Sci. Technol. Res.*, **7**, 262
- Yu, S., Blennhow, A., Bojko, M., Madisen, M., Cisen, C.F. and Engelsen, S.B. (2002) Physico-chemical characterization of Floridean starch of red algae. *Starch/Stärke*, **54**, 66–69.
- Yuan, H., Zhang, W., Lu, X., Li, H., Geo, X. and Song, J. (2005) Preparation and in vitro antioxidant activity of  $\kappa$ -carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives. *Carbohydr. Res.*, **340**, 685.
- Zvyagintsova, T.N. Shevehenko, N.M., Popivnieb, I.B., Iskov, V.V., Sundukova, V. and Elyakova, L.A. (2004) A new procedure for the separation of water-soluble polysaccharides from brown seaweed. *Carbohydr. Res.*, **322**, 32–38.
- Zvyagintseva, T.N., Shevchenkoa, N.M., Popivnichia, I.B., *et al.* (1999) A new procedure for the separation of water-soluble polysaccharides from brown seaweeds. **322**, 32–39.

# Index

- Acanthophora* 46  
acetaminophen toxicity 389–95  
acetogenins (C<sub>15</sub>) 275–6  
2-acetoxy-15-bromo-6,17-dihydroxy-3-palmitoyl-neoparguera-4(19), 9(11)-diene 18  
acid detergent fiber (ADF) 183  
adhesives from seaweeds 38  
agar 17, 184, 186, 501–2, 523  
agarans  
    applications 246  
    culinary 247  
    molecular biology 246  
    motility assays 246  
    plant biology 246–7  
agricultural applications 514  
agricultural uses for macroalgae 89  
*Agrobacterium tumefaciens* 13  
*Alcaligenes aquamarinus* 19  
aldose reductase inhibitor  
    rhodophyta 27  
*Alexandrium catanella* 14  
algae 3  
algicidal substances  
    phaeophyta 14  
alginates 68, 501, 522–3  
alginic acid 186  
 $\alpha$ -alkokainic acid 23  
aluminium content of seaweeds 131–7  
Alzheimer's disease 344  
amarouciaxanthin 322, 404  
amino acid score (AAS) 176, 286, 287  
amino acids 174–6, 504  
2-aminoanthracene 8  
amylase 184  
amylopectin 184  
3,6-anhydrogalactose 65  
animal feeds 486–7, 527  
    animal breeding studies using seaweed  
        extracts  
        lambs 483  
        pigs 483–4  
        rats 483  
    animal breeding studies using seaweed  
        meals 482  
    aquatic animals 483  
    cattle and lambs 483  
    pigs 483  
    poultry 482  
    rats 483  
    sheep 483  
aquaculture 484, 528  
biosorption enriched 484–5  
    biofortification 485  
    increasing bioavailability 485–6  
    microelement carriers 486  
    microelement hunger 485  
general aspects 481  
historical aspects 481  
nutraceuticals 482  
nutritional properties of seaweeds 482  
poultry 528  
terrestrial animals 527–8  
anthropogenic pressure, seaweeds as  
    indicators 106–7, 111–12  
inorganic carbon acquisition 110–11  
light absorption 107–8  
photosynthesis at sub- and saturating  
    irradiance 108  
role of bicarbonate use in green tides 111  
anti-allergenic substances 345–6  
anti-asthmatic substances 344–5  
antibacterial substances  
    biomedical applications 535  
    chlorophyta 7  
anticoagulants 249–50, 512  
antidiabetic substances 362–4, 371–2, 375  
active components  
    fucoidan 374  
    fucoxanthin 373–4  
    phlorotannins 374  
phaeophyta 17, 372, 409–10  
    *Ascophyllum nodosum* 373  
    *Hizikia fusiforme* 373  
    *Laminaria japonica* 373  
    *Petalonia binghamiae* 373  
    *Sargassum yendoense* 373  
    *Undaria pinnatifida* 372  
anti-elastase activity against porcine  
    pancreas elastase  
    rhodophyta 27  
antifeedent substances  
    phaeophyta 17  
    rhodophyta 26–7  
antifungal substances  
    chlorophyta 8  
    phaeophyta 13  
antihelminthic substances  
    rhodophyta 22  
anti-HIV substances 417, 421  
diterpenes 420  
lectins 420–1  
peptides 421  
phlorotannins 419–20  
sulfated polysaccharides 417–19  
antihypertensive substances  
    phaeophyta 17  
anti-inflammatory substances 512  
    chlorophyta 5  
    phaeophyta 13–14  
    phloroglucinol 381–2  
    rhodophyta 23  
    sulfated fucans (SFs) 250–1  
    sulfated galactans (SGs) 251  
antimicrobial substances 89  
    agriculture 89  
    aquaculture 89  
    rhodophyta 25–6  
antimony content of seaweeds 138–44  
    antimony species 161  
antimutagenic substances  
    chlorophyta 8

- anti-obesity substances 371–2, 375
  - active components
    - fucoidan 374
    - fucoxanthin 373–4
    - phlorotannins 374
- phaeophyta 372, 409–10
  - Ascophyllum nodosum* 373
  - Hizikia fusiforme* 373
  - Laminaria japonica* 373
  - Petalonia binghamiae* 373
  - Sargassum yezoense* 373
  - Undaria pinnatifida* 372
- antioxidant substances 87
  - biomedical applications 510–11
  - food components 506–8
  - food industry 398, 401
    - carotenoids 400–1, 403–10
    - phlorotannins 399
    - sulfated polysaccharides 399–400
  - mycosporine-like amino acids (MAAs) 88–9
  - phaeophyta 15–17
  - phenolic compounds 87
  - phloroglucinol 382
- antioxidants
  - biomedical applications 535
- antiparasitic substances 254
- anti-photoaging substances
  - phloroglucinol 382–3
- antiplasmodial substances
  - chlorophyta 7
- anti-rheumatoid arthritis (RA)
  - substances 345
- antithrombal agents 249–50
- antitumor substances 511–12
  - phloroglucinol 383–4
  - sulfated fucans (SFs) 253–4
  - sulfated galactans (SGs) 254
- antiviral substances
  - biomedical applications 535–6
  - chlorophyta 7–8
  - phaeophyta 14–15
  - rhodophyta 21–2
  - sulfated fucans (SFs)
    - HSV and influenza 247–8
  - sulfated galactans (SGs) 248–9
  - sulfated polysaccharides 247
- Aplysia californica* 20
- aplysiatoxin 3
- aplysin-9-ene 21
- apparent protein biological value (ABV) 286
- apparent protein digestibility (APD) 286
- aquacultural uses for macroalgae 89
  - feeds 484, 528
- arachidonic acid 26, 81, 182
- Arrainvilla rawsonii* 5
- arsenic content of seaweeds 131–7
  - arsenic species 155, 157–8
  - metallothionein binding 305–6
- Ascochyta salicorniae* 7
- Ascophyllum nodosum* 373
  - diabetes management 362–4
- ascorbic acid (vitamin C) 179–80
- ascosalipyrrolidinones A and B 7
- Asparagopsis armata*
  - polysaccharides 66–7
- astaxanthin 51, 55, 322–3, 404, 525–6
- Avrainvillea nigrans* 8
- Bacillus subtilis* 5, 25
- barium content of seaweeds 131–7
- Beckerella subcostatum* 25
- Betaphycus* 17
- 6,6'-bieckol 341, 342, 343
  - anti-HIV activities 419
- 6,8'-bieckol 341
- 8,4''-bieckol 14
  - anti-HIV activities 419
- 8,8'-bieckol 14, 341, 343
  - anti-HIV activities 419
- bifurcadiol 9
- bifurcane 72
- Bifurcaria bifurcata* 9, 11
- bioaccessibility studies 349
  - cell culture models 352
  - comparison between *in vivo* and *in vitro* methods 352
  - in vitro* methods 349–50
    - bioaccessible fraction 350–1
    - dialyzable fraction 351
  - in vivo* methods 349
    - animals 349
    - humans 349
- bioaccumulation 445
- bioactive metabolites 262–3, 280–1
  - chemical constituents
    - acetogenins (C<sub>15</sub>) 275–6
    - diterpenes 268–73
    - meroterpenoids 274
    - phlorotannins 277–8
    - sesquiterpenes 263–7
    - steroids 279
- biodiversity 59
- bioenergy 426–7, 453, 515–16
  - algal biomass characteristics 455
  - bioethanol types and characteristics 453–4
  - future direction 459
  - international industries and technologies 454–5
- red algae bioethanol production
  - technology
    - fermentation 457–9
    - overview 455–6
    - saccharification process 456–7
    - separation and distillation 459
- biofouling 58
  - antifouling from French brown seaweeds 80–1
    - Grateloupia turuturu* 79–80
  - screening 76–7
  - context 75–6
  - organisms 76
- bioinformatics 41
- biological treatment of wastes using seaweeds 46
- biomedical applications 510, 532–4
  - antibacterial effects 535
  - anticoagulant activity 512
  - anti-inflammatory activity 512
  - antioxidants 510–11, 535
  - antitumor and immunomodulatory activities 511–12
  - antiviral effects 535–6
  - heme-agglutinating properties 536
  - hepatoprotective properties 536
  - importance of seaweeds 534
    - treatments 534–5
    - ulcers and allergies 535
  - tissue engineering 512–13
  - weight control 536–7
- bioprocess technology 436–8
- biosorption 444–5
- biotechnology 424, 427
  - bioremediation and bioenergy 426–7
  - blue farming 424–5
  - chemical and pharmaceutical industries 425–6
  - heavy metal detoxification 447–8
  - blue farming 424–5
- Bostrychia* 50
- Bostrychia calliptera* 445–6
- brevetoxins 3, 4
- bromine content of seaweeds 131–7
  - bromine species 156–7, 158, 159–60
- 3-bromo-4-(2,3-dibromo-4,5-dihydroxybenzyl)-5-methoxymethylpyrocatechol 27
- 1,2-bis(3-bromo-4,5-dihydroxyphenyl)ethane 23
- bromobekerelide 25
- bromophycolides 20
- Bryopsis* 6–7
- 3-butyl-4-vinylcyclopentene 17

- Caco-2 cell culture digestibility method 291
- cadmium content of seaweeds 131–7  
metallothionein binding 304–5
- calcium content of seaweeds 118–30, 180
- Callophycus serratus* 20, 25
- callus culture 435–6
- Caloglossa lepriuriae* 50
- Calurepa prolifera* 7
- Candida albicans* 7, 13
- canthaxanthin 322, 526
- capillary electrophoresis (CE) 163
- capisterones A and B 8
- carbon-concentrating mechanisms (CCMs) 110–11
- carotene 37
- carotenoids (provitamin A) 181, 404–5, 525–6  
food antioxidants 400–1, 405–6  
brown seaweeds 406–9
- carrageen moss 17
- carrageenans 17, 65, 184, 186, 193–6, 501, 523  
applications 244–5  
disaccharide units 194–5  
health concerns 246  
polysaccharide composition and life cycle phase  
*Chondrus pinnulatus* 197, 200–1  
*Tichocarpus crinitus* 197–201  
rheological and viscosity properties 200–1  
sources in Russian Far East 196–7
- Catenella repens* 46, 50, 55
- caudoxirene 17
- Caulerpa* 46
- Caulerpa racemosa* 22
- Caulerpa taxifolia* 8
- caulerpals A and B 8
- cell suspension culture 435–6
- cellobiose 206
- cellulose 184–5, 206
- Ceratodictyon spongiosum* 23
- ceratospongamides 23
- chaetoglobosin O 6
- Chaetomorpha*  
inorganic carbon acquisition 110–11  
light absorption 107  
photosynthesis at sub- and saturating irradiance 108  
role of bicarbonate use 111
- Chaetomorpha aerea* 49
- chemical composition of seaweeds 173–4, 186  
components  
dietary fiber (DF) 182–6  
lipids 181–2  
minerals 176–9  
proteins and amino acids 174–6  
vitamins 179–81
- chemical industry applications 425–6
- chemical speciation  
importance of 154  
organometallic species, sources of 154  
organometallic species in algae 154–61
- chitin 210
- chitosan 210
- chlorine content of seaweeds  
chlorine species 156–7, 158, 159–60
- chloro Beckerleide 25
- chlorophylls 37, 524–5
- chlorophyta (green algae) 5, 37, 45  
biologically active components  
antibacterial activity 7  
antifungal activity 8  
anti-inflammatory substances 5  
antimutagenic activity 8  
antiplasmodial activity 7  
antiviral activity 7–8  
cytotoxic and immunosuppressive activities 5  
protein tyrosine phosphate 1B inhibitors (PTP1B) 8
- diterpenes 273
- sesquiterpenes 267
- sulfated glucans (SGs) 242
- cholesteryl formate 81
- Chondria arnata* 24
- Chondria atropurpurea* 22
- Chondria oppositoclada* 7
- chondriamide 22
- Chondrus crispus* 17, 73
- Chondrus pinnulatus*  
structural peculiarities of sulfated polysaccharides 193–6  
carrageenan sources in Russian Far East 196–7  
polysaccharide composition and life cycle phase 197
- Chorda tomentosa* 17
- chromium content of seaweeds 131–7
- chromium-contaminated wastewater treatment  
case study using *Hydrilla verticillata*  
adsorption isotherm study 475  
adsorption kinetics study 471–2  
characterization of absorbent 465–7
- effect of adsorbate concentration 470  
effect of adsorbate dose 470  
effect of contact time 468  
effect of pH 468–9  
effect of stirring speed 467–8  
effect of temperature 470–1  
materials 465  
method 465  
rate controlling mechanism 473–5
- harmful effects of Cr(VI) 461–2
- importance of chromium 461
- treatment methods 462  
adsorption 462–5
- ciguatoxins 3, 4
- circular dichroism (CD) 303
- Cladophora*  
green tides  
inorganic carbon acquisition 110–11  
light absorption 107  
photosynthesis at sub- and saturating irradiance 108  
role of bicarbonate use 111
- Cladophora fascicularis* 5
- Cladophora socialis* 8
- cobalamin (vitamin B<sub>12</sub>) 181
- cobalt content of seaweeds 131–7
- Codium iyengarri* 7
- communesins A and B 5
- copper content of seaweeds 131–7, 178
- cord grass 37
- cosmetics from seaweeds 86–7, 537  
cosmeceuticals 513–14  
osmolytes 87  
sulfated polysaccharides 242–4
- cotton fabric applications 205  
antimicrobial agents 208–9  
inorganic nonparticles 209  
organic substances 209  
oxygen bleach 209–10  
plant products 210
- chitin and chitosan 210
- seaweed nanoparticles 211  
antibacterial finishing 216–17  
bioactive compounds 211–12  
characterization 212–16  
extraction 212  
permanent finish 217
- textiles  
cotton fabric 207  
cotton fiber 205–6  
cotton yarn 206–7  
preparatory process 207–8
- crude fiber (CF) 183
- Culex pipens pallens* 24

- cupalaurenol 25  
 cycloartenol disulfate 5  
 cyclooudesmol 7  
 cymobarbatol 8  
*Cymopolia barbat* 8  
*Cystophora siliquosa* 17  
 cystophorene 17  
*Cystoseira crinita* 15  
*Cystoseira myrica* 11  
*Cystoseira tamariscifolia* 13  
 cystoseirol monoacetate 11  
 cytokinin 173  
 cytotoxic substances  
   chlorophyta 5  
   phaeophyta 9–12  
   rhodophyta 17–21
- dairy products from seaweeds 38  
 debromoepiapsinol 21  
 dehydrothyrsiferol 21  
*Dendrophiella salina* 13  
 9-deoxyelatal 25  
 deoxylapachol 13  
 deoxyparguerol-7-acetate 22  
 deoxyrepacifenol 24  
*Desmia hornemanni* 19  
 diabetes management 362–4  
 10,18-diacetoxy-8-hydroxy  
   2,6-dolabelladiene 14, 17  
 dialysis cell digestibility methods 289  
 2,3-dibromo-4,5-dihydroxybenzyl  
   alcohol 27  
 2,3-dibromo-4,5-dihydroxybenzyl  
   methyl ether 27  
 3,5-dibromo-4-  
   hydroxyphenylethylamine 27  
 3,5-dibromo-4-  
   hydroxyphenylethylamine 27  
 2-(2',4'-dibromophenoxy)-  
   4,6-dibromoanisol 5  
 dichloroacetamide 25  
 dictyol F monoacetate 11  
 dictyol J 14  
 dictyolactone 14  
 dictyone acetate 11  
 dictyopterene 17  
*Dictyopteris zonaroides* 13  
*Dictyota crenulata* 12  
*Dictyota dichotoma* 10, 14  
*Dictyota menstrualis* 14  
*Dictyota pfaffi* 14, 15, 17  
*Dictyota spinulosa* 12  
 dictyotins 10  
 dieckol 14, 17, 341, 342, 343  
 dietary fiber (DF) content of seaweeds  
   182–6
- Digenea simplex* 23  
 digestability 285, 297  
   contribution of seaweed to food and  
     feed digestibility 296–7  
   evaluation of seaweed digestibility  
     295–6  
   factors influencing seaweed  
     digestibility 291  
     chemical composition and  
       antinutritional factors 293–4  
     digestibility model organism 291–2  
     digestive enzyme systems 292  
     enzyme hydrolysis conditions 292–3  
     food processing 294–5  
   methods 287  
     *in situ* assessment 288  
     *in vitro* assessment 289–91  
     *in vivo* assessment 287–8  
   proteins 285–6  
     amino acid score (AAS) 286, 287  
     essential amino acid index (EAAI)  
       286–7  
 4,18-dihydroxydictyolactone 12  
 8 $\alpha$ ,11-dihydroxypachydictyol 12  
 1-(3',5'-dihydroxyphenoxy)-7-(2'',4'',6-  
   trihydroxyphenoxy)-2,4,9-  
   trihydroxydibenzo-1,4-dioxin  
   341, 342, 343  
*cis*-dihydroxytetrahydrofuran 13  
*Dilophus okamurae* 17  
 1,9-dimethyl-methylene blue (DMB) 231  
 3,5-dinitriguaiacol 25  
 dinophysistoxins 3, 4  
 dioxinodehydroeckol 341, 342, 343  
 1,1-diphenyl-2-picrylhydrazyl (DPPH)  
   radical scavengers 15, 16  
 diploretol 68  
 diploretolhydroxycarmalol 342, 343  
   anti-HIV activities 419  
 diterpenes 72, 268  
   anti-HIV activities 420  
     dolabellane skeleton 270  
     dolastane skeleton 270  
     hydroazulenoids 270  
     other skeletons 271  
     xenicane skeleton 269  
   green algae 273  
   red algae 272–3  
*Dityota dichotoma* 11  
 DNA methyl transferase-1 20  
 docosahexaenoic acid (DHA) 182, 329,  
   330  
   biosynthesis from  $\alpha$ -linolenic acid 333  
   hepatic enhancement by fucoxanthin  
     333–5  
   importance 331–2
- Dolabella californica* 15  
 dolabellane 10, 15  
 dolastatin 3, 4  
 dollabelladiene 14  
 dry matter digestibility (DMD) 290  
 dulse 17
- E. coli* 19  
*Ecklonia cava* 14, 16  
   health benefits 359–61  
*Ecklonia stolonifera* 14, 15, 17  
 eckol 14, 17, 341, 342, 343  
 eckstolonol 14, 15  
 ecological value of seaweeds 46  
 ectocarpene 17  
*Ectocarpus siliculosus* 73  
 edibility of seaweeds 146–7  
 eicosanoids 26  
 eicosapentaenoic acid (EPA) 83, 182,  
   329, 330  
   importance 331–2  
 elatal 25  
 electrospray ionization–mass  
   spectrometry (ESI-MS) 163, 303  
 eleganediol 72  
 eleganolone 73  
*Elysia rufescens* 6  
 emergent plants 37  
*Enteromorpha* 46  
*Enteromorpha compressa* 48  
*Enteromorpha intestinalis* 5, 6, 46, 48, 55  
*Enteromorpha prolifera* 46  
   green tides 106–7  
 environmental sources of organometallic  
   species 154  
 enzyme-assisted extraction and recovery  
   of bioactive compounds 221–2,  
   226  
   cell wall degrading enzymes 222  
   enzyme selection 222–3  
   importance 222  
   peptides 223–4  
   polyphenols and phlorotannins 224–5  
   polysaccharides 225–6  
   seaweeds 222  
 epiapsinol 21  
 epiphytism 73  
 epipolythiodioxopiperazine 9  
*Escherichia coli* 5, 13  
 essential amino acid index (EAAI)  
   286–7  
 ethylmethanesulfonate 8  
*Eupomocentrus leucostictus* 12  
 European Union (EU)  
   SEAPURA project 39–40  
 evolutionary analysis 42

- Farlowia mollis* 26  
 fatty acids 503–4, 526–7  
 feeding-deterrent substances  
   phaeophyta 12  
 fertilizers 478, 486–7, 528–9, 537  
   commercial seaweed fertilizers 479  
   general aspects 478–9  
   plant biostimulants 479  
   plant cultivation studies 479–80  
   seaweed extracts 479  
   value-added product from manure  
     480–1  
 flavin adenine dinucleotide (FAD) 180–1  
 flavin mononucleotide (FMN) 180–1  
 floating seaweeds 37  
 Floridean starch 65, 184  
 floridoside 26  
 fluconazole 8  
 fodder from seaweeds 46  
 food products from seaweeds 38, 46, 522,  
   529  
   antioxidants 398, 401, 506–8  
   carotenoids 400–1, 403–10  
   phlorotannins 399  
   sulfated polysaccharides 399–400  
   context 83  
   edible food coatings 510  
   functional foods 356–7, 365–6  
     *Ascophyllum nodosum* 361–4  
     *Ecklonia cava* 359–61  
   fucoidan polysaccharides 358  
   fucoxanthin 358–9  
   future directions 364–5  
   health benefits of phytochemicals  
     359  
   laminarin polysaccharides 358  
   microencapsulation of bacteria  
     505–6  
   prebiotics 505  
   functional foods, meat-based 491–2,  
     495–6  
   meat products 494–5  
   specific seaweed components 492–4  
 macroalgae-extracted compounds  
   agar 523  
   alginates 522–3  
   carrageenan 523  
   fucellaran 523–4  
   iridophycan 524  
   mannitol 524  
 microalgae-extracted compounds 524  
   fatty acids 526–7  
   pigments 524–6  
 polysaccharides  
   emulsions and foams 541–2  
   gelation 541  
   interaction with food components  
     542  
   major applications 542–51  
   water-binding 541  
   proteins from red seaweeds 84–5  
   regulatory and commercial aspects  
     551–2  
   seaweeds as vegetables 503  
     amino acids 504  
     antinutrients and toxic factors  
       504–5  
     fatty acids 503–4  
     minerals 504  
   sources of organometallic species 154  
   species identification 83–4  
   sulfated polysaccharides 242–4  
     agarans 247  
 France and territories coastal macroalgae  
   58–9, 89–90  
 against microorganisms 89  
   agriculture 89  
   aquaculture 89  
   brown seaweed biological activities 68  
   phenolic compounds 69–72  
   polysaccharides 68–9  
   terpenes 72–3  
 focus on red and brown macroalgae  
   64–5  
 French and Breton context 60–1  
 metabolites as chemomarkers for  
   taxonomy 81–3  
 metabolites for chemical defense  
   biofouling 75–81  
   biotic interactions 73–5  
 metabolites for industrial uses  
   foods 83–5  
   pharmaceuticals and cosmetics  
     85–9  
 red seaweed biological activities  
   65  
   phycoerythrin 67–8  
   polysaccharides 65–7  
 research network on bioactive  
   compounds 61  
   laboratories 61–2  
   national scale 63  
   regional scale 63–4  
 free radical scavengers  
   phaeophyta 15–17  
   rhodophyta 23  
 fucans, sulfated *see* sulfated  
   polysaccharides  
 fucodiphloroethol 16, 343  
 fucoidans 185–6, 502  
   antidiabetic and anti-obesity  
     properties 374  
 fucoxanthin (FX) 8, 181, 321, 327, 404  
   antidiabetic and anti-obesity  
     properties 373–4  
   health benefits 358–9  
   hepatic enhancement of DHA 333–5  
   *in vitro* and *in vivo* mutagenicity study  
     324–7  
   *in vivo* oral toxicity study 321–4  
 fucoxanthinol (FXOH) 321, 325, 404  
*Fucus vesiculosus* 25, 315  
   metallothionein 302–3  
     characterization 303–4  
     dynamic metallation studies  
       306–14  
     equilibrium metallation 304–6  
 functional foods *see under* food products  
   from seaweeds  
 fucellaran 523–4  
 furoflocamioid 19  
  
 galactans 184, 193  
 galactans, sulfated *see* sulfated  
   polysaccharides  
 galactoglycerolipids 81  
 $\alpha$ -D-galactopyranose 65  
 gamete-attracting pheromone  
   phaeophyta 17  
 Ganges Delta, eco-biochemical studies of  
   seaweeds  
   biochemical composition 51–5  
   commercial uses 46  
   Indian scenario 46–50  
   overview 45–6  
*Garacilaria tikvahiae* 51  
 gas chromatography (GC) 163  
 gas production digestibility methods  
   290–1  
*Gelidium* 17  
 giffordene 17  
*Gigartina tenella* 21  
 3- $\beta$ -D-glucopyranosylstititasta-5,25-  
   diene 5  
 glutamic acid 176  
 gold content of seaweeds 131–7  
 gracilamides 21  
*Gracilaria* 17, 46  
*Gracilaria asiatica* 21  
 gracilarioside 21  
*Grateloupia turuturu*  
   antifouling compounds 79–80  
   phycoerythrin 67–8  
 gravimetric and filtering methods 290  
 green tides 106–7, 111–12  
   inorganic carbon acquisition 110–11  
   light absorption 107–8

- green tides (*Continued*)  
 photosynthesis at sub- and saturating irradiance 108  
 role of bicarbonate use 111  
 $\alpha$ -L-guluronic acid 68
- Haemonchus contortus* 13  
*Halimeda lamouroux* 6  
*Halimeda tuna* 7  
 halimedalactone 6  
 halimediatrial 6  
 halitunal 7  
 halogenated  $\beta$ -bisabolene sesquiterpenes 22  
 health and safety considerations  
 carrageenans 246  
 heavy metal detoxification 441–2, 448  
 algal mechanisms 442  
 exclusion 443  
 extracellular binding polypeptides 442–3  
 internal detoxification 443  
 metal transformation 443–4  
 algal–bacterial consortia 445–6  
 algal–bacterial mechanisms 444  
 bioaccumulation 445  
 biosorption 444–5  
 biological treatment 446–7  
 biotechnological applications 447–8  
 heme-agglutinating properties 536  
 hepatoprotective properties 536  
 hepatoprotective substances  
 phaeophyta 14  
 herbal plants as antimicrobial agents 210  
 herbivorous animals, protection against  
 phaeophyta 15  
 Herpes simplex virus 2 (HSV-2) 22  
*Heterosigma akashiwo* 14  
 high performance liquid  
 chromatography (HPLC) 163  
 high-density lipoprotein (HDL) 182, 322  
*Hizikia fusiforme* 11, 373  
 glycoprotein protection against  
 acetaminophen-induced liver  
 damage 390–5  
*Hormosira hanksii* 17  
 hormosirene 17  
 human cytomegalovirus (HCMV) 16  
*Hydrilla verticillata*  
 chromium-contaminated wastewater  
 treatment  
 adsorption isotherm study 475  
 adsorption kinetics study 471–2  
 characterization of absorbent 465–7  
 effect of adsorbate concentration 470  
 effect of adsorbate dose 470  
 effect of contact time 468  
 effect of pH 468–9  
 effect of stirring speed 467–8  
 effect of temperature 470–1  
 materials 465  
 method 465  
 rate controlling mechanism 473–5  
*p*-hydroxybenzaldehyde 25  
 (6R)-6-hydroxydichototomo-3,14-diene-1,17-dial 14  
 hydroxydictyodial 12  
 12-(S)-hydroxyeicosapentaenoic acid 26  
 hydroxyisoavrainvilleol 8  
 10-hydroxykahukuene 25  
*Hypnea* 46  
*Hypnea musciformis* 27, 51  
*Hypnea valendiae* 23
- ichthyotoxins  
 phaeophyta 12  
 immunosuppressant substances  
 chlorophyta 5  
 phaeophyta 9–12  
 index of essential amino acids (IEAA) 176  
 India  
 seaweed farming 40–1, 46–50  
 indicator amino acid oxidation (IAAO)  
 method 288  
 inductively coupled plasma–mass  
 spectrometry (ICP-MS) 117, 163  
 inductively coupled plasma–optical  
 emission spectroscopy  
 (ICP-OES) 117  
 industrial applications 500, 516  
 phycocolloids  
 edible food coatings 510  
 extraction from seaweeds 508–9  
 food preparation 509  
 other applications 510  
 seaweed composition 500–1  
 mycosporine-like amino acids  
 (MAAs) 502–3  
 polyphenols 502  
 polysaccharides 501–2  
 inosine-5'-monophosphate  
 dehydrogenase inhibitor  
 (IMPDH) 5  
 insecticidal substances  
 rhodophyta 24  
 instrumental neutron activation analysis  
 (INAA) 117  
 Internet resources  
 seaweed databases 42  
 iodine content of seaweeds 131–7, 179  
 iodine species 156–7, 158, 159–60  
 5-iodo-5'-deoxy-tubercidin 23  
 iridophycan 524  
 iron content of seaweeds 131–7, 178  
*Ishige okamurai* 22  
 isocitrate lyase inhibitor  
 rhodophyta 27  
 4-isocymobarbatol 8  
 isodactyloxene 25  
 isodictyol monoacetate 11  
 isodomic acids 24  
 isoeptaondiol 16  
 isolaureatin 24  
 isolaurepinnacin 24  
 isolaurinterol 25  
 isorawsonol 5  
 isozonarol 12
- Jania rubens* 22
- kahalalide 6–7  
*Kappaphycus* 17  
*Karenia mikimotoi* 14  
 kelps 37  
 Kjeldahl method 174–5  
*Klebsiella pneumonia* 25
- laminarans 184, 502  
*Laminaria*  
 ecology and characteristics 357  
*Laminaria japonica* 373  
 glycoprotein stimulant effect 387–9  
*Landsburgia quercifolia* 13  
 lanosol enol ether 25  
 laureatin 24  
*Laurencia* 46  
*Laurencia brongniarti* 25  
*Laurencia elata* 25  
*Laurencia glandulifera* 26  
*Laurencia mariannensis* 25  
*Laurencia nipponica* 24  
*Laurencia obtusa* 18, 19, 20, 21, 24, 25, 27  
*Laurencia okamurai* 18  
*Laurencia pinnata* 24  
*Laurencia pinnatifida* 21  
*Laurencia scoparia* 22  
*Laurencia tristicha* 21  
*Laurencia venusta* 22  
*Laurencia viridis* 21  
*Laurencia yonaguniensis* 19  
*Laurencia brongniartii* 19  
*Laurencia microcladia* 20  
 laurenmariallene 25  
 laurepinacine 24

- laurinterol (LOEL) 18, 25  
 lead content of seaweeds 138–44  
 lectins  
   anti-HIV activities 420–1  
 leptosins 9–10  
*Leptosphaeria* species 9–10  
 ligand to metal charge transfer (LMCT)  
   303  
 light absorption 107–8  
 lignin 182  
*Lindra thalassiae* 8, 13  
 linoleic acid (LA) 329, 330  
 $\alpha$ -linolenic acid (LN) 329, 330  
   bioconversion to DHA 333  
 lipid content of seaweeds 181–2  
 lipooxygenase inhibitor  
   rhodophyta 26  
*Lobophora variegata* 13  
 lobophorolide 13  
*Lophocladia* 20  
 lopophorins 13  
 low-density lipoprotein (LDL) 182  
 lutein 526  
 lyengaroside A 7  
*Lyngbya majuscula* 3  
*Lytechinus variegatus* 17  
  
 magireols 19  
*Magnaporthe grisea* 27  
 magnesium content of seaweeds 118–30,  
   180  
 manganese content of seaweeds 138–44,  
   178  
 $\beta(1\rightarrow4)$  mannan 65, 184  
 mannitol 524  
 $\beta$ -D-mannuronic 68  
 manure from seaweeds 46  
*Marginisporium aberrans* 25  
 marine farming 39  
 marine resources  
   emergent plants 37  
   exploitation  
     French and Breton context 60–1  
     French research network on  
       bioactive compounds 61–4  
     International context 60  
   need for 36  
   producers 37  
   variety of 36–7  
*Mastocarpus stellatus* 17  
 mercury content of seaweeds 131–7  
   mercury species 160  
 meroditerpenes 72  
 meroterpenoids 9, 274  
 metagenomics 41  
 metal-binding polychelates 161–2  
  
 metallothionein (MT) 302–3, 315  
   characterization 303–4  
   dynamic metallation studies 306–14  
   equilibrium metallation  
     arsenic binding 305–6  
     cadmium binding 304–5  
 methicillin-resistant *Staphylococcus*  
   [\*aureus\*](#) (MRSA) 25  
 methoxybifurcarenone 13  
*N*-methyl-D-aspartate (NMDA)  
   receptors 20  
 microencapsulation of bacteria 505–6  
 micropropagation of seaweeds 434–5  
 mineral content of seaweeds 117,  
   176–9  
 molecular hybrid carrageenans 194–5  
 molybdenum content of seaweeds  
   138–44  
 murine coronavirus A59 7  
*Murrayella pericladus* 26  
 mycosporine-like amino acids (MAAs)  
   88–9  
  
 2,7-naphthyridine lophocladines 20  
 natural dyes 210  
 needle rush 37  
 nematocidal substances  
   phaeophyta 12–13  
 neoirietetraol 19  
 neorogiol diol 21  
 net protein utilization (NPU) 287  
 neurophysiological substances  
   rhodophyta 23–4  
 neutral detergent fiber (NDF) 183  
 nickel content of seaweeds 138–44  
 nigricanosides A and B 8  
*Nippostrongylus brasiliensis* 22  
 Nori 17  
 norxenicane 11  
*Notheia anomala* 12, 13  
 nutraceuticals 482  
  
*Odonthalia corymbifera* 27  
 oleic acid 182  
 organic matter digestibility (OMD) 290  
 organometallic species  
   in algae 154–6  
     arsenic 155, 157–8  
     sources of 154  
 osmolytes 86–7  
*Osmundaria serrata* 25  
 oxylipins 73–4  
  
*Padina* 46  
*Palmaria palmata* 17  
 palmitic acid 182  
  
*N*-palmitoyl-2-amino-1,3,4,5-  
   tetryhydroxyoctadecane  
   (sphingosine) 8  
 paper products from seaweeds 38  
*Pelvetia siliquosa* 17  
 penicillin-resistant *Streptococcus*  
   [\*pneumoniae\*](#) 25  
*Penicillus capitatus* 8  
 penochalasons 6  
 penostatins 5–6  
 peptides from seaweeds 223–6  
   anti-HIV activities 421  
 perforenol 20  
 perfuroplocamioid 19  
 perinidin 404  
*Perithalia capillaris* 12  
*Perithalia cadata* 17  
*Petalonia binghamiae* 373  
*Peyssonnelia* 22  
 peyssonols 22  
 phaeophyta (brown algae) 8, 37, 45–6  
   biologically active components 68  
     algicidal activity 14  
     antidiabetic activity 17, 409–10  
     antifeedent activity 17  
     antifungal activity 13  
     antihypertensive activity 17  
     anti-inflammatory activity 13–14  
     antiviral activity 14–15  
     cytotoxic and immunosuppressive  
       activity 9–12  
     free radical scavengers and  
       antioxidant activity 15–17  
     gamete-releasing, gamete-attracting  
       and sperm attracting pheromone  
       activity 17  
     hepatoprotective activity 14  
     ichthyotoxins and feeding-deterrent  
       substances 12  
     nematocidal activity 12–13  
     phenolic compounds 69–72  
     plant pathogen morphological  
       abnormality 17  
     polysaccharides 68–9  
     protection against herbivorous  
       animals 15  
     terpenes 72–3  
 commercial seaweeds 357  
   ecology and characteristics 357  
   health benefits 358–9  
 diterpenes  
   dolabellane skeleton 270  
   dolastane skeleton 270  
   hydroazulenoids 270  
   other skeletons 271  
   xenicane skeleton 269

- phaeophyta (brown algae) (*Continued*)  
   food antioxidants 406–9  
   lipids  
      $\omega$ -3 PUFAs 332–3  
   phenolic phytochemicals 359  
     *Ascophyllum nodosum* 362–4  
     *Ecklonia cava* 359–61  
     health benefits 359  
   restricted occurrence on sulfated fucans (SFs) 240–2  
   sesquiterpenes 268  
 pharmaceutical industry applications 425–6  
 pharmaceutical products from seaweeds 38, 46, 85–6  
 phenolic compounds  
   brown seaweeds 69  
     biological functions and variation 70–1  
     examples from French models 71–2  
     properties 69–70  
   7-phloroethol 342, 343  
   phlorofucofuroeckol 14, 17, 341, 342, 343  
   phloroglucinol 14, 69–72, 341, 342, 343  
     health benefits 378, 385  
       additional activities 384–5  
       anti-inflammatory activities 381–2  
       antioxidant activities 382  
       anti-photoaging activities 382–3  
       antitumor activities 383–4  
       brown seaweeds 378–81  
       MMP inhibition activities 384  
   phlorotannins 69–72, 224–5, 277–8  
     antidiabetic and anti-obesity properties 374  
     anti-HIV activities 419–20  
     food antioxidants 399  
     immune regulatory effects 340–2, 346  
       anti-allergenic effects 345–6  
       anti-asthmatic effects 344–5  
       anti-rheumatoid arthritis (RA) effects 345  
       macrophage cells 343–4  
       microglial cells 344  
   phosphorus content of seaweeds 117–30  
   phosphorus content of seaweeds 180  
   photosynthesis  
     at sub- and saturating irradiance 108  
     inorganic carbon acquisition 110–11  
     light absorption 107–8  
   photosynthetically active radiation (PAR) 197  
   pH-stat and pH-drop digestibility methods 289–90  
   phycobiliproteins 526  
   phycocolloids  
     industrial applications  
       edible food coatings 510  
       extraction from seaweeds 508–9  
       food preparation 509  
       other applications 510  
   phycocyanin 17, 526  
   phycoerythrin 17, 67, 526  
   red seaweeds  
     biological functions and variation 67  
     *Grateloupia turuturu* 67–8  
     properties 67  
   phylogeographical analysis 42  
   phytoplankton 37  
   P-I curves 107  
   pigments from seaweeds 524–6  
   pirene 19  
   plankton 37  
   plant pathogen morphological abnormality  
     phaeophyta 17  
   plants, emergent 37  
   *Plasmodium falciparum* 7, 27  
   plastoquinones 16  
   *Plocamium* 20  
   *Plocamium cartilagineum* 24  
   *Plocamium corallorhiza* 21  
   *Plocamium hamatum* 18  
   *Plocamium telfairia* 24  
   plocaralides 20  
   *Plocumium cartilagineum* 19  
   pollution biomonitoring  
     environmental monitoring 150–51  
     radioactive pollution 152–4  
     seaweeds as bioindicators 148–9  
       influence of algal division 149–50  
       influence of sampling time and sample part 149  
       influence of species 150  
   pollution detection and control 515  
   polybromindoles 19  
   polychelates 161–2  
   polyphenols 224–5  
   polysaccharides 225–6  
     brown seaweeds  
       biological functions and variation 68–9  
       examples from French models 69  
       properties 68  
     emulsions and foams 541–2  
     gelation 541  
     health benefits 358  
     interaction with food components 542  
     major applications 542–51  
   red seaweeds  
     *Asparagopsis armata* 66–7  
     biological functions and variation 65  
     properties 65  
     *Soliera chordalis* 65–6  
     water-binding 541  
   polysaccharides, sulfated *see* sulfated polysaccharides  
   *Polysiphonia urceolata* 8, 23  
   polyunsaturated fatty acids (PUFAs) 83, 329  
      $\omega$ -3 329–31  
       brown seaweed lipids 332–3  
       importance 331–2  
      $\omega$ -3 and  $\omega$ -6 181–2  
      $\omega$ -6 181–2, 330–1  
   *Porphyra* 46  
   *Porphyra yezoensis*  
     chemoprotection against drug toxicity 395–6  
   *Portiera hornemannii* 17, 20  
   *Posidonia*  
     inorganic carbon acquisition 110–11  
     light absorption 107  
     photosynthesis at sub- and saturating irradiance 108  
   potassium content of seaweeds 118–30  
   prebiotics 505  
   prenyl toluquinones 15  
   prevezol 21  
   primary colonisers in biofouling 77  
   protein efficiency ratio (PER) 287  
   protein tyrosine phosphate 1B inhibitors (PTP1B)  
     chlorophyta 8  
   proteins 174–6, 387  
     chemoprotection against  
       acetaminophen toxicity 389–95  
     chemoprotection against drug toxicity 395–6  
   digestibility 285–6  
     amino acid score (AAS) 286, 287  
     essential amino acid index (EAAI) 286–7  
     protein digestibility corrected amino acid score (PDCAAS) 288  
   glycoprotein protection against  
     acetaminophen-induced liver damage 390–5  
   glycoprotein protection against  
     drug-induced liver damage 395–6  
   glycoprotein stimulant from  
     *Laminaria japonica* 387–9  
   red seaweeds 84–5  
   *Pterocladia* 17

- ptilodene 26  
*Ptilotafilicina* 26  
*Pyricularia oryzae* 17
- radionuclides in seaweeds 148  
   as indicators of radioactive pollution 152–4  
 raw materials from seaweeds 46  
 recommended daily intake (RDI) of minerals 177–8  
 restriction fragment length polymorphism (RFLP) analysis 84  
*Rhizoclonium grande* 46  
*Rhizoclonium hookeri* 49  
*Rhizoclonium riparium* 49  
*Rhodomela confervoides* 20  
 rhodophyta (red algae) 17, 37, 46  
   biologically active components  
     aldose reductase inhibition activity 27  
     anti-elastase activity against porcine pancreas elastase 27  
     antifeedant activity 26–7  
     antihelmintic activity 22  
     anti-inflammatory activity 23  
     antimicrobial activity 25–6  
     antiviral activity 21–2  
     cytotoxic activity 17–21  
     free radical scavenger activity 23  
     insecticidal activity 24  
     isocitrate lyase inhibition activity 27  
     lipooxygenase inhibition 26  
     neurophysiological activity 23–4  
     phycoerythrin 67–8  
     polysaccharides 65–7  
 diterpenes 272–3  
 food products  
   proteins 84–5  
 sesquiterpenes  
   bisabolane skeleton 266  
   brasilane skeleton 266  
   chamigrane skeleton 263  
   cuparane skeleton 265  
   laurane skeleton 264  
   other skeletons 266  
   sulfated glucans (SGs) 242  
 riboflavin (vitamin B<sub>2</sub>) 180–1  
 ribofuranosides 11  
 ribulose-biphosphate carboxylase oxygenase (Rubisco) 110  
 rubber products from seaweeds 38  
 rubidium content of seaweeds 138–44
- Saccharomyces cerevisiae* 8, 25  
*Salmonella* 25  
*Salmonella typhimurium* 8  
 salt grass 37  
 sanadaol 14  
 sargachromanols 15  
 sargaol 16  
 sargaquinone 14  
*Sargassum micracanthum* 15, 16  
*Sargassum siliquastrum* 15  
*Sargassum* 46  
*Sargassum thunbergii* 16  
*Sargassum tortile* 9, 10  
*Sargassum yezoense* 373  
 sargols 9  
 sargothunbergol 16  
 sea lettuces 37  
 SEAPURA project (EU) 39–40  
 seaweed industry  
   France 60–1  
   International 60  
 Seaweed Metabolite Database (SWMD) 41  
 seaweed nanoparticles 211  
   antibacterial finishing  
     antibacterial property 217  
     antibacterial test 217  
     padding of extract 216–17  
   bioactive compounds 211–12  
     extraction methods 212  
     seaweed collection 211  
     solvent selection 211–12  
   characterization  
     Fourier transform infrared (FTIR) spectroscopy 213–15  
     transmission electron microscope (TEM) studies 215–16  
     UV-Vis spectroscopy 212–13  
   extraction  
     crude extract 212  
     nanoparticle extraction 212  
     permanent finish 217  
 seaweeds 36, 43, 348–9  
   as pollution bioindicators 148–9  
   environmental monitoring 150–51  
   influence of algal division 149–50  
   influence of sampling time and sample part 149  
   influence of species 150  
   radioactive pollution 152–4  
   biologically active components 4  
     chlorophyta 5–8  
     phaeophyta 8–17  
     rhodophyta 17–27  
   chemical speciation  
     importance of 154  
     organometallic species, sources of 154  
   organometallic species in algae 154–61  
   classification  
     chlorophyta 5  
     phaeophyta 8  
     rhodophyta 17  
   compound bioaccessibility studies 349  
   cell culture models 352  
   *in vitro* methods 349–51  
   *in vivo* methods 349  
   current research trends 41  
     bioinformatics 41  
     data storage and retrieval 41  
     information analysis 42  
     metagenomics 41  
     phylogeographical and evolutionary analysis 42  
   diversity 37  
   eco-biochemical studies in Lower Gangetic Delta  
     biochemical composition 51–5  
     commercial uses 46  
     Indian scenario 46–50  
     overview 45–6  
   edibility studies 146–7  
   floating 37  
   future prospects 42–3  
   marine farming 39  
     India 40–1  
   mineral content 117  
     *see also* trace and ultratrace elements  
     uses 37–9, 46  
   secondary colonisers in biofouling 77  
   selenium content of seaweeds 138–44  
     selenium species 159, 160–61  
   Semliki forest virus (SFV) 8  
   sesquiterpenes 72, 263  
     brown algae 267  
     green algae 268  
     red algae  
       bisabolane skeleton 266  
       brasilane skeleton 266  
       chamigrane skeleton 263  
       cuparane skeleton 265  
       laurane skeleton 264  
       other skeletons 266  
   ship fouling *see* biofouling  
   *Sigmatocia symbiotica* 23  
   snake bites 5  
   snyderol sesquiterpene 27  
   sodium content of seaweeds 118–30  
   *Soliera chordalis*  
     polysaccharides 65–6  
   sperm-attracting pheromone  
     phaeophyta 17

- sphingosine (*N*-palmitoyl-2-amino-1,3,4,5-tetrahydroxyoctadecane) 8
- Staphylococcus aureus* 5, 7
- Staphylococcus epidermidis* 25
- starch 184
- steroids 279
- streptozotocin (STZ)-induced diabetes 371
- Strongylocentrotus intermedius* 27
- strontium content of seaweeds 138–44
- stypodiol 16
- stypolactone 11
- stypoldione 12, 16
- Styopodium carpophyllum* 11, 17
- Styopodium zonale* 11, 12
- stypoquinonic acid 12
- sulfated polysaccharides (SPs) 193–6, 229–30, 255
- analysis
- detection, quantization and purity 231–3
  - molecular weight determination 233
  - structural characterization 233–8
- anti-HIV activities 417–19
- carrageenan sources in Russian Far East 196–7
- extraction methods
- isolation 230–1
- food antioxidants 399–400
- industrial applications
- carrageenans and agarans 244–7
  - food supplements and cosmetics 242–4
- pharmacological properties 247
- anti-inflammatory agents 250–1
  - antiparasitic activities 254
  - antithrombal agents 249–50
  - antitumor activities 253–4
  - antiviral actions 247–8
  - effect on cellular growth, migration and adhesion 254–5
  - pro- and antiangiogenic actions 251–2
- phylogenetic distribution 230
- polysaccharide composition and life cycle phase
- Chondrus pinnulatus* 197
  - Tichocarpus crinitus* 197–9
- rheological and viscosity properties 200–1
- structure and phylogenetic occurrence 239–40
- brown algae 240–2
  - green algae 242
  - red algae 242
- sulquinovosyldiacylglycerol 21
- Symphycladia latiuscula* 22, 23, 27
- taondiol 16
- Taonia atomaria* 12, 14, 17
- telfairine 24
- terpenes
- brown seaweeds 72
  - biological functions and variations 73
  - properties 72–3
- tertiary colonisers in biofouling 78
- 2,20,3,30-tetrabromo-4,40,5,50-tetrahydroxydiphenylmethane 27
- 2,3,5,6-tetrabromoindol 25
- tetrachlorinated cyclohexane 19
- tetrahydrofuran 13
- tetrameric acid contiguous metabolites 7
- tetraprenyltoluquinols 16
- teurilene 18
- textile products from seaweeds 38
- textiles 205
- cotton fabric 207
  - preparatory process 207–8
  - cotton fiber 205–6
  - structure and chemical reactivity 206
  - cotton yarn 206–7
- thiamine (vitamin B<sub>1</sub>) 180
- thunbergols 16
- thyresenols 21
- thysiferol 22
- thysiferyl 23-acetate 18, 22
- tichocarpols 26
- Tichocarpus crinitus* 26
- structural peculiarities of sulfated polysaccharides 193–6
  - carrageenan sources in Russian Far East 196–7
  - environmental influence 199–200
  - polysaccharide composition and life cycle phase 197–9
  - rheological and viscosity properties 200–1
- tin content of seaweeds
- tin species 161
- tissue culture 431–2, 438
- seaweed production 432–3
  - active chemicals 433
  - bioprocess technology 436–8
  - callus and cell suspension culture 435–6
  - growth regulators 434
  - micropropagation 434–5
  - polyamines as growth promoters 433–4
- tissue engineering 512–13
- tocopherol (vitamin E) 181
- total dietary fiber (TDF) 183
- trace and ultratrace elements 116–17
- analytical chemistry 162
  - sample preparation 162–3
  - separation and determination procedures 163–4
- chemical speciation
- importance of 154
  - organometallic species, sources of 154
  - organometallic species in algae 154–61
- edibility studies 146–7
- legislation
- European 117–146
  - US 146–7
- pollution biomonitoring
- environmental monitoring 150–51
  - radioactive pollution 152–4
  - seaweeds as bioindicators 148–9
- radionuclides 148
- 2,20,3-tribromo-30,4,40,5-tetrahydroxy-60-hydroxymethyldiphenylmethane 27
- 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether 22
- (2R)-2-(2,3,6-tribromo-4,5-dihydroxybenzyl) cyclohexanone 23
- tributyltin (TBT) 76
- Trichostrongylus colubriformis* 13
- trifluhalol 68
- triphloroethol 342, 343
- true protein biological value (TBV) 286
- true protein digestibility (TPD) 286
- Turbinaria* 46
- Turbinaria conoides* 11
- Turbinaria ornate* 10
- turbinaric acid 10
- Tydemania expeditionis* 5
- tyrosine kinase p56lck 7
- ultratrace elements *see* trace and ultratrace elements
- Ulva* 46
- green tides
  - inorganic carbon acquisition 110–11
  - light absorption 107

- photosynthesis at sub- and saturating irradiance 108
- role of bicarbonate use 111
- Ulva fasciata* 8
- Ulva lactuca* 5, 46, 49, 55
- ulvans 184, 502
- Undaria*
  - ecology and characteristics 357
- Undaria pinnatifida* 372
- uranium content of seaweeds 138–44
- uses of seaweeds 37–9, 46
- vanadium content of seaweeds 138–44
- vancomycin-resistant *Enterococcus faecalis* and *E. faecium* 25
- Vaucheria* 50
- venustatriol 22
- vesicular stomatitis virus (VSV) 22
- Vidalia obtusiloba* 23
- vidalols 23
- vitamins 38, 179–81
- wastewater, biological treatment of 46, 537–8
- weight control 536–7
- World Summit on Sustainable Development (2002) 58
- xanthophylls 8, 37
- xenicane 11
- xylans 184
- $\beta$ -D-xylopyranose 65
- zinc content of seaweeds 138–44, 178
- zonarol 12, 13
- zonarone 12
- Zostera*
  - inorganic carbon acquisition 110–11
  - light absorption 107
  - photosynthesis at sub- and saturating irradiance 108