Recent Advances in Polyphenol Research

VOLUME 3

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

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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

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Volume 3

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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*.

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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

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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).

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 (λ_{max} ca 260 and 280 nm, respectively)'. Swain's opinion was that flavonoids (λ_{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

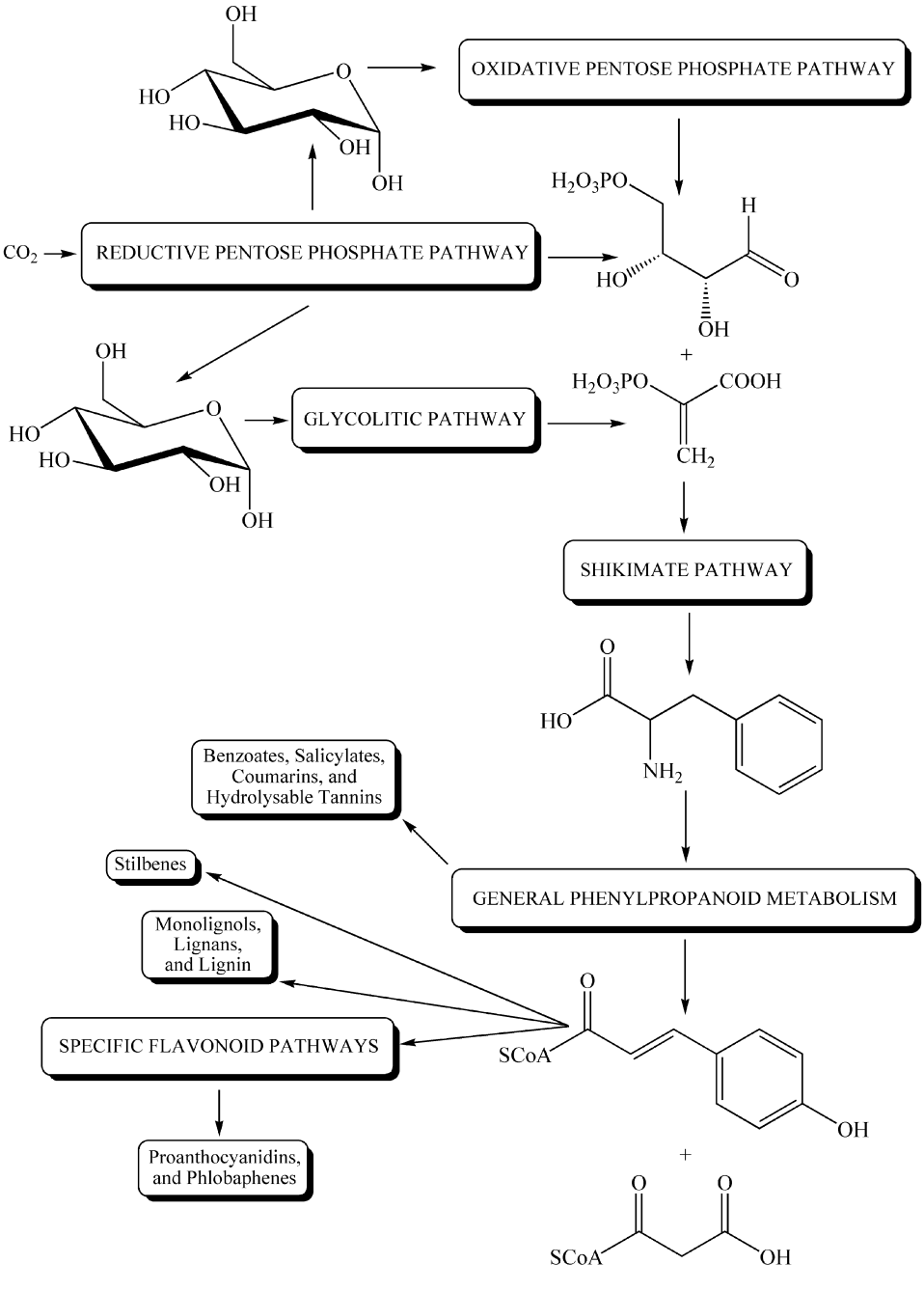
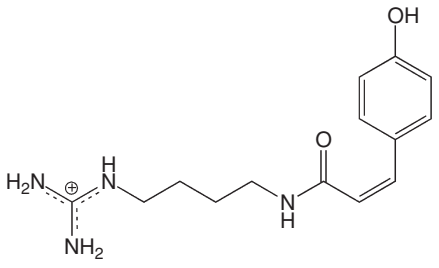


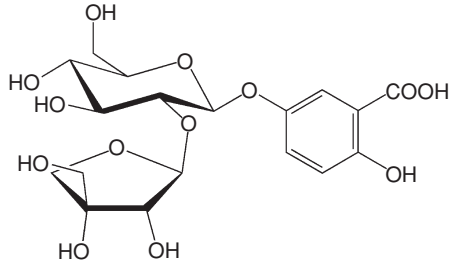
Fig. 1.1 Carbon fluxes towards the phenolic metabolism.

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 ancestor of modern bryophytes. This suggested that metabolic pathways leading to flavonoid synthesis appeared quite early in the evolutionary record of plants, perhaps even before the development of vascular tissues (Markham & Porter, 1979). Bryophyte lines that mainly synthesised flavones and flavonols, branched off within populations of pioneering land plants. Within other populations of early land plants, the evolution of the enzymes unique to the lignin pathway permitted the evolution of vascular plants, the tracheophytes. Proanthocyanidins and flavan-3-ols became widespread in some fern groups, while these and 3-hydroxyanthocyanidins became dominant flavonoids in gymnosperms and, especially, in angiosperms. Proanthocyanidins remained as major constitutive defence compounds in leaves of long-lived woody plants, but became relatively rare in short-lived herbaceous angiosperms, except in the seed coats of some of these plants. The pterocarpan pathways producing inducible phytoalexins for chemical defence purposes were evolved in a few angiosperm taxons (Stafford, 1991). Broadly, it is now well known that charophyte green algae can inhabit extreme habitats (highly saline and acidic waters with high levels of heavy metals) and that green algae are also common on land. Terrestrial algae grow in some of the most difficult habitats on earth, such as desert soils. Morphological and molecular analyses of some of these charophyte green algae have indicated multiple transitions to arid habitats from aquatic ancestors. 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. In addition to a bulk of phenolic substances with cell wall structural roles, an amazing diversity of non-structural constituents was also formed, having such various roles as defending plants, determining the durability of different woods and barks, establishing flower colour and contributing substantially to certain flavours. In addition, phenolics – and ultimately flavonoids – were also selected for their protection against ultraviolet damage and autotoxicity. All these diverse functions performed by the different classes of phenolic compounds are essential for the continued survival of all types of vascular plants (Lowry *et al*., 1980; Cooper-Driver & Bhattacharya, 1998; Flechtner *et al*., 1998; Croteau *et al*. 2000; Bieza & Lois, 2001; Lewis & Mccourt, 2004; Teklemariam & Blake, 2004; Caldwell *et al*., 2007; Lattanzio *et al*., 2008).

However, it is not true that all plants lack mobility, although, plants are generally rooted and unable to move from place to place by themselves. Some plants are now known to be able to move in certain ways; some plants are known to open their leaves in the daytime and 'sleep' at night with their leaves folded. This circadian rhythmic leaf movement known as nyctinasty is widely observed in leguminous plants. It was thought that nyctinastic movement was controlled by Schildknecht's turgorins (chemical factors controlling the turgor changes in plants which induce turgor-controlled movements including nyctinasty), which induce leaf-closing movement of the plants (Schildknecht & Schumacher, 1982; Schildknecht, 1983). Ueda and his collaborators found that nyctinastic plants have a pair of endogenous bioactive substances that control nyctinastic leaf movement (Ueda & Yamamura



cis-p-Coumaroylagmatine
leaf-opening factor (*Albizzia julibrissin* Durazz)



Gentisic acid 5-*O*-[β-D-apiofuranosyl-(1→2)-
β-D-glucopyranoside] leaf-closing factor
(*Mimosa pudica* L.)

Fig. 1.2 Leaf-movement factors from nyctinasic plants.

2000; Ueda & Nakamura 2006). One of these is a leaf-opening factor that 'awakens' plant leaves, and the other is a leaf-closing factor that reverses this process, so that the plant leaves 'sleep' (Fig. 1.2). All leaf-opening factors, which are effective under physiological pH and in a physiological concentration, have the common structural feature of *p*-coumaroyl moiety, and this result suggests that this structural feature is deeply involved in the common mechanism for leaf-opening (Ueda & Nakamura, 2010).

The highly ordered interactions between plants and their biotic and abiotic environments have been a major driving force behind the emergence of specific natural products. The accumulation of phenolics in plant tissues is considered a common adaptive response of plants to adverse environmental conditions, 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. Large increases in the amount of phenolic compounds can occur in stressed plants and those undergoing mechanical damage. Plant phenolics are considered to have a key role as defence compounds when environmental stresses such as bright light, low temperatures, pathogen infection, herbivores and nutrient deficiency can lead to increased production of free radicals and other oxidative species in plants. A growing body of evidence suggests that plants respond to these biotic and abiotic stress factors by increasing their capacity to scavenge reactive oxygen species. In addition, in order to establish a protective role for a given metabolite, it is necessary to monitor concentrations over the life cycle of the plant, to survey plant populations, to determine specific localisation within tissues and to carry out bioassays against insects and microorganisms. Finally, changes in secondary chemistry may also occur during ontogeny and protection may be restricted to the most vulnerable plant organs (Robbins *et al.*, 1985; Harborne, 1990; Lattanzio *et al*., 1994; Dixon & Paiva, 1995; Facchini, 1999; Winkel-Shirley, 2002, Blokhina *et al*., 2003).

The bewildering array of phenolic compounds produced by plant tissues (several thousand different chemical structures have been characterised) belong to various classes, such as esters, amides and glycosides of hydroxycinnamic acids, glycosylated flavonoids, especially flavonols, proanthocyanidins and their relatives and the polymeric lignin and

suberin. Some soluble phenolics, for example chlorogenic acid, are widely distributed, but the distribution of many other structures is restricted to specific genera or families making them convenient biomarkers for taxonomic studies. Even if the potential value of plant secondary metabolites to taxonomy has been recognised for nearly 200 years, their practical application has been restricted to the twentieth century and predominantly to the last 40 years. The use of secondary compounds has clear advantages over the use of primary compounds in establishing phylogenetic relationships because differences in the complement of secondary compounds are qualitative differences whereas differences in the concentrations of primary compounds are quantitative differences, and these are subject to both environmental and genetic control. Phenolic compounds are often similar within members of a clade and therefore the existence of a common pattern of secondary compounds may indeed provide much clearer evidence of common ancestry than morphological similarities attributable either to common ancestry or to convergent evolution (Bell, 1980; Lattanzio *et al*., 1996; Wink, 2003).

1.2 Effect of non-freezing low temperature stress on phenolic metabolism in crop plants

Of the various environmental stresses, exposure to non-freezing low temperatures is one of the most important abiotic stress factors for plants. The precise way in which plants adapt to low temperature is obviously of scientific interest, but there are also practical and economic aspects. Many important crop plants of tropical and subtropical origin are, in general, sensitive to low non-freezing temperatures less than 10°C to 12°C. Several studies have suggested that exposure to low temperatures usually triggers a variety of biochemical, physiological and molecular changes that allow the plants to adjust to stress conditions and this response is characterised by a greater ability to resist injury or survive an otherwise lethal low temperature stress. This process is known as cold acclimation (Lyons, 1973; Graham & Patterson, 1982; Janas *et al*., 2000; Sharma *et al*., 2005). Lowering temperatures will thermodynamically reduce the kinetics of metabolic reactions. Exposure to low temperatures will shift the thermodynamic equilibrium so that there is an increased likelihood of non-polar side chains of proteins becoming exposed to the aqueous medium of the cell. This leads to a disturbance in the stability of proteins, or protein complexes and also to a disturbance of the metabolic regulations. Lower temperatures induce rigidification of membranes, leading to a disturbance of all membrane properties (permeability, electric field, cation concentration and water ordering, and this leads to disturbance of the conformation and thus the activity, of membrane-bound enzymes). Chilling is also associated with the accumulation of reactive oxygen species (ROS). The activities of the scavenging enzymes will be lowered by low temperatures, and the scavenging systems will then be unable to counterbalance the ROS formation that is always associated with mitochondrial and chloroplastic electron transfer reactions. The accumulation of ROS has deleterious effects, especially on membranes. Some plants are able to adapt through mechanisms based on protein synthesis, membrane composition changes, and activation of active oxygen scavenging systems. There is an increasing body of evidence that many of these biochemical and physiological

changes are regulated by low temperature through changes in gene expression. In recent years, a number of low temperature-responsive genes have been cloned from a range of both dicotyledon and monocotyledon species (Wolfe, 1978; Howarth & Ougham, 1993; Hughes & Dunn, 1996; Thomashow, 1998; Siddiqui & Cavicchioli, 2006; Ruelland *et al*., 2009).

Low temperature stress induces accumulation of phenolic compounds that protect chilled tissues from damage by free radical-induced oxidative stress. It has also been observed that cold stress increases the amount of water-soluble phenolics and their subsequent incorporation into the cell wall either as suberin or lignin (Chalker-Scott & Fuchigami, 1989; Ippolito *et al*., 1997). Many papers report the effects of low temperature on phenolic metabolism, and these have shown that phenolic metabolism is enhanced under chill stress and that the behaviour of the same metabolism is further dependent on the storage temperature. There is a low critical temperature below which an increase of phenylpropanoid metabolism is stimulated during the storage of plant tissues and this temperature varies from commodity to commodity. The threshold temperature for increasing phenolic metabolism is related to the threshold temperature at which chilling injury is also induced and it has been shown that low temperature treatments stimulate phenylpropanoid metabolism as well as flavonoid metabolism in various plant tissues, including artichoke, carrot, gherkin, maize, olive, pea, pear, potato, tomato and watermelon (Rhodes & Wooltorton, 1977, 1978; Rhodes *et al*., 1981; Blankenship & Richardson, 1985; Lattanzio & Van Sumere, 1987; Lattanzio *et al*., 1989; Christie *et al.*, 1994; Leyva *et al*., 1995; Chalker-Scott, 1999; Solecka *et al*., 1999; Gil-Izquierdo *et al*., 2001; Golding *et al*., 2001; Rivero *et al*., 2001; Ortega-García & Peragón, 2009). Figure 1.3a shows changes in the total flavonoid (quercetin and phloretin glycosides) content in Golden Delicious apple skin during storage at 2°C. During the first 60 days of cold storage, there is a relevant increase in flavonoid content, but flavonoid content gradually decreases in fruits stored for a longer period. Similar changes have been observed in the levels of phenolic compounds, mono- and di-caffeoylquinic acids, in artichoke heads stored at 4°C (Fig. 1.3b). The timing of the observed peak in the phenol level during cold storage depends on the species or cultivar, the harvesting time and the storage conditions (Lattanzio *et al*., 1989, 2001; Lattanzio, 2003a, 2003b).

In connection with the increased synthesis of phenolic compounds at low temperatures, some studies have been carried out on some enzymes of phenolic metabolism,

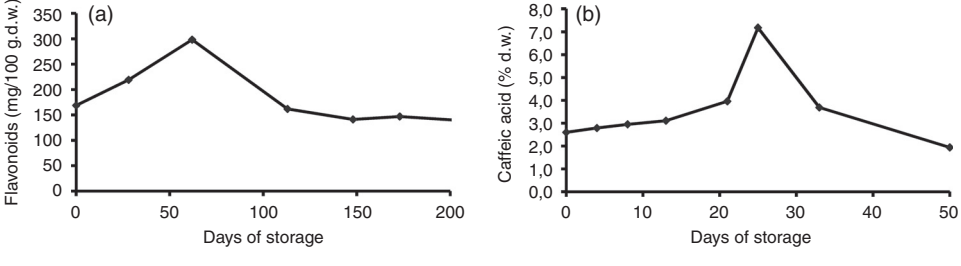


Fig. 1.3 Changes in the total flavonoid content in apple skin during storage at 2°C (a) and in the levels of mono- and di-caffeoylquinic acids (as % of caffeic acid on dry weight) in artichoke heads stored at 4°C (b).

phenylalanine ammonia lyase (PAL, EC 4.3.1.5), cinnamic acid 4-hydroxylase (CA4H) (1.14.13.11), *p*-coumarate CoA ligase (4CL, EC 6.2.1.12), hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase (HQT, EC 2.3.1.133) and chalcone synthase (CHS, EC 2.3.1.74). Generally, this low temperature effect on the phenol level involves a cold-induced stimulation of PAL, the branch point enzyme between primary (shikimate pathway) and secondary (phenolic) metabolism. It is well known that activity of this key enzyme of phenolic biosynthesis is induced in response to different external stimuli including low temperature stress (Engelsma 1970; Camm & Towers, 1973; Engelsma, 1974; Jones, 1984; Shaw *et al*., 1990; Orr *et al*. 1993; Leyva *et al*., 1995; Liu & McClure, 1995; Sarma & Sharma, 1999; Campos-Vargas and Saltveit, 2002; Gomez-Vasquez *et al*., 2004; Tattini *et al*., 2005). An enhanced PAL activity has been observed during cold storage of tomato and potato (Rhodes & Wooltorton, 1977; Rhodes *et al*., 1981), citrus fruits (Sanchez-Ballesta *et al*., 2000a; Lafuente *et al*., 2001), olive (Ortega-García & Peragón, 2009) and onion (Benkeblia, 2000). PAL activity increased about fivefold in stored artichoke heads during the first days of storage at 4°C, and thereafter this activity decreased again to a low level (Lattanzio *et al*., 1989).

The observed increases in PAL activity induced by low temperature might involve both enzyme *de novo* synthesis and release of PAL from a pre-existing but inactive enzyme–inhibitor complex. In any case, stimulation of PAL activity and, in turn, of phenylpropanoid pathway has been considered as a part of the response mechanism of fruits and vegetables to cold stress (Siriphanich & Kader, 1985a; Lattanzio & Van Sumere, 1987; Christie *et al*., 1994; Dixon & Paiva, 1995; Leyva *et al*., 1995; Janas *et al*., 2000; Sanchez-Ballesta *et al.*, 2000a, 2000b; Lattanzio *et al*., 2001; Hannah *et al*., 2006; Olsen *et al*., 2009; Ortega-García & Peragón, 2009). It is likely that endogenous ethylene, produced in plant tissue exposed to low temperature stress, promotes the induction of PAL activity and this is consistent with data showing that cold-induced PAL activity is reduced by inhibitors of ethylene production or by inhibitors of the action of ethylene. The onset of ethylene production in stressed plant tissues occurs at approximately the same time as an increase in PAL activity. Moreover, the effect of exogenously-added ethylene on most tissues is to cause increased production of PAL. The concentration of ethylene that affects PAL levels varies in different plants (Hyodo & Yang, 1971; Rhodes & Wooltorton, 1971; Chalutz, 1973; Hyodo *et al*., 1978; Blankenship & Richardson, 1985; Blankenship & Unrath, 1988; Ke & Saltveit, 1989; Nigro *et al*., 2000; Lafuente *et al*., 2001).

Low temperature induction of PAL activity alone in plant tissues does not produce a corresponding increase in phenol production. At low temperatures, it is possible that the subsequent steps in the biosynthesis of phenolic compounds may limit their formation. In this connection, reference must be made to some excellent papers showing that other enzymes important in the phenolic biosynthetic pathway (e.g. CA4H, CQT, 4CL and CHS) can be stimulated by low temperature treatments. This phenomenon is largely dependent on the plant material studied, the storage temperature and the controlled or modified atmosphere used. In tomatoes stored at 2°C, besides PAL activity, during the first days of storage, a sizeable increase was observed in the activity of CQT, an enzyme involved in chlorogenic acid metabolism. A similar pattern of changes was observed in the enzymes CQT and *p*-coumarate CoA ligase in potato tubers stored at 0°C (Rhodes & Wooltorton, 1977, 1978;