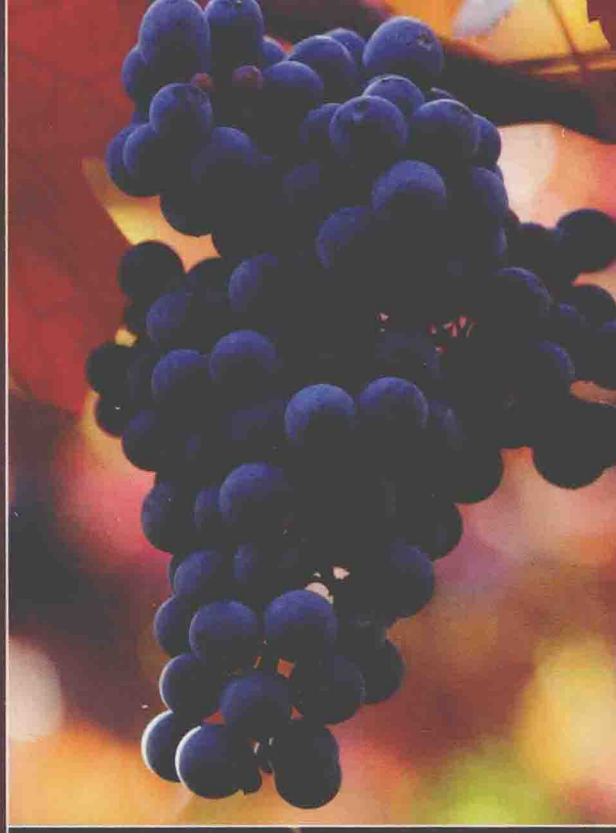
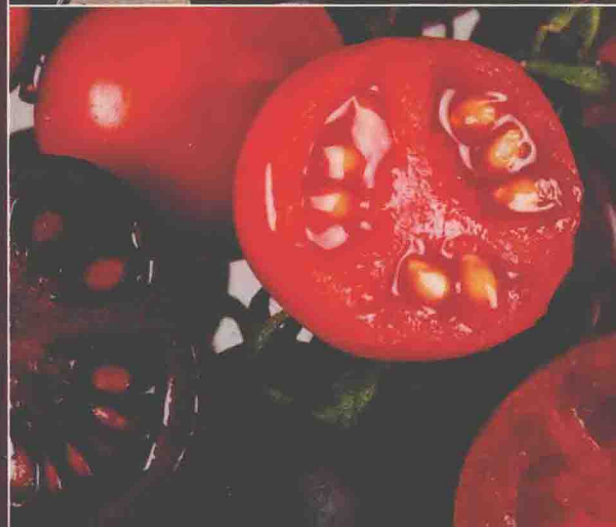
Recent Advances in Polyphenol Research

VOLUME 2

Edited by
Celestino Santos-Buelga,
Maria Teresa Escribano-Bailon
and Vincenzo Lattanzio





WILEY-BLACKWELL

Recent Advances in Polyphenol Research

Volume 2

Edited by

Celestino Santos-Buelga
Professor, Food Chemistry
Department of Analytical Chemistry, Nutrition and Food Science
Faculty of Pharmacy
University of Salamanca, Spain

Maria Teresa Escribano-Bailón
Lecturer, Food Technology
Area of Food Technology
Technical School of 'Zamora'
University of Salamanca, Spain

Vincenzo Lattanzio
Professor, Plant Biochemistry and Physiology
Department of Agro-Environmental Sciences, Chemistry and Plant Protection
Faculty of Agricultural Sciences
University of Foggia, Italy

WILEY-BLACKWELL

A John Wiley & Sons, Ltd., Publication

This edition first published 2010

Blackwell Publishing was acquired by John Wiley & Sons in February 2007. Blackwell's publishing programme has been merged with Wiley's global Scientific, Technical, and Medical business to form Wiley-Blackwell.

Registered office
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

Editorial offices
9600 Garsington Road, Oxford, OX4 2DQ, United Kingdom
2121 State Avenue, Ames, Iowa 50014-8300, 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.

Library of Congress Cataloging-in-Publication Data is available

ISBN 9781405193993

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

Set in 10/13 pt Times New Roman PS MT by MPS Limited, A Macmillan Company
Printed in Singapore by Fabulous Printers Pte Ltd

1 2010

Contributors

Cristina Andres-Lacueva, *Nutrition and Food Science Department, XaRTA, INSA, Pharmacy Faculty, University of Barcelona, Av. Joan XXIII, s/n. 08028, Barcelona, Spain.*

Yves Baissac, *University of Montpellier 2, UMR-188 DIA-PC, Rhizogenesis team, Laboratory of Plant Biochemistry and Physiology, CC 024, Bât.15, Place Eugène Bataillon, F-34095 Montpellier cedex 05, France.*

Luc P.R. Bidel, *INRA, UMR-188 DIA-PC, Rhizogenesis group, University Montpellier 2, CC 024, Place E. Bataillon, F-34095 Montpellier cedex 05, France.*

Arnaud G. Bovy, *Plant Research International, P.O. Box 16, 6700AA Wageningen, The Netherlands and Centre for BioSystems Genomics (CBSG), P.O. Box 98, 6700PB, Wageningen, The Netherlands.*

Raymond Brouillard, *Laboratoire de Chimie des Polyphénols, Faculté de Chimie (CNRS-UMR 7177), Université Strasbourg 1, 4, rue Blaise Pascal, 67070 Strasbourg, France.*

Stefan Chassaing, *LSPCMIB-Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse cedex 09, France.*

Marc Coumans, *University of Montpellier 2, UMR-188 DIA-PC, Rhizogenesis team, Laboratory of Plant Biochemistry and Physiology, CC 024, Bât.15, Place Eugène Bataillon, F-34095 Montpellier cedex 05, France.*

Patrick Doumas, *INRA, UMR-188 DIA-PC, Rhizogenesis group, Institut de Recherche pour le Développement (IRD), 911, Avenue Agropolis, F-34394 Montpellier cedex 05, France.*

Paulo Figueiredo, *Universidade Atlântica CEIDSS, Antiga Fábrica da Pólvora de Barcarena, Oeiras, 2730-036 Barcarena, Portugal.*

Victor A.P. de Freitas, *Chemistry Investigation Centre, Department of Chemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal.*

Yves Glories, *Institut des Sciences de la Vigne et du Vin, Bordeaux-Aquitaine (UMR-INRA 1219), Université de Bordeaux, 210 Chemin de Leysotte CS 50008, 33882 Villenave d'Ornon, France.*

Victoria Gómez-Roldán, *Plant Research International, P.O. Box 16, 6700AA Wageningen, The Netherlands and Netherlands Consortium for Systems Biology (NCSB), Kruislaan 318, 1098SM Amsterdam, The Netherlands.*

Kevin S. Gould, *School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand.*

Robert D. Hall, *Plant Research International, P.O. Box 16, 6700AA Wageningen, The Netherlands, Centre for BioSystems Genomics (CBSG), P.O. Box 98, 6700PB, Wageningen, The Netherlands, and Netherlands Consortium for Systems Biology (NCSB), Kruislaan 318, 1098SM Amsterdam, The Netherlands.*

Géraldine Isorez, *Laboratoire de Chimie des Polyphénols, Faculté de Chimie (CNRS-UMR 7177), Université Strasbourg 1, 4, rue Blaise Pascal, 67070 Strasbourg, France.*

Christian Jay-Allemand, *University of Montpellier 2, UMR-188 DIA-PC, Rhizogenesis team, Laboratory of Plant Biochemistry and Physiology, CC 024, Bât.15, Place Eugène Bataillon. F-34095 Montpellier cedex 05, France.*

James A. Joseph, *USDA-ARS, Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111, USA.*

Michael Jourdes, *Institut des Sciences de la Vigne et du Vin, Bordeaux-Aquitaine (UMR-INRA 1219), Université de Bordeaux, 210 Chemin de Leysotte CS 50008, 33882 Villenave d'Ornon, France.*

Yoshichika Kawai, *Department of Food Science, Graduate School of Nutrition and Bioscience, the University of Tokushima, Kuramoto-cho 3 Tokushima, Japan.*

Lea Kontio, *Department of Chemistry, Organic Chemistry Laboratory, University of Helsinki, 00014 Helsinki, Finland.*

Marie Kueny-Stotz, *Laboratoire de Chimie des Polyphénols, Faculté de Chimie (CNRS-UMR 7177), Université Strasbourg 1, 4, rue Blaise Pascal, 67070 Strasbourg, France.*

João Laranjinha, *Center for Neurosciences and Cell Biology and Faculty of Pharmacy, University of Coimbra, Health Sciences Campus, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.*

Dorothée Lefeuvre, *Université de Bordeaux, Institut des Sciences Moléculaires (CNRS-UMR 5255), Institut Européen de Chimie et Biologie, 2 rue Robert Escarpit, 33607 Pessac cedex, France.*

Nuno Mateus, *Chemistry Investigation Centre, Department of Chemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal.*

Kaeko Murota, *Department of Food Science, Graduate School of Nutrition and Bioscience, The University of Tokushima, Kuramoto-cho 3 Tokushima, Japan.*

Eeva A. Mustonen, *Department of Production Animal Medicine, University of Helsinki, Paroninkuja 20, 04920 Saarentaus, Finland.*

Yoko Nakamura, *Department of Chemistry, Faculty of Science, Tohoku University, Aramaki-aza Aoba, Aoba-ku, Sendai 980-8578, Japan.*

Patrick Pardon, *Université de Bordeaux, Institut des Sciences Moléculaires (CNRS-UMR 5255), Institut Européen de Chimie et Biologie, 2 rue Robert Escarpit, 33607 Pessac cedex, France.*

Stéphane Quideau, *Université de Bordeaux, Institut des Sciences Moléculaires (CNRS-UMR 5255), Institut Européen de Chimie et Biologie, 2 rue Robert Escarpit, 33607 Pessac cedex, France.*

Ilkka Saastamoinen, *Department of Production Animal Medicine, University of Helsinki, Paroninkuja 20, 04920 Saarentaus, Finland.*

Hannu Saloniemi, *Department of Production Animal Medicine, University of Helsinki, Paroninkuja 20, 04920 Saarentaus, Finland.*

Cédric Saucier, *Institut des Sciences de la Vigne et du Vin, Bordeaux-Aquitaine (UMR-INRA 1219), Université de Bordeaux, 210 Chemin de Leysotte CS 50008, 33882 Villenave d'Ornon, France.*

Barbara Shukitt-Hale, *USDA-ARS, Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111, USA.*

Juhani Taponen, *Department of Production Animal Medicine, University of Helsinki, Paroninkuja 20, 04920 Saarentaus, Finland.*

Pierre-Louis Teissedre, *Institut des Sciences de la Vigne et du Vin, Bordeaux-Aquitaine (UMR-INRA 1219), Université de Bordeaux, 210 Chemin de Leysotte CS 50008, 33882 Villenave d'Ornon, France.*

Junji Terao, *Department of Food Science, Graduate School of Nutrition and Bioscience, The University of Tokushima, Kuramoto-cho 3 Tokushima, Japan.*

Minoru Ueda, *Department of Chemistry, Faculty of Science, Tohoku University, Aramaki-aza Aoba, Aoba-ku, Sendai 980-8578, Japan.*

Aila Vanhatalo, *Department of Animal Science, University of Helsinki, P.O. Box 28, 00014 Helsinki, Finland.*

Nigel C. Veitch, *Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK.*

Kristiina Wähälä, *Department of Chemistry, Organic Chemistry Laboratory, University of Helsinki, P.O. Box 55, 00014 Helsinki, Finland.*

Lauren M. Willis, *USDA-ARS, Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111, USA.*

Raul Zamora-Ros, *Nutrition and Food Science Department, XaRTA, INSA, Pharmacy Faculty, University of Barcelona, Av. Joan XXIII, s/n. 08028, Barcelona, Spain.*

Preface

Plant phenolics are secondary metabolites that constitute one of the most common and widespread group of substances in plants and that have been considered for a long time waste products of primary metabolism. Nowadays, plant phenols and polyphenols are considered to have a large and diverse array of beneficial effects on both plants and humans. The ability to synthesize secondary compounds has been selected throughout the course of evolution in different plant lineages when such compounds addressed specific needs. Secondary metabolites apparently act as defence (against herbivores, microbes, viruses, or competing plants) and signal compounds (to attract pollinating or seed-dispersing animals), as well as protect the plant from ultraviolet radiation and oxidants. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution. In addition, biomedical research has revealed that dietary phenolics, because of their antioxidant and free radical scavenging properties, play important roles in the prevention of many of the major contemporary chronic diseases.

The diversity of structure and activity of phenolic compounds resulted in the multiplicity of research areas such as chemistry, biotechnology, ecology, physiology, nutrition, medicine, and cosmetics. The International Conference on Polyphenols, organized under the auspices of *Groupe Polyphénols*, is a unique opportunity for scientists in these and other fields to get together every other year and exchange their ideas and new findings.

The last edition of the conference (the 24th edition) was hosted by the University of Salamanca, Spain, from July 8 to 11, 2008, and covered five topics:

1. *Chemistry*: Structure, reactivity, physicochemical properties, analytical methods, synthesis ....
2. *Biosynthesis and metabolic engineering*: Molecular biology, omics, enzymology, gene expression and regulation, biotechnology ....
3. *Roles in Plant Ecophysiology and Environment*: Plant growth and development, biotic and abiotic stress, resistance, sustainable development, by-products valorization ....
4. *Food and Beverages*: Composition, organoleptic properties, impact of processing and storage, functional foods, nutraceuticals ....
5. *Health and Disease*: Medicinal properties, mode of action, bioavailability and metabolism, cosmetics ....

Some 450 participants from 41 countries attended Salamanca's Conference, where over 370 presentations were made, including 330 posters, 31 selected oral communications,

and 12 invited lectures made by acknowledged experts. The present second volume in the series includes chapters from the guest speakers and some invited contributors.

The 24th International Conference on Polyphenols would not have been possible without the generous support of public and private donors such as the Spanish *Ministerio de Ciencia e Innovación*, *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria* (INIA), *Junta de Castilla y León*, and *Caja Duero*. Furthermore, we are also indebted to the Natraceutical Group, Indena, "Viñas del Jaro" wine cellars, and Phytolab that also sponsored the conference. Our sincere thanks to all of them.

Celestino Santos-Buelga,
Maria Teresa Escribano-Bailon,
Vincenzo Lattanzio

Contents

Contributors *xiv*
Preface *xviii*

1 The Visible Flavonoids or Anthocyanins: From Research to Applications 1
Raymond Brouillard, Stefan Chassaing, Géraldine Isorez, Marie Kueny-Stotz, and Paulo Figueiredo

1.1 Introduction 1
1.2 Copigmentation of anthocyanins 5
1.3 Formation of inclusion complexes 6
1.4 Ion-pair formation 7
1.5 Metalloanthocyanins 7
1.6 *Z*-Chalcones: unexpected open cavities for the ferric cation 11
1.7 Anthocyanin biological activity 14
1.8 Some thoughts on applications 15
1.9 References 17

2 Flavonoid Chemistry of the Leguminosae 23
Nigel C. Veitch

2.1 Introduction 23
2.1.1 Classification and nomenclature of the Leguminosae: a brief synopsis 24
2.2 Flavonoid structures in the Leguminosae: trends and distribution 26
2.2.1 Occurrence of 5-deoxyflavonoids in the Leguminosae 28
2.2.2 Isoflavonoids in subfamily Papilionoideae 30
2.2.2.1 Recent advances in biosynthetic studies 32
2.2.2.2 Isoflavonoid glycosides 35
2.2.2.3 Isoflavone glucosyltransferases 35
2.2.2.4 Acylated isoflavone glycosides 36
2.2.3 Leguminosae anthocyanins: malonyltransferases of *Clitoria ternatea* 38
2.3 Advances in analytical methodology applied to Leguminosae flavonoids 38
2.3.1 Hyphenated MS techniques 40

2.3.2 Hyphenated NMR techniques and miniaturization 41
2.3.3 Chiroptical methods 43
2.4 Leguminosae flavonoids and chemosystematics 44
2.4.1 The disputed position of the Swartzieae: subfamily Caesalpinioideae or Papilionoideae? 44
2.4.2 Generic delimitation in the aldinoid clade of swartzioid legumes: *Cordyla* and *Dupuya* 47
2.4.3 Species-level studies of the isoflavonoid chemistry of *Cicer* 50
2.5 Concluding remarks 52
2.6 Acknowledgments 52
2.7 References 52

3 Updating Wine Pigments 59
Victor A.P. de Freitas and Nuno Mateus

3.1 General overview 59
3.2 Factors that affect wine color intensity and stability 60
3.3 Chemical transformations of flavonoids 63
3.3.1 Condensation between anthocyanins and flavanols mediated by aldehydes 65
3.3.2 Reaction between flavanols and aldehydes 67
3.3.3 Direct condensation between flavanols and anthocyanins 68
3.3.4 Pyranoanthocyanins 69
3.3.4.1 Reaction between anthocyanins and vinyl compounds 70
3.3.4.2 Yeast metabolites involved in anthocyanin transformations 72
3.3.5 Vinylpyranoanthocyanins (portisins) 74
3.4 Final remarks 75
3.5 Acknowledgments 76
3.6 References 76

4 Ellagitannins – An Underestimated Class of Plant Polyphenols: Chemical Reactivity of *C*-Glucosidic Ellagitannins in Relation to Wine Chemistry and Biological Activity 81
Stéphane Quideau, Michael Jourdes, Dorothée Lefeuvre, Patrick Pardon, Cédric Saucier, Pierre-Louis Teissedre, and Yves Glories

4.1 Ellagitannins: an underestimated class of bioactive plant polyphenols 81
4.2 *C*-Glucosidic ellagitannins: a special subclass of ellagitannins 95
4.2.1 Major *C*-glucosidic ellagitannins in oak and chestnut heartwoods 100
4.2.2 Complex *C*-glucosidic ellagitannins 102

4.2.3 Biosynthesis of *C*-glucosidic ellagitannins 107
4.2.4 Chemical reactivity of vescalagin and castalagin 110
4.2.5 Diastereofacial differentiation of the vescalagin-derived benzylic cation 113
4.3 Implications of *C*-glucosidic ellagitannins in wine chemistry 114
4.3.1 Hemisynthesis of acutissimins and their occurrence in wine 115
4.3.2 Condensation reaction between vescalagin and glutathione 118
4.3.3 Hemisynthesis of anthocyano-ellagitannins: possible influence on wine color 119
4.3.4 Oxidative conversion of acutissimin A into mongolicain A 120
4.4 Biological activity of *C*-glucosidic ellagitannins 122
4.4.1 Antiviral activity of *C*-glucosidic ellagitannins 123
4.4.2 Antitumor activity of *C*-glucosidic ellagitannins 124
4.5 Conclusion 125
4.6 Acknowledgments 126
4.7 References 126

5 Strategies to Optimize the Flavonoid Content of Tomato Fruit 138
Arnaud G. Bovy, Victoria Gómez-Roldán, and Robert D. Hall

5.1 Introduction 138
5.2 The metabolic route to flavonoids in tomato fruit 140
5.3 The natural biodiversity of flavonoids in tomato 141
5.3.1 Flavonoid biodiversity I: commercially available genotypes 142
5.3.2 Flavonoid biodiversity II: wild tomato species 142
5.3.3 Flavonoid biodiversity III: information from specific tomato mutants 143
5.4 Metabolic engineering of the flavonoid pathway 145
5.4.1 Exploitation of the transgenic approach using upregulation of structural genes 145
5.4.2 Using RNAi to block targeted steps in the flavonoid pathway 146
5.4.3 Production of novel tomato flavonoids by introducing new branches of the flavonoid pathway: flavonoid-related stilbenes 147
5.4.4 Production of novel tomato flavonoids by introducing new branches of the flavonoid pathway: deoxychalcones 148
5.4.5 Production of novel tomato flavonoids by introducing new branches of the flavonoid pathway: flavones, isoflavones, and aurones 149
5.4.6 Modifying the flavonoid pathway using regulatory genes 150
5.5 Metabolomics-assisted breeding 154
5.6 Conclusions and future prospects 156
5.7 Acknowledgments 156
5.8 References 156

6 Biological Activity of Phenolics in Plant Cells 163
Luc P.R. Bidel, Marc Coumans, Yves Baissac, Patrick Doumas, and Christian Jay-Allemand

6.1 Introduction 163
6.2 Synthesis and transports 164
6.2.1 Metabolic channeling at the endoplasmic reticulum (ER) level 164
6.2.2 Endomembrane carriers 165
6.2.3 Vesicle trafficking 166
6.2.4 Long-distance transport 166
6.3 Phenolics interact with plasmalemma components 167
6.3.1 Biophysical interactions with phospholipid bilayers 167
6.3.2 Interactions with plasma membrane-associated proteins 169
6.3.3 Flavonoids prevent and alleviate oxidative burst 172
6.3.4 Phenolics modulate plasma membrane carriers 172
6.4 Phenolics in apoplast 175
6.4.1 Phenolics as a major player in mechanical tissue rigidification 175
6.4.2 Phenolics as major components of apoplastic chemical protection 175
6.4.3 Phenolics as apoplastic allelochemical signals 177
6.5 Phenolics in hyaloplasm 177
6.5.1 Phenolics interact with cytoskeleton 178
6.5.2 Phenolics inhibit carbohydrate catabolism 178
6.5.3 Many flavonoids prevent and alleviate oxidative and nitrosative stresses 178
6.5.4 Salicylic acid promotes oxidative stress signaling pathway 179
6.6 Phenolics in vacuoles 180
6.6.1 Sunscreen role for vacuolar phenolics 180
6.6.2 Are vacuolar phenolics effective buffers? 180
6.6.3 Are vacuolar phenolics effective chelators? 182
6.7 Phenolics in mitochondria and chloroplasts 183
6.7.1 Inhibitory effects 183
6.7.2 Protecting effects 183
6.7.3 Putative phenolic photoreceptors 183
6.8 Phenolics have many emergent roles within the nucleus 184
6.8.1 Presence of phenolics within the nucleus 184
6.8.2 Flavonoids prevent DNA damages 184
6.8.3 Prooxidative actions of phenolics on DNA 186
6.8.4 Flavonoids affect histone acetylation and phosphorylation 186
6.8.5 Flavonoids inhibit DNA methylation 187
6.8.6 Phenolics affect cell cycle 187
6.8.7 Phenolics inhibit replication 188
6.8.8 Phenolics promote or repress transcription 189
6.9 Conclusion 190
6.10 References 191

7 Muriel Wheldale Onslow and the Rediscovery of Anthocyanin Function in Plants 206
Kevin S. Gould

7.1 Introduction 206
7.1.1 Muriel Wheldale Onslow: a brief biography 208
7.2 Functional hypotheses for anthocyanins in vegetative tissues 211
7.3 A modern spin on some old ideas 213
7.3.1 Photoprotection revisited 213
7.3.2 Anthocyanins, sugars, and autumn leaves 217
7.4 Concluding remarks 218
7.5 Acknowledgments 219
7.6 References 219

8 Plant Phenolic Compounds Controlling Leaf Movement 226
Minoru Ueda and Yoko Nakamura

8.1 Introduction 226
8.2 Endogenous bioactive substance controlling nyctinasty 227
8.3 The chemical mechanism of the rhythm in nyctinasty 228
8.4 Bioorganic studies of nyctinasty using functionalized leaf-movement factors as molecular probes 230
8.4.1 Fluorescence studies on nyctinasty 230
8.4.2 Photoaffinity labeling of the target protein for the leaf-movement factor 231
8.4.3 Are leaf-movement target proteins common to the same plant genus? 234
8.5 References 235

9 Red Clover Derived Isoflavones: Metabolism and Physiological Effects in Cattle and Sheep and their Concentration in Milk Produced for Human Consumption **238**
Juhani Taponen, Eeva A. Mustonen, Lea Kontio, Ilkka Saastamoinen, Aila Vanhatalo, Hannu Saloniemi, and Kristiina Wähälä

9.1 Introduction 238
9.2 Phytoestrogens in ruminant feeds 238
9.3 Red clover as a source of isoflavones 239
9.4 Metabolism of isoflavones in ruminants 241
9.5 Equol: the most important metabolite 243

9.6 Physiological effects and regulatory mechanisms of endogenous estrogens 245
9.7 Effects of phytoestrogens in sheep reproduction 247
9.7.1 Classical clover disease 247
9.7.2 Temporary subfertility 247
9.7.3 Permanent infertility 247
9.8 Effects of phytoestrogens in cattle reproduction 248
9.9 Antioxidant capacity of isoflavones 249
9.10 New outlook 249
9.11 References 250

10 Polyphenols as Biomarkers in Nutrition Research: Resveratrol Metabolome a Useful Nutritional Marker of Moderate Wine Consumption 255
Raul Zamora-Ros and Cristina Andrés-Lacueva

10.1 Introduction 255
10.2 Characteristics of nutritional biomarkers 256
10.3 Strengths and limitations of biological biomarkers over dietary estimation 261
10.4 Resveratrol: a useful biomarker of wine consumption 262
10.5 References 265

11 Translation of Chemical Properties of Polyphenols into Biological Activity with Impact on Human Health 269
João Laranjinha

11.1 Introduction 269
11.2 Polyphenols as antioxidants: the earlier notions 270
11.2.1 The influence of redox potentials 270
11.2.2 Redox cycles of polyphenols with vitamins E and C: the influence of solubility 272
11.3 Beyond "global" antioxidation: alternate biological activities for polyphenols with impact on human health 274
11.3.1 Modulation of redox signaling pathways 274
11.3.2 Modulation of nitric oxide metabolism 276
11.4 References 278

12 Mitigation of Oxidative Stress and Inflammatory Signaling by Fruit and Walnut Polyphenols: Implications for Cognitive Aging **283**
James A. Joseph, Barbara Shukitt-Hale, and Lauren M. Willis

12.1 Introduction 283
12.2 Oxidative stress/inflammatory interactions 284
12.2.1 Oxidative stress 284
12.2.2 Inflammation 284
12.2.3 Intracellular signaling 285
12.2.4 Calcium buffering capacity 286
12.2.5 Neurogenesis 286
12.2.6 Membrane changes 287
12.3 Nutritional interventions 287
12.3.1 Fruit polyphenols as neuroprotective agents 287
12.3.2 Polyunsaturated fatty acids and cognition: animal studies 289
12.4 References 291

13 Antiatherosclerotic Effects of Dietary Flavonoids: Insight into their Molecular Action Mechanism at the Target Site 299
Junji Terao, Kaeko Murota, and Yoshichika Kawai

13.1 Introduction 299
13.2 Flavonoids in the diet and their antioxidant/prooxidant activity 300
13.3 Absorption and metabolism of dietary flavonoids in the digestive system 304
13.4 Oxidative LDL theory and antioxidant activity of flavonoids in plasma 307
13.5 Antioxidant and "beyond" antioxidant activity of flavonoids in the artery 309
13.6 Activated macrophages as potential targets of dietary flavonoids as antiatherosclerotic factors 312
13.7 Conclusion 313
13.8 References 314

Index 319

Color plate section follows page 108

Chapter 1

The Visible Flavonoids or Anthocyanins: From Research to Applications

Raymond Brouillard, Stefan Chassaing, Géraldine Isorez, Marie Kueny-Stotz, and Paulo Figueiredo

1.1 Introduction

Anthocyanins are polyphenolic pigments responsible for most of the color diversity found in plants. Here the *in vivo* color expression and the stability of anthocyanins are interpreted by extrapolation of the results acquired *in vitro* with model solutions of pigments obtained through plant extraction or laboratorial synthesis. Behavior of anthocyanins is explained in terms of molecular interactions of the chromophore units with parts of the pigments themselves and/or with some constituents of the plant cell. These include, among others, diverse polyphenols, metal cations, and inorganic salts. Attention is also given to the biophysicochemical environment found in plant vacuoles that plays a fundamental role on the intermolecular and intramolecular associations displayed by anthocyanins. For example, anthocyanin *Z*-chalcones (retrochalcones) provide an unexpected open cavity for the ferric cation. Medicinal, nutritional, and industrial applications of anthocyanins are proposed.

Colors are conferred to plants by chlorophylls, carotenoids, and flavonoids (Britton, 1983). Chlorophylls are responsible for the green colors displayed by the leaves, whereas carotenoids provide some of the red-orange hues often found in fruits, flowers, and other plant constituents. Flavonoids belong to a larger family, the polyphenols, and can be found in most flowers and fruits (Brouillard & Dangles, 1993; Andersen & Jordheim, 2006). They include the principal elements responsible for the color diversity found in the plant world, the anthocyanins (Fig. 1.1). In fact, these pigments are the only polyphenols that possess the ability to absorb light both in the ultra-violet and in all the visible range (from yellow-orange to bluish-green) (Goto & Kondo, 1991). It is well known that anthocyanins are at the origin of plants' most brilliant colors, a phenomenon particularly visible from flowers. Nevertheless, there exists only one chromophore – the flavylium nucleus – whose subtle interactions with vacuole biochemicals, including water, are capable of providing all kind of colors.

Anthocyanins are stored in an organized aqueous medium in the cell vacuoles. A slightly acidic environment (pH 3–5; Stewart *et al.*, 1975) rich in inorganic ions and other polyphenols is essential for the transformations in these pigments that enable the formation of molecular complexes and subsequent color changes and stabilization (Brouillard & Dangles, 1993).