

# Recent Advances in Polyphenol Research

Volume 5

Edited by

Kumi Yoshida,  
Véronique Cheynier  
and Stéphane Quideau



WILEY Blackwell



# **Recent Advances in Polyphenol Research**

## **Recent Advances in Polyphenol Research**

A series for researchers and graduate students whose work is related to plant phenolics and polyphenols, as well as for individuals representing governments and industries with interest in this field. Each volume in this biennial series focuses on several important research topics in plant phenols and polyphenols, including chemistry, biosynthesis, metabolic engineering, ecology, physiology, food, nutrition, and health.

### **Volume 5 Editors:**

Kumi Yoshida, Véronique Cheynier, and Stéphane Quideau

### **Series Editor-in-Chief:**

Stéphane Quideau (University of Bordeaux, France)

### **Series Editorial Board:**

Oyvind Andersen (University of Bergen, Norway)

Luc Bidel (INRA, Montpellier, France)

Véronique Cheynier (INRA, Montpellier, France)

Catherine Chèze (University of Bordeaux, France)

Gilles Comte (University of Lyon, France)

Fouad Daayf (University of Manitoba, Winnipeg, Canada)

Olivier Dangles (University of Avignon, France)

Kevin Davies (Plant & Food Research, Palmerston North, New Zealand)

Maria Teresa Escribano-Bailon (University of Salamanca, Spain)

Ann E. Hagerman (Miami University, Oxford, OH, USA)

Victor de Freitas (University of Porto, Portugal)

Johanna Lampe (Fred Hutchinson Cancer Research Center, Seattle, WA, USA)

Vincenzo Lattanzio (University of Foggia, Italy)

Virginie Leplanquais (LVMH Research, Christian Dior, France)

Stephan Martens (Fondazione Edmund Mach, IASMA, San Michele all'Adige, Italy)

Nuno Mateus (University of Porto, Portugal)

Annalisa Romani (University of Florence, Italy)

Pascale Sarni-Manchado (INRA, Montpellier, France)

Celestino Santos-Buelga (University of Salamanca, Spain)

Katy Schwinn (Plant & Food Research, Palmerston North, New Zealand)

David Vauzour (University of East Anglia, Norwich, UK)

# Recent Advances in Polyphenol Research

Volume 5

*Edited by*

**Kumi Yoshida**

*Professor, Natural Product and Bioorganic Chemistry*

*Graduate School of Information Science*

*Nagoya University, Japan*

**Véronique Cheynier**

*Research Director, Plant and Food chemistry*

*Institut National de la Recherche Agronomique*

*UMR1083 Sciences pour l'Œnologie*

*Montpellier, France*

**Stéphane Quideau**

*Professor, Organic and Bioorganic Chemistry*

*Institut des Sciences Moléculaires, CNRS-UMR 5255*

*University of Bordeaux, France*

**WILEY** Blackwell

This edition first published 2017 © 2017 by John Wiley & Sons, Ltd.

*Registered Office*

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

*Editorial Offices*

9600 Garsington Road, Oxford, OX4 2DQ, UK

The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, 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 UK 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.

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.

**Limit of Liability/Disclaimer of Warranty:** While the publisher and author(s) have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. It is sold on the understanding that the publisher is not engaged in rendering professional services and neither the publisher nor the author shall be liable for damages arising herefrom. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

*Library of Congress Cataloging-in-Publication Data*

ISBN: 9781118883266

Recent advances in polyphenol research

ISSN 2474-7696

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

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

Cover image: The ICP2014 Organizing Committee

Set in 10/13pt Times by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

## **Dedication**

To **Michel Bourzeix**—one of the founders of Groupe Polyphénols and its secretary from 1972 to 1995—who devoted his career to promoting research on polyphenols and supported GP activities and conferences with dedication and enthusiasm

To **Dieter Treutter**—a faithful member of the Groupe Polyphénols board for many years and the organiser of ICP2000

*in memoriam*

The editors wish to thank all of the members of the “Groupe Polyphénols” Board Committee (2012–2014) for their guidance and assistance throughout this project.

*Groupe Polyphénols Board 2012–2014*

Prof. Oyvind Andersen

Dr. Luc Bidel

Dr. Véronique Cheynier

Dr. Catherine Chèze

Prof. Olivier Dangles

Prof. Ann E. Hagerman

Dr. Johanna Lampe

Prof. Vincenzo Lattanzio

Dr. Virginie Leplanquais

Dr. Nuno Mateus

Dr. Gary Reznik

Prof. Celestino Santos-Buelga

Dr. Katy Schwinn

Dr. David Vauzour

Prof. Kristiina Wähälä

Prof. Kumi Yoshida





# Contents

<i>Contributors</i>	xv
<i>Preface</i>	xix
<b>1 The Physical Chemistry of Polyphenols: Insights into the Activity of Polyphenols in Humans at the Molecular Level</b>	<b>1</b>
<i>Olivier Dangles, Claire Dufour, Claire Tonnelé and Patrick Trouillas</i>	
1.1 Introduction	1
1.2 Molecular complexation of polyphenols	4
1.2.1 Polyphenol–protein binding	4
1.2.1.1 Interactions in the digestive tract	5
1.2.1.2 Interactions beyond intestinal absorption	6
1.2.2 Interactions with membranes	9
1.3 Polyphenols as electron donors	11
1.3.1 The physicochemical bases of polyphenol-to-ROS electron transfer	12
1.3.1.1 Thermodynamics descriptors	12
1.3.1.2 Kinetics of hydrogen atom transfer	14
1.3.1.3 Kinetics and mechanisms	15
1.3.2 ROS scavenging by polyphenols in the gastrointestinal tract	20
1.4 Polyphenols as ligands for metal ions	21
1.4.1 Interactions of polyphenols with iron and copper ions	22
1.4.2 A preliminary theoretical study of iron–polyphenol binding	25
1.4.2.1 Charge states, spin states, and geometries	25
1.4.2.2 Oxidation of the bideprotonated catechol	26
1.5 Conclusions	27
References	28
<b>2 Polyphenols in Bryophytes: Structures, Biological Activities, and Bio- and Total Syntheses</b>	<b>36</b>
<i>Yoshinori Asakawa</i>	
2.1 Introduction	36
2.2 Distribution of cyclic and acyclic bis-bibenzylys in Marchantiophyta (liverworts)	37

2.3	Biosynthesis of bis-bibenzyls	39
2.4	The structures of bis-bibenzyls and their total synthesis	50
2.5	Biological activity of bis-bibenzyls	58
2.6	Conclusions	60
	Acknowledgments	61
	References	61
<b>3</b>	<b>Oxidation Mechanism of Polyphenols and Chemistry of Black Tea</b>	<b>67</b>
	<i>Yosuke Matsuo and Takashi Tanaka</i>	
3.1	Introduction	67
3.2	Catechin oxidation and production of theaflavins	71
3.3	Theasinensins	73
3.4	Coupled oxidation mechanism	75
3.5	Bicyclo[3.2.1]octane intermediates	77
3.6	Structures of catechin oxidation products	78
3.7	Oligomeric oxidation products	82
3.8	Conclusions	84
	Acknowledgments	85
	References	85
<b>4</b>	<b>A Proteomic-Based Quantitative Analysis of the Relationship Between Monolignol Biosynthetic Protein Abundance and Lignin Content Using Transgenic <i>Populus trichocarpa</i></b>	<b>89</b>
	<i>Jack P. Wang, Sermsawat Tunlaya-Anukit, Rui Shi, Ting-Feng Yeh, Ling Chuang, Fikret Isik, Chenmin Yang, Jie Liu, Quanzi Li, Philip L. Loziuk, Punith P. Naik, David C. Muddiman, Joel J. Ducoste, Cranos M. Williams, Ronald R. Sederoff and Vincent L. Chiang</i>	
4.1	Introduction	90
4.2	Results	94
4.2.1	Production of transgenic trees downregulated for genes in monolignol biosynthesis	94
4.2.2	Absolute quantification of protein abundance	95
4.2.3	Variation in protein abundance in wild-type and transgenic plants	96
4.2.4	Variation in lignin content	96
4.2.5	Relationship of lignin content and protein abundance	98
4.3	Discussion	101
4.4	Materials and methods	102
4.4.1	Production of transgenic trees	102
4.4.2	Proteomic analysis	103

---

4.4.3	Lignin quantification	104
4.4.4	Statistical analysis	104
	References	104

## **5 Monolignol Biosynthesis and Regulation in Grasses 108**

*Peng Xu and Laigeng Li*

5.1	Introduction	108
5.2	Unique cell walls in grasses	109
5.3	Lignin deposition in grasses	110
5.4	Monolignol biosynthesis in grasses	111
5.4.1	Proposed pathway for monolignol biosynthesis	111
5.4.2	Monolignol biosynthetic genes in grasses	112
5.4.3	Functional genomics of monolignol biosynthesis in grass species	114
5.5	Regulation of monolignol biosynthesis in grasses	114
5.5.1	Lignin regulation in secondary cell wall biosynthesis	114
5.5.2	Repressor genes of monolignol biosynthesis in grasses	117
5.5.3	Regulation of monolignol biosynthesis under stress	118
5.6	Remarks	119
	Acknowledgments	119
	References	120

## **6 Creation of Flower Color Mutants Using Ion Beams and a Comprehensive Analysis of Anthocyanin Composition and Genetic Background 127**

*Yoshihiro Hase*

6.1	Introduction	127
6.2	Induction of flower color mutants by ion beams	129
6.3	Mutagenic effects and the molecular nature of the mutations	131
6.4	Comprehensive analyses of flower color, pigments, and associated genes in fragrant cyclamen	131
6.5	Mutagenesis and screening	133
6.5.1	Yellow mutants	134
6.5.2	Red–purple mutants	135
6.5.3	White mutants	135
6.5.4	Deeper color mutants	136
6.6	Genetic background and the obtained mutants	136
6.7	Carnations with peculiar glittering colors	137
6.8	Conclusions	139
	Acknowledgments	140
	References	140

<b>7 Flavonols Regulate Plant Growth and Development through Regulation of Auxin Transport and Cellular Redox Status</b>	<b>143</b>
<i>Sheena R. Gayomba, Justin M. Watkins and Gloria K. Muday</i>	
7.1 Introduction	143
7.2 The flavonoids and their biosynthetic pathway	144
7.3 Flavonoids affect root elongation and gravitropism through alteration of auxin transport	146
7.4 Mechanisms by which flavonols regulate IAA transport	149
7.5 Lateral root formation	151
7.6 Cotyledon, trichome, and root hair development	152
7.7 Inflorescence architecture	154
7.8 Fertility and pollen development	154
7.9 Flavonols modulate ROS signaling in guard cells to regulate stomatal aperture	155
7.10 Transcriptional machinery that controls synthesis of flavonoids	157
7.11 Hormonal controls of flavonoid synthesis	160
7.12 Flavonoid synthesis is regulated by light	161
7.13 Conclusions	162
Acknowledgments	162
References	163
<b>8 Structure of Polyacylated Anthocyanins and Their UV Protective Effect</b>	<b>171</b>
<i>Kumi Yoshida, Kin-ichi Oyama and Tadao Kondo</i>	
8.1 Introduction	171
8.2 Occurrence and structure of polyacylated anthocyanins in blue flowers	173
8.2.1 Searching for polyacylated anthocyanins	175
8.2.2 Isolation and structural determination of polyacylated anthocyanins	176
8.2.2.1 Structural determination of phacelianin and tecophilin	177
8.3 Molecular associations of polyacylated anthocyanins in blue flower petals	178
8.3.1 Intermolecular associations of anthocyanins	179
8.3.2 Intramolecular associations of anthocyanins	180
8.3.3 Coexistence of inter- and intramolecular associations involved in the blue coloration	182

8.4	UV protection of polyacylated anthocyanins from solar radiation	183
8.4.1	<i>E,Z</i> -isomerization of cinnamoyl derivative residues in polyacylated anthocyanins	184
8.4.2	UV protective effect of polyacylated anthocyanins	186
8.5	Conclusions	187
	References	188
<b>9</b>	<b>The Involvement of Anthocyanin-Rich Foods in Retinal Damage</b>	<b>193</b>
	<i>Kenjiro Ogawa and Hideaki Hara</i>	
9.1	Introduction	193
9.2	Anthocyanin-rich foods for eye health	195
9.3	Experimental models to mimic eye diseases and the effect of anthocyanin-rich foods	196
9.3.1	3-(4-Morpholinyl) sydnonimine hydrochloride (SIN-1)-induced and <i>N</i> -methyl-D-aspartate receptor (NMDA)-induced retinal ganglion cell damage models to mimic glaucoma <i>in vitro</i> and <i>in vivo</i>	196
9.3.2	Vascular endothelial growth factor (VEGF)-induced angiogenesis models that mimic diabetic retinopathy <i>in vitro</i> and <i>in vivo</i>	198
9.3.3	Light-induced retinal damage models to mimic AMD <i>in vitro</i> and <i>in vivo</i>	199
9.4	Conclusions	201
	References	203
<b>10</b>	<b>Prevention and Treatment of Diabetes Using Polyphenols via Activation of AMP-Activated Protein Kinase and Stimulation of Glucagon-like Peptide-1 Secretion</b>	<b>206</b>
	<i>Takanori Tsuda</i>	
10.1	Introduction	206
10.2	Activation of AMPK and metabolic change	207
10.2.1	Activation of AMPK	207
10.2.2	Dietary factors that exert diabetes-preventing and -suppressing effects through the activation of AMPK	208
10.2.2.1	Blueberry (bilberry)	209
10.2.2.2	Black soybean	210
10.3	GLP-1 action and diabetes prevention/suppression	212
10.3.1	GLP-1 action	212
10.3.2	Dietary factors that promote GLP-1 secretion	213

10.3.2.1	Curcumin	214
10.3.2.2	Edible young leaves of sweet potato (culinary sweet potato leaves)	217
10.3.2.3	Delphinidin 3-rutinoside (D3R)	218
10.4	Future issues and prospects	220
	References	222

**11 Beneficial Vascular Responses to Proanthocyanidins: Critical Assessment of Plant-Based Test Materials and Insight into the Signaling Pathways** **226**  
*Herbert Kolodziej*

11.1	Introduction	227
11.2	Appraisal of test materials	228
11.2.1	Analytical challenges of proanthocyanidin composition	229
11.2.2	Chemical data on proanthocyanidin-containing materials	230
11.3	Endothelial dysfunction	233
11.4	<i>In vitro</i> test systems	234
11.5	Vasorelaxant mechanisms	235
11.5.1	Endothelium-dependent vasorelaxation	235
11.5.2	eNOS-NO-cGMP signaling pathway	235
11.5.2.1	Key role of the NO-cGMP signaling pathway	236
11.5.2.2	Activation of eNOS via the phosphatidylinositol-3-kinase (PI3K)/Akt pathway	242
11.5.2.3	Role of reactive oxygen species and redox-sensitive kinases	243
11.5.3	Eicosanoid-mediated vasorelaxation	245
11.5.4	Endothelium-derived hyperpolarizing signaling cascade	245
11.5.4.1	Modulation of K <sup>+</sup> channel functions	247
11.5.4.2	Ca <sup>2+</sup> signaling events and modulation of Ca <sup>2+</sup> channel functions	248
11.6	Bioavailability and metabolic transformation: the missing link in the evidence to action in the body	249
11.7	Conclusions	250
	References	251

**12 Polyphenols for Brain and Cognitive Health** **259**  
*Katherine H. M. Cox and Andrew Scholey*

12.1	Introduction	259
12.2	Studies of total polyphenols and cognition	260
12.2.1	Tea	262
12.2.2	Cocoa	265

12.2.3	Wine and grapes	267
12.2.4	Soy	269
12.3	Pine bark	272
12.4	Discussion and conclusions	283
References		283

### **13 Curcumin and Cancer Metastasis** **289**

*Ikuo Saiki*

13.1	Introduction	290
13.1.1	Antimetastatic mechanisms	291
13.1.2	Curcumin, a polyphenol from <i>Curcuma longa</i>	292
13.2	Effects of curcumin on intra-hepatic metastasis of liver cancer	293
13.2.1	Effect of curcumin on the growth of the implanted HCC and intrahepatic metastasis	293
13.2.2	Effect of curcumin on tumor invasion and expression of invasion-related molecules	293
13.2.3	Effect of curcumin on tumor cell adhesion to fibronectin, laminin, and poly-L-lysine substrates	295
13.2.4	Effect of curcumin on the expression of some integrin subunits	295
13.2.5	Effect of curcumin on the haptotactic migration	295
13.2.6	Effect of curcumin on the formation of actin stress fibers	297
13.3	Effects of curcumin on lymph node metastasis of lung cancer	298
13.3.1	Comparison of metastatic properties of Lewis lung carcinoma (LLC) and its metastatic variant cell line	298
13.3.2	Effect of curcumin on the growth of the inoculated tumor and lymph node metastasis of orthotopically implanted LLC cells	299
13.3.3	Combined effect of curcumin and CDDP ( <i>cis</i> -diamine-dichloroplatinum) in the lung cancer model	299
13.3.4	Effect of curcumin on the growth and invasion of LLC cells <i>in vitro</i>	300
13.3.5	Anti-AP-1 transcriptional activity of curcumin in LLC cells	301
13.3.6	Effect of curcumin on the expression of mRNAs for u-PA and u-PAR in LLC	301
13.4	Effects of curcumin on tumor angiogenesis	303
13.4.1	Curcumin inhibits the formation of capillary-like tubes in rat lymphatic endothelial cells (TR-LE)	303
13.4.2	Inhibition of IKK is independent of the inhibitory effect of curcumin	304
13.4.3	Involvement of Akt's inhibition in curcumin's activities	304
13.4.4	Involvement of MMP-2 in lymphangiogenesis	306
13.5	Conclusions	307
References		307

<b>14</b>	<b>Phytochemical and Pharmacological Overview of <i>Cistanche</i> Species</b>	<b>313</b>
	<i>Hai-Ning Lv, Ke-Wu Zeng, Yue-Lin Song, Yong Jiang and Peng-Fei Tu</i>	
14.1	Introduction	313
14.2	Chemical constituents of <i>Cistanche</i> species	314
14.2.1	Phenylethanoid glycosides (PhGs)	315
14.2.2	Benzyl glycosides	315
14.2.3	Iridoids	315
14.2.4	Monoterpenoids	315
14.2.5	Lignans	321
14.2.6	Polysaccharides	322
14.2.7	Other types of compounds	322
14.3	Bioactivities of the extracts and pure compounds from <i>Cistanche</i> species	322
14.3.1	Antioxidation	323
14.3.2	Neuroprotection	324
14.3.2.1	Anti-Parkinson's disease (PD)	324
14.3.2.2	Cognitive improvement	328
14.3.2.3	Sedation	331
14.3.3	Vasorelaxation	331
14.3.4	Antifatigue and longevity promotion	331
14.3.5	Anti-inflammation and immunoregulation	332
14.3.6	Antitumor	333
14.3.7	Defecation promotion	333
14.3.8	Hepatoprotection	333
14.3.9	Antimyocardial ischemia	333
14.3.10	Radiation resistance	334
14.3.11	Tissue repairing	334
14.4	Conclusions	334
	References	334
	<i>Index</i>	342



# Contributors

**Yoshinori Asakawa**, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, Japan.

**Vincent L. Chiang**, Forest Biotechnology Group, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA.

**Ling Chuang**, Forest Biotechnology Group, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA.

**Katherine H. M. Cox**, Centre for Human Psychopharmacology, School of Health Sciences, Swinburne University, Melbourne, Victoria, Australia.

**Olivier Dangles**, UMR 408 INRA, Sécurité et Qualité des Produits d'Origine Végétale, University of Avignon, Avignon Cedex 9, France.

**Joel J. Ducoste**, Civil, Construction and Environmental Engineering, North Carolina State University, Raleigh, NC, USA.

**Claire Dufour**, UMR 408 INRA, Sécurité et Qualité des Produits d'Origine Végétale, Centre de Recherche PACA, University of Avignon, Avignon Cedex 9, France.

**Sheena R. Gayomba**, Department of Biology and Center for Molecular Signaling, Wake Forest University, Winston-Salem, NC, USA.

**Hideaki Hara**, Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, Gifu, Japan.

**Yoshihiro Hase**, Ion Beam Mutagenesis Research Group, Quantum Beam Science Directorate, Japan Atomic Energy Agency, Takasaki, Gunma, Japan.

**Fikret Isik**, NCSU Cooperative Tree Improvement Program, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA.

**Yong Jiang**, State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, China.

**Herbert Kolodziej**, Institute of Pharmacy, Pharmaceutical Biology, Freie Universität Berlin, Berlin, Germany.

**Tadao Kondo**, Graduate School of Information Science, Nagoya University, Chikusa, Nagoya, Japan.

**Laigeng Li**, National Key Laboratory of Plant Molecular Genetics and CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China.

**Quanzi Li**, State Key Laboratory of Tree Genetics and Breeding, Chinese Academy of Forestry, Beijing, China.

**Jie Liu**, Forest Biotechnology Group, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA.

**Philip L. Loziuk**, W.M. Keck Fourier Transform Mass Spectrometry Laboratory, Department of Chemistry, North Carolina State University, Raleigh, NC, USA.

**Hai-Ning Lv**, State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, China.

**Yosuke Matsuo**, Department of Natural Product Chemistry, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan.

**Gloria K. Muday**, Department of Biology and Center for Molecular Signaling, Wake Forest University, Winston-Salem, NC, USA.

**David C. Muddiman**, W.M. Keck Fourier Transform Mass Spectrometry Laboratory, Department of Chemistry, North Carolina State University, Raleigh, NC, USA.

**Punith P. Naik**, Civil, Construction and Environmental Engineering, North Carolina State University, Raleigh, NC, USA.

**Kenjirou Ogawa**, Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, Gifu, Japan.

**Kin-ichi Oyama**, Research Center for Materials Science, Nagoya University, Chikusa, Nagoya, Japan.

**Ikuo Saiki**, Division of Pathogenic Biochemistry, Institute of Natural Medicine (INM), University of Toyama, Toyama, Japan.

**Andrew Scholey**, Centre for Human Psychopharmacology, School of Health Sciences, Swinburne University, Melbourne, Victoria, Australia.

**Ronald R. Sederoff**, Forest Biotechnology Group, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA.

**Rui Shi**, Forest Biotechnology Group, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA.

**Yue-Lin Song**, Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China.

**Takashi Tanaka**, Department of Natural Product Chemistry, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan.

**Claire Tonnelé**, Chimie des Matériaux Nouveaux, University of Mons, Mons, Belgium.

**Patrick Trouillas**, UMR 850 INSERM, Faculté de Pharmacie, University of Limoges, Limoges Cedex, France.

**Takanori Tsuda**, College of Bioscience and Biotechnology, Chubu University, Kasugai, Aichi, Japan.

**Peng-Fei Tu**, State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, China.

**Sermsawat Tunlaya-Anukit**, Forest Biotechnology Group, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA.

**Jack P. Wang**, Forest Biotechnology Group, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA.

**Justin M. Watkins**, Department of Biology and Center for Molecular Signaling, Wake Forest University, Winston-Salem, NC, USA.

**Cranos M. Williams**, Electrical and Computer Engineering, North Carolina State University, Raleigh, NC, USA.

**Peng Xu**, National Key Laboratory of Plant Molecular Genetics and Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China.

**Chenmin Yang**, Forest Biotechnology Group, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA.

**Ting-Feng Yeh**, Forestry and Resource Conservation, National Taiwan University, Taipei, Taiwan.

**Kumi Yoshida**, Natural Product and Bioorganic Chemistry, Graduate School of Information Science, Nagoya University, Chikusa, Nagoya, Japan.

**Ke-Wu Zeng**, State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, China.

# Preface

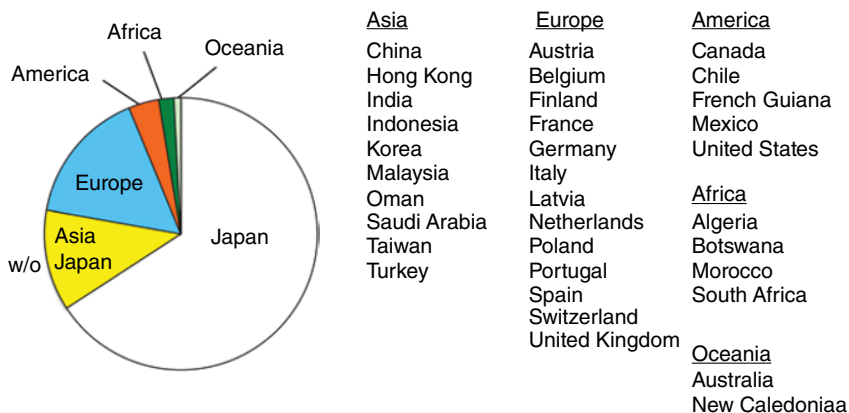
Polyphenols are secondary metabolites that are variously distributed in the plant kingdom and characterized by a wide diversity of chemical structures. On behalf of the international scholarly society “*Groupe Polyphénols*,” which organizes the biennial conference, “*International Conference on Polyphenols*” (ICP), we define the term “polyphenol” as related to plant products exclusively derived from the shikimate/phenylpropanoid and/or the polyketide pathway, featuring more than one phenolic unit and deprived of nitrogen-based functions (<http://www.groupepolyphenols.com/the-society/why-bother-with-polyphenols/>). The number of known plant polyphenols is quite large, from structurally simple compounds such as the stilbenoid resveratrol or the flavonoid quercetin to complex macromolecules such as the proanthocyanidin oligomers or the lignin polymer. It is thus not surprising that their functions in plant and physicochemical properties are also quite varied. In the early 20th century, investigations on polyphenols were mainly dedicated to the determination of their structures and their roles in traditional medicines, as well as in vegetable tanning. Nowadays, research on plant polyphenols concerns a much wider area of science with novel and multidisciplinary efforts made toward the understanding of their properties and exploitation thereof in *inter alia* the development of new materials, the innovation in agriculture and food products, including the development of new crops and flowers, the higher fixation of carbon dioxide, and the formulation of functional foods with human health benefits, as well as the discovery of new pharmaceutical medicines.

This book series “*Recent Advances in Polyphenol Research*” began its publication in 2008 on the occasion of the 24th ICP in Salamanca, Spain. The content of this first volume was already mostly based on review articles written by plenary lecturers of the previous ICP, which had taken place in Winnipeg, Canada. Since then, this flagship publication of the *Groupe Polyphénols* has been released without any discontinuity every 2 years to provide the reader with authoritative updates on various topics of polyphenol research written by ICP plenary lecturers and by invited expert contributors.

This book, the fifth volume of the series, is concerned with the topics that were covered during the 27th ICP, which was organized jointly with the 8th edition of the *Tannin Conference* in September 2014 in Nagoya, Japan. In more than 40 years of the history of the *Groupe Polyphénols*, it was the first time that the *International Conference on*

*Polyphenols* took place in Asia. Six different main topics of the polyphenol science were selected for the scientific program of this memorable ICP2014 edition:

- 1) **Chemistry, Physicochemistry, and Materials Science**, covering structures, reactivity, organic synthesis, molecular modeling, fundamental aspects, chemical analysis, spectroscopy, molecular associations, and interactions of polyphenols.
- 2) **Biosynthesis, Genetics, and Metabolic Engineering**, covering molecular biology, genetics, enzymology, gene expression and regulation, trafficking, biotechnology, horticultural science, and molecular breeding related to polyphenols.
- 3) **Plants and Ecosystems, Lignocellulose Biomass**, covering plant growth and development, biotic and abiotic stress, resistance, ecophysiology, sustainable development, valorization, plant environmental system, forest chemistry, and lignin and lignan.
- 4) **Food, Nutrition, and Health**, covering food ingredients, nutrient components, functional food, mode of action, bioavailability and metabolism, food processing, influence on food and beverages properties, cosmetics, and antioxidant activity of polyphenols.
- 5) **Natural Medicine and Kambo**, a special session for this first conference held in Asia covering oriental traditional medicine, herbal medicine, Chinese herbal medicine, folklore, mode of action, metabolism, natural products chemistry, and drug discovery.
- 6) **Tannins and Their Functions**, another special session on the occasion of this joint meeting with the *Tannin Conference* covering research topics related to condensed tannins, hydrolyzable tannins, tea, wine, persimmon, seed-coat color, mode of action, and enzymatic reactions.



More than 500 scientists from 35 countries attended the conference, with 321 paper contributions that comprised 61 oral communications and 260 poster presentations. The fifth volume of “*Recent Advances in Polyphenol Research*” contains chapters from 14 guest speakers of the conference. The support and assistance of the *Groupe Polyphénols*, the *Tannin Conference* Group, several Japanese academic associations and foundations, notably the Nagoya University, the City of Nagoya and the Nagoya Convention & Visitors Bureau, and numerous private sponsors are gratefully acknowledged, as the great success of these joint editions of the *International Conference on Polyphenols* and the *Tannin*

*Conference* would not have been possible without their contributions. As a final note, we would also like to deeply thank all of the plenary, communication, and poster presenters for the quality of their contributions, from basic science to more applied fields, and all of the attendees, with a special thank to the numerous Asian researchers for their first participation in the ICP and for expressing their eagerness to attend the next ICP meetings.

Kumi Yoshida  
Véronique Cheynier  
Stéphane Quideau





## Chapter 1

# The Physical Chemistry of Polyphenols: Insights into the Activity of Polyphenols in Humans at the Molecular Level

*Olivier Dangles, Claire Dufour, Claire Tonnelé and Patrick Trouillas*

**Abstract:** This chapter reviews the following versatile physicochemical properties of polyphenols in relation with their potential activity in humans:

- 1) Interactions with proteins and lipid–water interfaces. These interactions must be qualified with respect to the current knowledge on polyphenol bioavailability and metabolism. They are expected to mediate most of the cell signaling activity of polyphenols.
- 2) A general reducing capacity that may be expressed in the gastrointestinal tract submitted to postprandial oxidative stress and also in cells, for example, by direct scavenging of reactive oxygen species, especially if preliminary deconjugation of metabolites takes place
- 3) The complex relationships with transition metal ions involving binding and/or electron transfer in close connection with the antioxidant versus pro-oxidant activity of polyphenols

**Keywords:** polyphenol, flavonoid, Health effectsbiological activity, mechanism, antioxidant, protein, membrane, metal ion, gastrointestinal tract, DFT methods.

## 1.1 Introduction

The activity, functions, and structural diversity of polyphenols in plants, food, and humans reflect the remarkable diversity of their physicochemical properties: UV–visible absorption, electron donation, affinity for metal ions, propensity to develop molecular interactions (van der Waals, hydrogen bonding) with proteins and lipid–water interfaces, and

nucleophilicity. This chapter aims to exemplify how polyphenols act to promote health in humans at the molecular level. It rests on two common assumptions based on epidemiological evidence and food analysis (Manach *et al.*, 2005; Crozier *et al.*, 2010; Del Rio *et al.*, 2013):

- The consumption of fruit and vegetables helps prevent chronic diseases and, in particular, favors cardiovascular health.
- Phenolic compounds, from the simple hydroxybenzoic and hydroxycinnamic acids to the complex condensed and hydrolyzable tannins, constitute the most abundant class of plant secondary metabolites in our diet and take part in this protection.

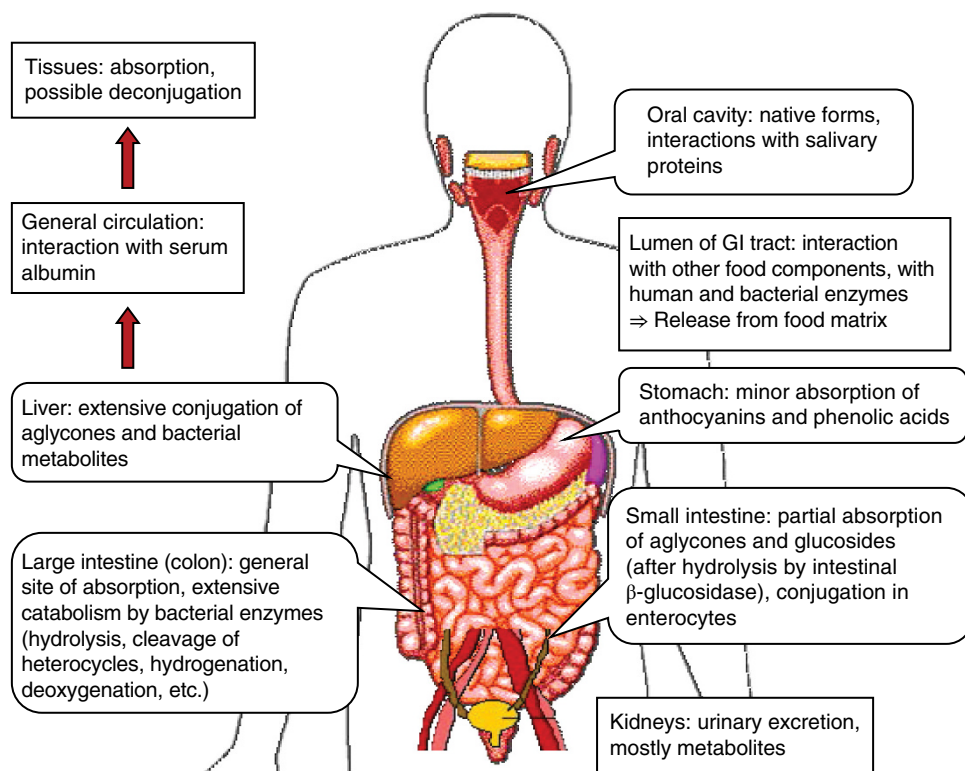
By contributing to the sensorial properties of food, for example, color and astringency, native polyphenols and their derivatives obtained after technological and domestic processing can directly influence the consumer's choice. Moreover, polyphenols undergo only minimal enzymatic conversion in the oral cavity and in the gastric compartment although their release from the food matrix (bioaccessibility) is an important issue. Thus, intact food polyphenols may directly promote health benefits in the upper digestive tract, in particular by fighting postprandial oxidative stress resulting from an unbalanced diet (Sies *et al.*, 2005; Kanner *et al.*, 2012). Beyond the gastric compartment, polyphenol bioavailability<sup>1</sup> (Fig. 1.1) must be considered as a priority to tackle any biological effects (Manach *et al.*, 2005; Crozier *et al.*, 2010; Del Rio *et al.*, 2013). Indeed, even for polyphenols that can be partially absorbed in the upper intestinal tract (aglycones, glucosides), most of the dietary intake reaches the colon where extensive catabolism by the microbiota takes place: hydrolysis of glycosidic and ester bonds, release of flavanol monomers from proanthocyanidins, hydrogenation of the C=C double bond of hydroxycinnamic acids, deoxygenation of aromatic rings, cleavage of the central heterocycle of flavonoids, and so on. Conjugation of polyphenols and their bacterial metabolites in intestinal and liver cells eventually results in a complex mixture of circulating polyphenol *O*- $\beta$ -D-glucuronides and *O*-sulfo forms (less rigorously called sulfates). When present, catechol groups are also partially methylated.

The concentration of circulating polyphenols is usually evaluated after treatment by a mixture of glucuronidases and sulfatases that release the aglycones and their *O*-methyl ethers. This concentration is usually quite low (barely higher than 0.1  $\mu$ M) and much lower than that of typical plasma antioxidants such as ascorbate (>30  $\mu$ M). At first sight, this does not argue in favor of nonspecific biological effects, such as the antioxidant activity by radical scavenging or chelation of transition metal ions to form inert complexes. This seems all the more true that the catechol group, displayed by many common dietary polyphenols and which is a critical determinant of the electron-donating and metal-binding capacities, is generally either absent in the circulating metabolites (bacterial deoxygenation) or at least

---

<sup>1</sup>Bioavailability: the fraction of ingested polyphenol (native form+metabolites) that enters the general blood circulation and is thus potentially available for health effects.

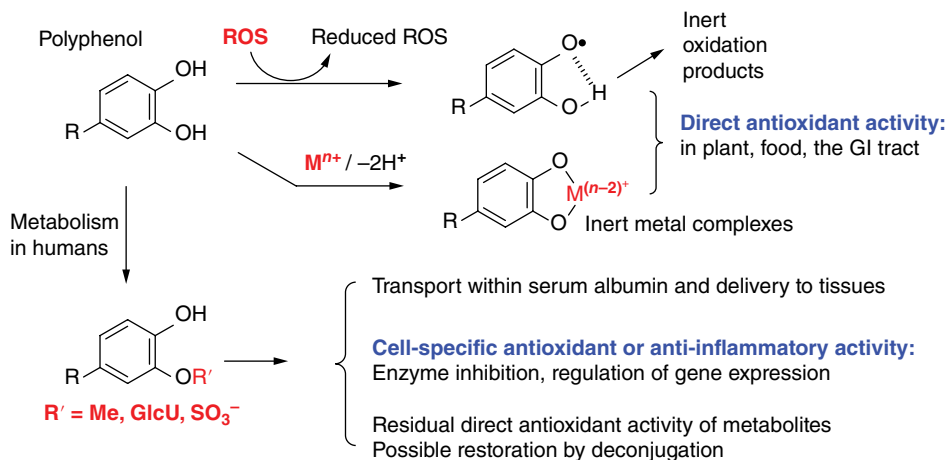
Bioaccessibility: the first step of bioavailability, the fraction of ingested polyphenol (native form+metabolites) that is released from the food matrix and is thus potentially available for intestinal absorption.



**Fig. 1.1** A simplified view of polyphenol bioavailability. (See insert for color representation of the figure)

partially conjugated. However, the claim that *in vivo* polyphenol concentrations are low should be nuanced for the following reasons:

- 1) The complete assessment of polyphenol bioavailability must include the bacterial catabolites and their conjugates, some being much more abundant in the circulation than the parent phenol. A spectacular example can be found in the case of anthocyanins. Indeed, after consumption of blood orange juice, the total amount of native cyanidin 3-*O*- $\beta$ -D-glucoside (C3G) in plasma is 0.02% of the ingested dose versus 44% for (unconjugated) protocatechuic acid (PCA), its main catabolite (Vitaglione *et al.*, 2007). When the fecal content is also taken into account, PCA eventually represents ca. 73% of the metabolic fate of ingested C3G. Its absence in urine (unlike C3G) also suggests that it takes part in the antioxidant protection and is thus oxidized in tissues.
- 2) The circulating concentration and its time dependence say nothing concerning either the possibility of polyphenol metabolites accumulation at a much higher local concentration at specific sites of inflammation and oxidative stress or their deconjugation into more active forms.



**Fig. 1.2** Health effects expressed by polyphenols.

For instance, when quercetin is continuously perfused through the vascular wall of arteries, it rapidly undergoes oxidative degradation into PCA, whereas the fraction retained in the wall is much more stable and partially methylated (Menendez *et al.*, 2011). By contrast, quercetin 3-*O*- $\beta$ -D-glucuronide (Q3G), the main circulating metabolite, is not oxidized upon perfusion but slowly converted into quercetin. The kinetics of quercetin release parallels the inhibition in the contractile response of the artery. Thus, the biological effect can be ascribed to quercetin released from its glucuronide, which basically appears as a stable storage form. A schematic view for the bioactivity of polyphenols is summed up in Fig. 1.2.

## 1.2 Molecular complexation of polyphenols

The phenolic nucleus can be regarded as a benchmark chemical group for molecular interactions as it combines an acidic OH group liable to develop hydrogen bonds (both as a donor and as an acceptor) and an aromatic nucleus for dispersion interactions (the stabilizing component of van der Waals interactions).

### 1.2.1 Polyphenol–protein binding

Polyphenol–protein binding of nutritional relevance can be classified as follows:

- Binding processes within the gastrointestinal (GI) tract, that is, with food proteins, mucins, and the digestive enzymes, with an impact on the bioaccessibility of polyphenols and the digestibility of macronutrients
  - Interactions with plasma proteins, with an impact on transport and the rate of clearance from the general circulation
  - Interactions with specific cell proteins (enzymes, receptors, transcription factors, etc.) that would mediate the nonredox health effects of polyphenols

As the last two situations lie downstream the intestinal absorption and passage through the liver, they concern the circulating polyphenol metabolites. However, some exceptions may be found. For instance, epigallocatechin 3-*O*-gallate (EGCG), the major green tea flavanol, is a rare example of a polyphenol entering the blood circulation mostly in its initial (nonconjugated) form (Manach *et al.*, 2005). No less remarkable, EGCG is also one of the rare polyphenols for which a specific receptor has been identified, namely the 67-kDa laminin receptor (67LR) that is expressed on the surface of various tumor cells (Umeda *et al.*, 2008). EGCG-67LR binding leads to myosin phosphatase activation and actin cytoskeleton rearrangement, thus inhibiting cell growth. It provides a strong basis for interpreting the *in vivo* anticancer activity of EGCG and its anti-inflammatory activity in endothelial cells (Byun *et al.*, 2014).

It is not the authors' purpose to provide the reader with an exhaustive updated report on polyphenol–protein binding processes (see Dangles and Dufour (2008) for a specific review on this topic). Only a few recent important examples will be discussed with an emphasis on works dealing with polyphenol metabolites.

#### 1.2.1.1 Interactions in the digestive tract

In the postprandial phase, black tea drinking leads to vasorelaxation as evidenced by flow-mediated dilation experiments in humans and a strong increase in the activity of endothelial nitric oxide synthase (eNOS) (Lorenz *et al.*, 2007). However, these effects are completely abolished when 10% milk is added to black tea. Experiments with isolated fractions of milk proteins show that caseins are actually responsible for this inhibition. It can thus be proposed that caseins bind and probably precipitate black tea polyphenols in the GI tract, thereby preventing their intestinal absorption. This is a spectacular example of how food proteins may sequester oligomeric polyphenols and cancel their bioaccessibility and downstream biological effects.

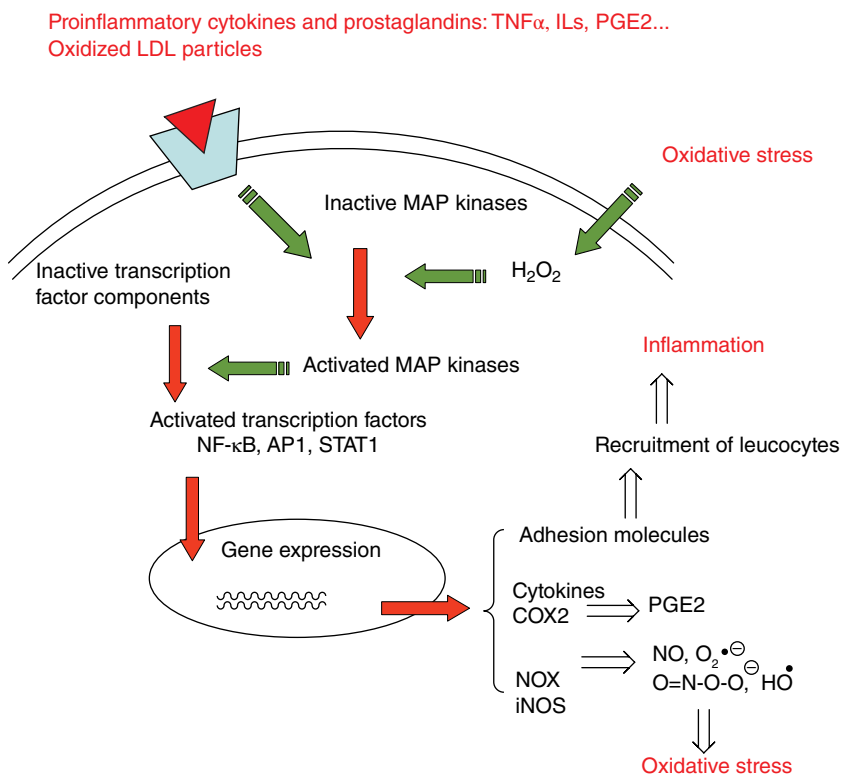
The binding between dietary polyphenols and the digestive enzymes is best evidenced with large polyphenols such as oligomeric proanthocyanidins (OPAs). For instance, OPAs inhibit pancreatic elastase, a serine protease, proportionally to their mean degree of polymerization (Bras *et al.*, 2010). A  $K_i$  value of ca. 0.5 mM was estimated for a catechin tetramer. However, a mixture of *n*-mers ( $n=2-6$ ) rich in 3-*O*-galloyl flavanol units binds much more tightly ( $K_i \approx 14 \mu\text{M}$ ). Similar data were obtained with trypsin (Goncalves *et al.*, 2007). By slowing down the digestion, such interactions could prolong the sensation of satiety and help fight weight gain and obesity. By contrast, simple phenols were shown to mildly enhance pepsin activity at pH 2 in the following order: resveratrol  $\geq$  quercetin  $>$  EGCG  $>$  catechin (Tagliazucchi *et al.*, 2005). Tannins are known to inhibit pancreatic lipase (McDougall *et al.*, 2009), thereby possibly contributing to lowering fat intake. Polyphenol-rich berry extracts also inhibit pancreatic  $\alpha$ -amylase (thus decreasing starch digestibility) and intestinal  $\alpha$ -glucosidase, with tannins and anthocyanins being, respectively, the main contributors to the observed inhibition (McDougall *et al.*, 2005). These mild inhibitory effects could help regulate the circulating D-glucose concentration.

### 1.2.1.2 Interactions beyond intestinal absorption

In the circulating blood, polyphenol metabolites likely travel in association with serum albumin, the most abundant plasma protein, which displays several binding sites for the transport of drugs, free fatty acids, and other nutrients. Our recent work (Khan *et al.*, 2011) has shown that flavanone glucuronides (conjugation at the A- or B-ring) are moderate serum albumin ligands ( $K_b = 3\text{--}6 \times 10^4 \text{ M}^{-1}$ ) that bind site 2 (subdomain IIIA), in contrast to the more planar flavones and flavonols, which bind site 1 (subdomain IIA).

Once delivered to tissues, polyphenol metabolites are expected to bind specific cell proteins to express their biological effects, in particular their well-documented anti-inflammatory activity (Pan *et al.*, 2010; Spencer *et al.*, 2012; Wu & Schauss, 2012). Inflammation is an adaptive response to deleterious stimuli, activating the immune system. What is at stake with dietary polyphenols is the inhibition of chronic low-grade inflammation (in contrast to acute inflammation following microbial infection) associated with the development of degenerative diseases, such as type 2 diabetes and cardiovascular disease. Indeed, this pathological state is deeply influenced by lifestyle and environmental factors, especially dietary habits.

At the cell level, inflammation involves complex signaling pathways and cascades (Fig. 1.3). In particular, mitogen-activated protein kinases (MAPKs, e.g., ERK, JNK, and



**Fig. 1.3** Pathways of inflammation and oxidative stress in cells. Kinases, proinflammatory transcription factors, and pro-oxidant enzymes are possible target proteins for polyphenols and their metabolites.