# Recent Advances in Polyphenol Research

VOLUME 3

Edited by
Véronique Cheynier,
Pascale Sarni-Manchado and
Stéphane Quideau

WILEY-BLACKWELL

# Contents

**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 will focus on several important research topics in plant phenols and polyphenols, including chemistry, biosynthesis, metabolic engineering, ecology, physiology, food, nutrition, and health.

**Volume 3 editors:**

Véronique Cheynier, Pascale Sarni-Manchado, and Stéphane Quideau

Virginie Leplanquais (LVMH Research, Christian Dior, France)
Stephan Martens (Fondazione Edmund Mach, IASMA, San Michele all'Adige, Italy)
Anna-Lisa Romani (University of Florence, Italy)
Pascale Sarni-Manchado (INRA, Montpellier, France)
Celestino Santos-Buelga (University of Salamanca, Spain)
Dieter Treutter (Technical University of Munich, Freising, Germany)
Kristiina Wähälä (University of Helsinki, Finland)

# Recent Advances in Polyphenol Research

Volume 3

*Edited by*
Véronique Cheynier
*Research Director, Food Chemistry*
*Institut National de la Recherche Agronomique*
*UMR1083 Sciences pour l'Œnologie*
*Montpellier, France*

Pascale Sarni-Manchado
*Research Associate, Plant and Food Biochemistry*
*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*
*Institut Européen de Chimie et Biologie*
*Université de Bordeaux, France*

WILEY-BLACKWELL
A John Wiley & Sons, Ltd., Publication

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

*Editorial offices:* 9600 Garsington Road, Oxford, OX4 2DQ, UK
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
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*

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.

# Dedications

*To Jean-Jacques Macheix—a board member of Groupe Polyphénols for many years and its President from 1986 to 1990—whose career has been devoted to phenolic compounds in plants.*

*To Ismaïl El-Hadrami—an active and enthusiastic member of the Groupe Polyphénols board for many years, and a member of the editorial board of the RAPR series—in memoriam.*

# *Acknowledgments*

The editors wish to thank all of the members of the Groupe Polyphénols Board Committee
(2008--2010) for their guidance and assistance throughout this project.

*Groupe Polyphénols Board 2008--2010*

Dr. Catherine Chèze
Prof. Gilles Comte
Dr. Kevin Davies
Prof. Ismaïl El-Hadrami
Dr. Sylvain Guyot
Prof. Victor de Freitas
Dr. Hélène Fulcrand
Dr. Paul A. Kroon
Dr. Virginie Leplanquais
Dr. Stephan Martens
Prof. Stéphane Quideau
Prof. Anna-Lisa Romani
Dr. Pascale Sarni-Manchado
Prof. Celestino Santos-Buelga
Prof. Dieter Treutter
Prof. Kristiina Wähälä

# Contributors

**Lorne R. Adam,** Department of Plant Science, University of Manitoba, 66 Dafoe Road, Winnipeg, MB, R3T 2N2, Canada

**Elena Bernardini,** Biblioteca Centrale di Farmacia, Università degli Studi di Milano, Via Balzaretti 9, 20133 Milano, Italy

**Andrés Bohórquez-Restrepo,** Plant Biotechnology Center, 206 Rightmire Hal, 1060 Carmack Road, Columbus, OH 43210, USA

**Alain-Michel Boudet,** Laboratoire Surfaces Cellulaires et Signalisation chez les Végétaux—UMR-CNRS 5546, Università Paul Sabatier, Pôle de Biotechnologies Végétale, 24 chemin de Borde Rouge, B.P. 42617, Auzeville, 31326 Castanet-Tolosan, France

**Eugenio Butelli,** John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

**Angela Cardinali,** Istituto di Scienze delle Produzioni Alimentari—CNR, Via Amendola 122/O, 70126-Bari, Italy

**Véronique Cheynier,** INRA, UMR1083 Sciences pour l'œnologie, 2 place Viala, F-34060 Montpellier Cedex 1, France

**Fouad Daayf,** Department of Plant Science, University of Manitoba, 66 Dafoe Road, Winnipeg, MB, R3T 2N2, Canada

**Holly Derksen,** Department of Plant Science, University of Manitoba, 66 Dafoe Road, Winnipeg, MB, R3T 2N2, Canada

**Montserrat Dueñas,** Grupo de Investigación de Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

**Ahmed F. El-Bebany,** Department of Plant Science, University of Manitoba, 66 Dafoe Road, Winnipeg, MB, R3T 2N2, Canada; and Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Aflaton Street, El-Shatby, 21545, Alexandria, Egypt

**Abdelbasset El Hadrami**, Laboratoire de Biotechnologies, Protection et Valorisation des Ressources Végétales (Biotec-VRV), Faculté des Sciences Semlalia, Université Cadi Ayyad, B.P. 2390, 40 000 Marrakech, Morocco

**Ismaïl El-Hadrami,** Laboratoire de Biotechnologies, Protection et Valorisation des Ressources Végétales (Biotec-VRV), Faculté des Sciences Semlalia, Université Cadi Ayyad, B.P. 2390, 40 000 Marrakech, Morocco

**Susana González-Manzano,** Grupo de Investigación de Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

**Ana M. González-Paramás,** Grupo de Investigación de Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

**Antonio Granell,** IBMCP-CSIC-UPV, Universidad Politécnica de Valencia, Avda Los Naranjos SN, 46022 Valencia, Spain

**Erich Grotewold,** Department of Molecular Genetics and Plant Biotechnology Center, 206 Rightmire Hal, 1060 Carmack Road, Columbus, OH 43210, USA

**Ann E. Hagerman,** Department of Chemistry & Biochemistry, Miami University, 701 E. High Street, Hughes Laboratories, Oxford, OH 45056, USA

**Maria A. Henriquez,** Department of Plant Science, University of Manitoba, 66 Dafoe Road, Winnipeg, MB, R3T 2N2, Canada

**Yung-Fen Huang,** INRA, UMR1083 Sciences pour l'Œnologie, and INRA, UMR AGAP (Amélioration Génétique et Adaptation des Plantes), 2 place Viala, F-34060 Montpellier Cedex 1, France

**Niloufer G. Irani,** Department of Plant Systems Biology, Flanders Institute for Biotechnology, and Department of Plant Biotechnology and Genetics, Ghent University, 9052 Ghent, Belgium

**Jonathan D.G. Jones,** Sainsbury Laboratory, Norwich Research Park, Colney, Norwich NR4 7UH, UK

**Kalyani Kallam**, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

**Tadao Kondo,** Graduate School of Bioagricultural Science & Graduate School of Information Science, Nagoya University, Chikusa, Nagoya 464-8601, Japan

**Vincenzo Lattanzio,** Dipartimento di Scienze Agro-Ambientali Chimica e Difesa Vegetale, Facoltà di Agraria, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy

**Vito Linsalata,** Istituto di Scienze delle Produzioni Alimentari—CNR, Via Amendola 122/O, 70126-Bari, Italy

**Jie Luo,** Department of Metabolic Biology, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK; and National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

**Andrew Marston,** Chemistry Department, University of the Free State, Nelson Mandela Drive, Bloemfontein 9300, South Africa

**Cathie Martin,** John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

**Diego Orzaez,** IBMCP-CSIC-UPV, Universidad Politécnica de Valencia, Avda Los Naranjos SN, 46022 Valencia, Spain

**Kin-ichi Oyama,** Chemical Instrumentation Facility, Research Center for Materials Science, Nagoya University, Chikusa, Nagoya 464-8601, Japan

**Lucille Pourcel,** Département de Botanique et Biologie Végétale, Université de Genève, Sciences III, 30 quai Ernest-Ansemet, 1211 Genève, Switzerland

**Celestino Santos-Buelga,** Grupo de Investigación de Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

**Scott A. Snyder,** Department of Chemistry, Columbia University, 3000 Broadway, Havemeyer Hall, NY 10027, USA

**Angelique Stalmach,** College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

**Nancy Terrier,** INRA, UMR1083 Sciences pour l'œnologie, 2 place Viala, F-34060 Montpellier Cedex 1, France

**Laurence Tomlinson,** Sainsbury Laboratory, Norwich Research Park, Colney, Norwich, NR4 7UH, UK

**Francesco Visioli,** Laboratory of Functional Foods, IMDEA-Food, Campus de Cantoblanco, 28049 Madrid, Spain

**Gary Williamson,** School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK

**Zhen Yao,** Department of Plant Science, University of Manitoba, 66 Dafoe Road, Winnipeg, MB, R3T 2N2, Canada

**Kumi Yoshida,** Graduate School of Information Science, Nagoya University, Chikusa, Nagoya 464-8601, Japan

**Yang Zhang,** John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

# Preface

Plant polyphenolics are secondary metabolites that constitute one of the most common and widespread groups of substances in plants. They are structurally diverse, from rather simple compounds (e.g., anthocyanins, flavonols, isoflavones, catechins, and resveratrol) to highly complex polymeric species, and exhibit a large and diverse array of biological properties, for both plants and humans. Synthesis of polyphenolic compounds, which contribute to the pigmentation of flowers, fruits, leaves, or seeds, and play protective roles against biotic and abiotic stresses, is part of the adaptative strategies of plants. Polyphenolic compounds also contribute to the development of color and taste properties of plant-based foods and beverages, such as tea, wine, or chocolate, and they may play a part in the health protecting effects associated with the dietary consumption of such food products, although the actual benefit and mechanisms involved are yet to be proven. Finally, they are potentially helpful as therapeutic agents against various pathologies.

The list of plant (poly) phenolic compounds is constantly expanding, and, in spite of recent progress in the development of analytical methods, in particular for metabolomics, these molecules still present a considerable challenge to the analyst. Biological studies are aimed at understanding their role and status *in planta*, but also their fate *in vivo* after ingestion from food and beverages. Most of the work is sustained by the analysis of their chemical characteristics and physicochemical properties. There has been much effort over the last years to understand polyphenol biosynthesis and build the knowledge required to engineer or better harness their production in plants. Alternative strategies rely on organic synthesis to prepare polyphenolic target compounds in sufficient quantities to explore their properties and use them in various applications.

The diversity of structure and activity of (poly) phenolic compounds resulted in a 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,” every other year, is a unique opportunity for scientists in these and other fields to get together and exchange their ideas and new findings.

The 25th edition of this conference (ICP2010) was held in Montpellier, France, from August 24 to 27, 2010, and organized by the Polyphenols and Interactions group of UMR1083—Sciences pour l’Oenologie (INRA Montpellier), in partnership with UMR47—Diversité, Adaptation et Développement des Plantes (Université Montpellier II). Five topics were covered:

**1.** *Chemistry and physicochemistry*: structure, reactivity, physicochemical properties, synthesis, …

**2.** *Biosynthesis, genetics, and metabolomic engineering*: molecular biology, enzymology, gene expression and regulation, transport, biotechnology, …

**3.** *Roles in plants and ecosystems*: plant growth and development, plant–insect relationships, biotic and abiotic stress, resistance, …

**4.** *Health and nutrition*: medicinal properties, bioavailability and metabolism, mode of action, nutraceuticals, cosmetics, …

**5.** *Analysis and metabolomics*: analytical methods, omics, …

Some 365 participants, from government institutional research and private business, representing 44 countries from all over the world, attended ICP2010, where 40 oral communications and 300 posters were presented. The present and third volume of *Recent Advances in Polyphenol Research* (RAPRIII), a series initiated by Groupe Polyphenols in 2008, includes chapters from the 11 guest speakers and some invited contributors. Essential complement to Polyphenols Communications 2010, the proceedings of ICP2010, RAPRIII offers in-depth knowledge on selected aspects of current polyphenol research, pursuing the role of ICP in being a base for debates and exchange on all research topics related to plant polyphenols.

In conclusion, we are pleased to observe that research advances in polyphenol science, enabling progress of our understanding of polyphenols at both the chemical and biological levels, are based on different approaches from different research areas and interactions between them. This would not be possible without the constant involvement of "Groupe Polyphénols" in maintaining ICP and coordinating this book series. So, we wish to thank deeply its Board and the scientific committee of ICP2010 for their contribution to the advancement of polyphenol research worldwide.

This 25th International Conference on Polyphenols would not have been possible without the generous support of public donors such as the French *Région Languedoc Roussillon, Montpellier Agglomération, INRA,* and *Université Montpellier II.* Grants from *Groupe Polyphénols* and from the *Phytochemical Society of Europe* for junior and senior attendees are also gratefully acknowledged. Other sponsors included Agilent Technology, GlaxoSmithKine, Indena, L'Oréal, PhenoFarm, Sanofi Aventis, and Waters.

Last, but not least, ICP2010 and RAPRIII would not be without the members of the local organizing committee, as well as many other "volunteers," whose dedicated

effort and support ensured a smooth and eventless scientific and logistic organization. Our sincere thanks to all of them.

Véronique Cheynier
Pascale Sarni-Manchado
Stéphane Quideau

# *Chapter 1*

# *Plant Phenolics: A Biochemical and Physiological Perspective*

**Vincenzo Lattanzio, Angela Cardinali and Vito Linsalata**

**Abstract:** The plant polyphenols are a very heterogeneous group, some universally and others widely distributed among plants, and often present in surprisingly high concentrations. During the evolutionary adaptation of plants to land, the biosynthesis of different phenolics classes in plants has evolved in response to changes in the external environment. Besides a bulk of phenolic substances having cell wall structural roles, a great diversity of non-structural constituents was also formed, having such various roles as defending plants, establishing flower colour and contributing substantially to certain flavours. The accumulation of phenolics in plant tissues is considered a common adaptive response of plants to adverse environmental conditions, therefore increasing evolutionary fitness. In addition, these secondary metabolites may still be physiologically important as a means of channelling and storing carbon compounds, accumulated from photosynthesis,

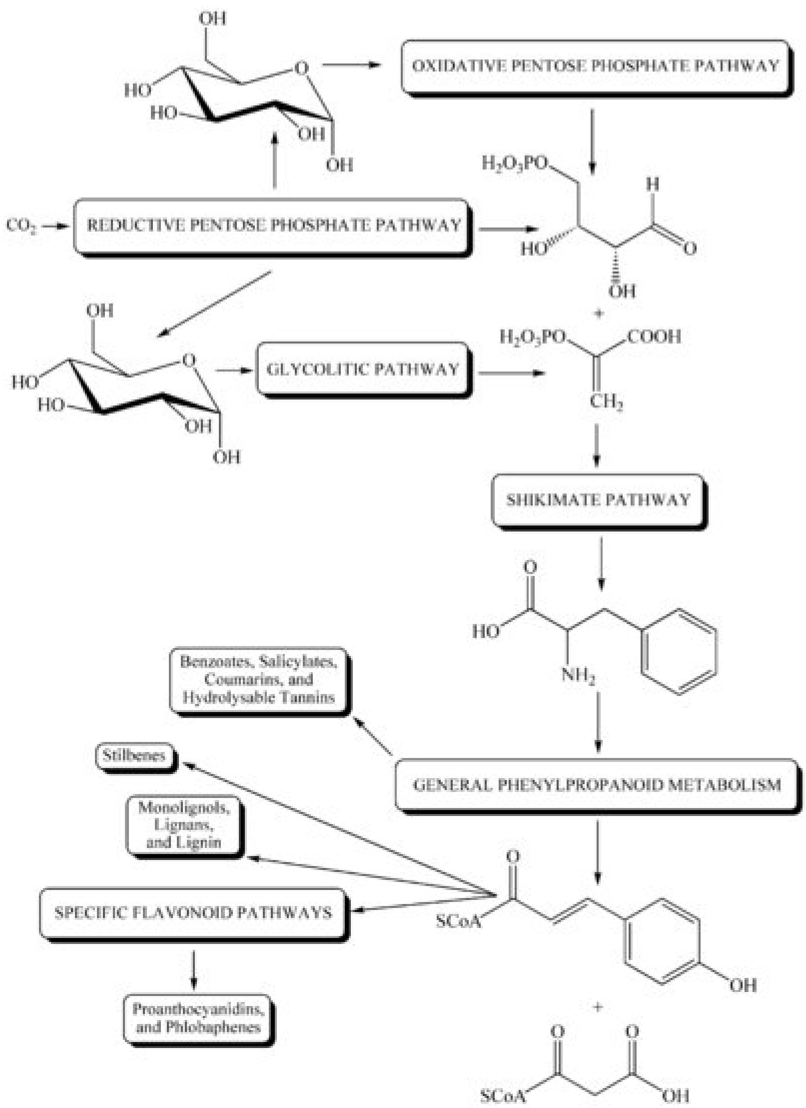
during periods when nitrogen is limiting or whenever leaf growth is curtailed.

**Keywords:** phenolics; abiotic/biotic stress; primary/secondary metabolism relationships; metabolic costs of resistance

# 1.1 The general phenolic metabolism in plants

Phenolic compounds are found throughout the plant kingdom but the type of compound present varies considerably according to phylum. Phenolics are uncommon in bacteria, fungi and algae, and few classes of phenols are recorded: flavonoids are almost completely absent. Bryophytes are regular producers of polyphenols including flavonoids, but it is in the vascular plants that the full range of polyphenols is found (Swain, 1975; Harborne, 1980; Stafford, 1991). The plant polyphenols are a very heterogeneous group; some are universally and others widely distributed among plants, and they are often present in surprisingly high concentrations. They are not distributed evenly throughout the plant – either quantitatively or qualitatively – in space and in time. The pattern of secondary metabolites in a given plant is complex because it changes in a tissue- and organ-specific way. Differences can regularly be seen between different developmental stages (e.g. organs important for survival and reproduction have the highest and most potent secondary metabolites), and between individuals and populations and these differences are subject to environmental as well as genetic control (Swain, 1977; Harborne, 1980; Wink, 1988; Osbourn *et al.*, 2003; Wink, 2003; Noel *et al*., 2005; Singh & Bharate, 2006; Yu & Jez, 2008). Phenolic metabolism in plants is a complex process resulting from the interaction of at least five different pathways. The glycolytic pathway that produces phosphoenolpyruvate; the pentose phosphate pathway that produces erythrose-4-phosphate; the shikimate pathway that synthesises phenylalanine; the general phenylpropanoid metabolism that produces the activated cinnamic acid derivatives and the plant structural component lignin, and the diverse specific flavonoid pathways (Boudet *et al*., 1985; Hrazdina, 1994; Schmid & Amrhein, 1995; Winkel-Shirley, 2001; Austin & Noel, 2003) (Fig. 1.1). Phenolic metabolism must be regarded as a dynamic system involving steady-state concentrations of the various phenolic compounds, which during certain phases of growth and development are subject to substantial qualitative and quantitative changes. This turnover may involve three types of reactions: (i) interconversions which are involved in biosynthetic sequences; (ii) catabolic reactions where the products are converted to primary metabolic constituents and (iii) oxidative polymerisation reactions leading to insoluble structures of high molecular weight (Barz & Hoesel, 1975, 1979).

**Fig. 1.1** Carbon fluxes towards the phenolic metabolism.




Plants, as sessile organisms, evolve and exploit metabolic systems to produce a vast and diverse array of phenolic and polyphenolic compounds with a variety of ecological and physiological roles. The ability to synthesise phenolic compounds has been selected throughout the course of evolution in different plant lineages when such compounds addressed specific needs, thus permitting plants to cope with the constantly changing environmental challenges over evolutionary time (Pichersky & Gang, 2000; Noel *et al.*, 2005). For example, the successful adaptation to land by some higher members of the Charophyceae – which are regarded as prototypes of amphibious plants that presumably preceded true land plants when they emerged from an aquatic environment onto the land – was achieved largely by massive formation of 'phenolic UV light screens' (Swain, 1975; Lowry *et al*., 1980; Stafford, 1991; Graham *et al*., 2000). Regarding the structure of phenolic compounds involved in this photoprotective role of plant phenolics, there was an exciting discussion between Tony Swain and Brian Lowry. Lowry's speculative viewpoint was that 'when plants invaded the land habitat and were exposed to solar-ultraviolet radiation more intense than that found today, an early obvious protective adaptation strategy used by plants would be the accumulation of substituted cinnamic acids from the deamination of aromatic amino acids' (Lowry *et al*., 1980). Swain's objection to this speculative hypothesis was that 'cinnamic acids absorbing at 310–325 nm do not have the right absorption characteristics to enable them to act efficiently in this way and thus prevent UV photodestruction of either nucleic acids or proteins ($\lambda_{max}$ ca 260 and 280 nm, respectively)'. Swain's opinion was that flavonoids ($\lambda_{max}$ ca 260 and 330 nm), cell wall polysaccharide acylation by cinnamic acids and suberin could all presumably have aided in the success of land plants (Swain, 1981). Lowry's reply was that, 'given the presence of even trace amounts of ozone in the atmosphere during the time leading up to the Silurian and early Devonian (starting some 420 million years ago), it is extremely unlikely that terrestrial organisms would have been exposed to UV-C radiation (less than 280 nm)' and that DNA and proteins are both damaged by radiation in the UV-B region (280–315 nm) (Lowry *et al*., 1983). A wide array of flavones have been reported for *Takakia lepidozioides*, believed to be amongst the most primitive of extant liverworts and the possible