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Analysis and Antioxidant Capacity of Anthocyanin Pigments. Part II: Chemical Structure, Color, and Intake of Anthocyanins

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Anthocyanins belong to a large group of secondary plant metabolites collectively known as flavonoids, a subclass of the polyphenol family. They are a group of very efficient bioactive compounds that are widely distributed in plant food. Anthocyanins occur in all plant tissues, including leaves, stems, roots, flowers, and fruits. Research on phenolic compounds through the last century, from the chemical, biochemical, and biological points of view, has focused mainly on the anthocyanins. Anthocyanins have structures consisting of two aromatic rings linked by three carbons in an oxygenated heterocycle (i.e., a chromane ring bearing a second aromatic ring in position 2). The basic chromophore of anthocyanins is the 7-hydroxyflavylium ion. Anthocyanin pigments consist of two or three chemical units: an aglycon base or flavylium ring (anthocyanidin), sugars, and possibly acylating groups. Only six of the different anthocyanidins found in nature occur frequently and are of dietary importance: cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin. Each aglycon may be glycosylated or acylated by different sugars and aromatic or aliphatic acids, yielding over 600 different anthocyanins reported from plants. The sugar moiety is typically attached at the 3-position on the C-ring or the 5-position on the A-ring. The chromophore of eight conjugate double bonds carrying a positive charge on the heterocyclic oxygen ring is responsible for the intensive red-orange to blue-violet color produced by anthocyanins under acidic conditions. Anthocyanins occur in solution as a mixture of different secondary structures: flavylium ion, a quinoidal base, a carbinol base, and a chalcone pseudobase. Self-association, intermolecular, and intramolecular co-pigmentation of anthocyanins leads to the formation of tertiary structures through varying stabilization mechanisms. Anthocyanin composition has been used as a botanical tool for taxonomic classification of plants. In addition, anthocyanin profiles of fruits and vegetables allow detecting adulteration of anthocyanin-based products and are indicators of product quality. Anthocyanins are common components of the human diet, as they are present in many foods, fruits, and vegetables, especially in berries. Moreover, anthocyanins have an antioxidant activity, depending to a large extent upon their chemical structure. Many epidemiological studies have shown the benefits of a diet rich in fruit and vegetables to human health, and for the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular diseases. Anthocyanin-rich extracts are increasingly attractive to the food industry as natural alternatives to synthetic FD&C dyes and lakes, because of their coloring properties. Anthocyanins are also one of the nine European Union-designated natural color classes. Various adverse effects on health have frequently been attributed to synthetic antioxidants. For these reasons, currently, there is a trend towards relying on antioxidants derived from natural products. Anthocyanins act as antioxidants both in the foodstuffs in which they are found and in the organism after intake of these foods. This review, like the first one of the series, intends to reflect the interdisciplinary nature of the research that is currently carried out in this prolific area.

Keywords anthocyanins, short history, chemical structure, color, anthocyanin intake

INTRODUCTION

Anthocyanins are prominent members of the secondary plant metabolite class of flavonoid compounds that belong to the super family of antioxidants named phenolics or polyphenolics (Buono, submitted; Quideau et al., 2011; Ferretti et al., 2010; Daayf and Lattanzio, 2008). A great interest of botanists, chemists, and biochemists has been always aroused by these vegetal chameleons, as they were called by the eminent botanist Tswett (1914), discoverer of the adsorption column chromatography (Sakodinskii, 1972). Flavonoids are perhaps the most important single group of phenolics in foods; they comprise a group of phytochemicals predominant in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa, and cereals (Buono, submitted; Raghvendra et al., 2011; Wallace, 2011; Andersen and Markham, 2006). Anthocyanins are the largest group of phenolic pigments and the most important group of water-soluble pigments in plants, responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers, being odorless and nearly flavorless and contributing to taste as a moderately astringent sensation (He and Giusti, 2010; Gould et al., 2009; Motohashi, 2008; Veitch and Grayer, 2008; Andersen and Jordheim, 2006; Escribano-Bailón et al., 2004; Vidal et al., 2004; Williams and Grayer, 2004; Harborne and Williams, 2000; Harborne and Grayer, 1988). Generally, the structures of the anthocyanins isolated from fruits and vegetative tissues are simpler than those found in flowers (Andersen and Jordheim, 2010).

Anthocyanins are almost universally found in higher plants (occurring in about 30 families), but in general anthocyanins seem to be absent in the liverworts, algae, and other lower plants, although some of them have been identified in mosses and ferns (Gould et al., 2009; Delgado-Vargas et al., 2000). Anthocyanin biosynthesis was one of the first branches of the general propanoid metabolism, for which biosynthetic enzymes and corresponding transcription factors were identified, given the ease of visualization and control of mutants and genetic imbalances (Hichri et al., 2011; He et al., 2010; Vogt, 2010; Tanaka et al., 2009).

Anthocyanins are effective donors of hydrogen. Owing to anthocyanin's positive charge (Figure 1), the number and arrangement of aromatic hydroxyl groups, the extent of structural conjugation, and the presence of electron-donating and electron-withdrawing substituents in the ring structure, these compounds can easily donate protons to highly reactive free radicals, thereby preventing further radical formation (Ignat et al., 2011; Leopoldini et al., 2011). This protects cells from oxidative damage, which leads to aging and various diseases, such as cancer, neurological, and cardiovascular diseases, inflammation, diabetes, and bacterial infections (Chahar et al., 2011; Cisowska et al., 2011; Siva et al., 2011; Ncube et al., 2008; Charlebois, 2007; Nichenametla et al., 2006; Fernández-Pachón et al., 2005; Ghosh, 2005). Laboratory-based evidence was provided of the potential health benefits of anthocyanins (Raghvendra et al., 2011; Wallace, 2011). Consumption of diets rich in natural bioactive components (i.e., fruits and vegetables) as an alter-

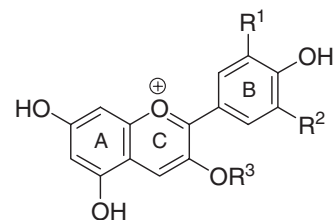


FIG. 1. Structure of the major anthocyanins-3-O-glucoside present in fruits (Freitas and Mateus, 2006; Kähkönen and Heinonen, 2003; Giusti et al., 1999).

native to pharmaceutical medication has been a subject of considerable interest to researchers (Rao and Snyder, 2010; Yadav et al., 2009; Motohashi and Sakagami, 2008; Rajasekaran et al., 2008; Wu and Prior, 2008; Shahidi and Nacz, 2006; Antal et al., 2003). However, much work remains to achieve definitive conclusions and the need for additional basic and applied research in this area is evident (Wallace, 2011; Ndhala et al., 2010; Zhang and Wang, 2010; Thomasset et al., 2009).

The anthocyanins' health properties are due to their peculiar chemical structure, as they are reactive towards reactive oxygen species (ROS), such as superoxide (O_2^-), singlet oxygen (1O_2), peroxide ($RCOO^{\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{\cdot}), and reactive nitrogen species in a terminator reaction, which breaks the cycle of generation of new radicals, because of their electron deficiency (Magalhaes et al., 2008; Choe and Min, 2006; Kong et al., 2003; Min and Bolf, 2002). The antioxidant capacity of phenolic compounds is also attributed to chelate metal ions involving in the production of free radicals, thereby reducing metal-induced peroxidation (Liu, 2010).

Anthocyanins have long been the subject of investigation by botanists and plant physiologists because of their roles as pollination attractants and phytoprotective agents (Giusti and Jing, 2008; Grotewold, 2006b; Delpech, 2000). It is believed that anthocyanins are dissolved in the cell sap found in the vacuole of a plant's epidermis cells and a major function of these compounds is to provide color to most flowers and fruits (Mlodzinska, 2009; Jordheim, 2007; Brouillard, 1983). Many other flavonoid constituents accompany anthocyanins in plant cell vacuoles. Anthocyanins are probably the most important group of visible plant pigments besides chlorophyll (Bechtold and Mussak, 2009). Flavonoids/anthocyanins and carotenoides are often present in the same organs, their combination increasing color variety (Tanaka et al., 2008).

In recent years, synthetic food dyes have been banned in many countries because of their toxicity and carcinogenicity. Anthocyanin-colored natural compounds easily obtained from fruits and vegetables may be considered as potential substitutes for the banned food dyes (Ignat et al., 2011; Scotter, 2010; Motohashi and Sakagami, 2009). In fact, they have bright attractive colors, while their high solubility in water allows their easy incorporation into aqueous food systems (Melo et al., 2009; Mercadante and Bobbio, 2008). Moreover, the proved

TABLE 1
Major anthocyanins-3-*O*-glucoside present in fruits

Anthocyanidin	Abbrev.	R ₁	R ₂	λ_{\max} (nm)*		Some of the produced colors
				R ₃ = H	R ₃ = gluc	
Delphinidin	Dp	OH	OH	546	541	Purple, mauve, and blue
Petunidin	Pt	OH	OCH ₃	543	540	Purple
Malvidin	Mv	OCH ₃	OCH ₃	542	538	Purple
Cyanidin	Cy	OH	H	535	530	Magenta and crimson
Peonidin	Pn	OCH ₃	H	532	528	Magenta
Pelargonidin	Pg	H	H	520	516	Orange salmon

*Methanol-hydrochloric acid (0.01%).

antioxidant activity of anthocyanins, related to the prevention of a number of degenerative diseases, as shown above, provides additional benefits to the food dyed with these natural substances (Bueno, submitted; Fraga, 2010; Jaganath and Crozier, 2010; Pascual-Teresa and Sánchez-Ballesta, 2008; Ghosh and Konishi, 2007). Their contribution is likely to be additive or synergistic in combination with the many other phytochemicals present in, for example, berries (Jing and Giusti, 2011; Pascual-Teresa et al., 2010; Lila, 2009; Lila et al., 2005; Schwinn and Davies, 2004).

Daily intake of anthocyanins is high (estimated at ~200 mg/d); the consumption of other phytonutrients such as carotenoids, vitamin E, and vitamin C are estimated at 5, 12, and 90 mg/d, respectively (Scalbert et al., 2005). The estimated intake (20–25 mg/d) of other dietary flavonoids such as genistein and quercetin is lower than that of anthocyanins (Wallace, 2011). However, regular consumers of red wine are likely to have significantly higher intakes (Cordova and Sumpio, 2009; Mateus and De Freitas, 2009; Pascual-Teresa and Sánchez-Ballesta, 2008).

The analytical chemistry of polyphenols has been the subject of study in this journal from a different point of view (Escudero,

2011; Biesaga and Pyrzynska, 2009; Chrzascik, 2009; Peckova et al., 2009; Pohl et al., 2009; Zima et al., 2009; Harnly et al., 2007; Marszall and Kaliszan, 2007; Schoefs, 2004; Escarpa and González, 2001).

ANTHOCYANINS

Chemically, anthocyanins are glycosylate polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrilium (Andersen and Jordheim, 2010; He and Giusti, 2010; Castañeda-Ovando et al., 2009; Gould et al., 2009; Ghosh and Konishi, 2007), usually with molecular weights ranging from 400 to 1200 (medium-size biomolecules), and containing two benzyl rings (A and B), i.e., anthocyanins are heteroxides of an aglycone unit (anthocyanidin), which is a derivative of flavylum ion. The major anthocyanins are shown in Figure 1 and Table 1 (Kähkönen and Heinonen, 2003; Giusti et al., 1999), and the most commonly accepted nomenclature numbering of their carbon atoms is shown inside the structure in Figure 2. The red, violet, or blue colors of numerous fruits, flowers, and vegetables are due to the presence of anthocyanins, as seen in Table 2 (Melo et al., 2009;

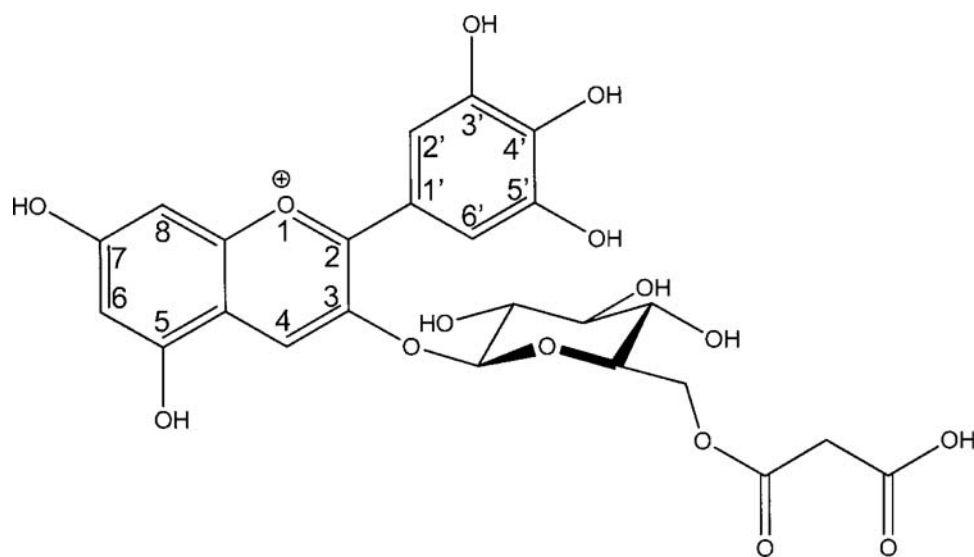


FIG. 2. Structure of delphinidin-3-malonylglucoside.

TABLE 2
Major anthocyanins from selected plant sources

Plant sources	Anthocyanins
Strawberry, banana, red radish, potato	Pelargonidin
Apple, blackberry, elderberry, peach, pear, fig, cherry, red onion, gooseberry, red cabbage, rhubarb	Cyanidin
Black currant, blood orange, gooseberry, red cabbage, purple carrot, seed coat of soybean	Cyanidin and delphinidin
Passion fruit, eggplant, green bean, pomegranate	Delphinidin
Common cranberry, plum, sweet cherry, purple sweet potato	Cyanidin and peonidin
Bilberry, red grape	Petunidin and malvidin
Mango	Peonidin

Delgado-Vargas and Paredes-López, 2003; Clifford, 2000; Timberlake, 1980; Shrikhande and Francis, 1976). In general the anthocyanin concentration in most fruits and vegetables goes from 0.1 up to 1% d.w. (Delgado-Vargas and Paredes-López, 2003; Delgado-Vargas et al., 2000). Synthetic flavylum salts (Bülow and Wagner, 1901), anthocyanins (Willstätter and Everest, 1913), and natural flavylum compounds (Chapman et al., 1927; Pratt et al., 1927), discovered in this order, all have the 2-phenyl-1-benzopyrylium chromophore in common, and their history has been intertwined since the beginning (Pina et al., 2011; Melo et al., 2009). Anthocyanins are omnipresent in our diet, have little or no known toxicity, and are usually quite water soluble, making them particularly attractive as natural substitutes for synthetic pigments and antioxidants (Zafra-Stone et al., 2007; Wrolstadt et al., 2005; Wrolstadt, 2004; Asuero et al., 2002; Kuskoski et al., 2003, 2006; Kuskoski et al., 2002; Francis and Markakis, 1989; Jackman et al., 1987b).

In fruits, anthocyanins mainly exist in glycosidic forms, and with the exception of blueberries, fruits usually contain anthocyanin derived from only one or two of the aglycone bases. Grapes offer a richer anthocyanin profile than many other fruits (McCallum et al., 2007). Various berries and black currants are the fruits richest in anthocyanins (Clifford, 2000). There is only one common vegetable that contains high level of anthocyanins, the eggplant (Horbowicz et al., 2008). Although most species, such as apples, plums, and pears, contain a limited number of anthocyanin pigments, red grapes may contain a mixture of more than 20 pigments, offering a richer anthocyanin profile than many other fruits (Jaganath and Crozier, 2010; Clifford, 2000; Mazza and Francis, 1995). Anthocyanins are found mainly in the skin, except for certain types of red fruit (Pandey and Rizvi, 2009; Manach et al., 2004), in which they also occur in the flesh (cherries and strawberries). The flavonoids present in leaves are completely hidden by the ubiquitous green of chlorophylls, in

contrast with the very visible flavonoids present in flower petals (Harborne and Williams, 2000).

A SHORT DISCUSSION OF THE EARLY HISTORY

The term *anthocyan* derives from the Greek *anthos*, meaning flower, and *kyanos*, meaning blue, and was introduced (Marquardt, 1835; Lee and Gould, 2002) by the German pharmacist Ludwig Clamor Marquardt (1804–1886) to designate the blue pigments present in flowers (cornflower *Centaurea cyanus* being studied originally). Nevertheless, it was shown later that not only the blue color but practically all the red, blue, and purple shades of flowers, fruits, stems, leaves, and roots are due to such pigments, and this resulted in the extension of the term to all pigments of this group (all natural flavylum salts regardless of color). However, when it became apparent that in the plant these pigments were combined with sugar and thus occurred as glucosides, the ending “in” was attached to the word (Huntress, 1928a) to emphasize this chemical classification.

The word first coined by Marquardt for these substances has been retained in the same sense to the present day. Other rival terms now obsolete, such as crythropyll, cyanophyll, and cianin, have been also used from time to time (Onslow, 1925). Note that we retain other more common uses of the root *anthos* in *anthology* (a collection of literature, i.e., a selection of poems) and *anthomania* (an extravagant fondness for flowers) (Huntress, 1928a). As a side anecdote, Marquardt accepted Fresenius (who was apprenticed to an apothecary) into his private laboratory (Szabadvary, 2008) and suggested that he publish in a book the qualitative analysis system of cations and anions that he had devised (*Anleitung zur qualitativen chemischen Analyse*; Fresenius, 1841). Fresenius dedicated the first edition of the book to Marquardt (the second appeared with a preface by Liebig). The book, a basic reference in analytical chemistry history, was translated into English, French, Italian, Dutch, Russian, Spanish, Hungarian, and Chinese.

Anthocyanins are easily soluble in water or alcohol but insoluble in other organic solvents. They were first obtained in a crystalline condition in 1903 and shown to be glucosides in 1906 (Russell, 2004). Molish discovered the important fact that anthocyanins in solution create products that crystallize well when treated with acids, and this fact was then used in the isolation and purification of these pigments. The anthocyanins are usually represented as flavylum cations, which of course must also associated with anions (Timberlake, 1980), because they were first isolated in these forms as chlorides from strongly acidic solutions.

Willstätter and Everest identified the first anthocyanin in 1913, from the blue cornflower *Centaurea cyanus*. Arthur E. Everest had taken his Ph.D. that same year at Basel under the direction of Willstätter and was then a lecturer in chemistry at University College, Reading. When first isolated by chemists, many anthocyanins were named after the colorful flowers from which they were extracted (Raghvendra et al., 2011), such as petunidin (petunia), rosinidin (rose), and peonidin (peonies).

The British biochemist Muriel Wheldale Onslow, a member of William Bateson school of genetics (Richmond, 2001, 2007; Creese, 1991; Geissman, 1949), tried in the early twentieth century to establish the linkage between the inheritance of genetic factors and the production of pigments, the anthocyanins, performing plant breeding experiments and also investigating the corresponding chemistry of flower pigments of the plants (like Erwing Bauer in Germany). J. B. Haldane used the research of Onslow to conclude that genes controlled the formation of large molecules, such as pigment molecules (Rayner-Canham and Rayner-Canham, 2002). The early history of anthocyanins to the 1970s is summarized in Table 3 (Andersen and Markham, 2006; Sakata et al., 2006; Russell, 2004; Cooper-Driver, 2001; Whiting, 2001; Jackman et al., 1987a; Scott-Moncrieff, 1981; Heines, 1972; Robinson, 1953; Mayer and Cook, 1943; Huntress, 1928a, 1928b; Onslow, 1925; Perkin and Everest, 1918).

R. W. Willstätter, working in the Kaiser Wilhelm Institute of Dahlem at Berlin, laid the foundations for effective progress in a work so masterly that it received the Nobel Prize in chemistry in 1915 (Willstätter, 1920). Through the efforts of P. Karrer (Nobel Prize in 1937) and his students at the Technische Hochschule at Zürich, the fundamental work of Willstätter was greatly extended and clarified (Karrer and Widmer, 1927a, 1927b; Karrer et al., 1927). Sir Robert Robinson (Nobel Prize in 1947) made extensive contributions to the chemistry of anthocyanidins and anthocyanins, and he synthesized the most important examples (Livingstone, 1987; Todd, 1987; Todd and Cornforth, 1976; Robinson and Robinson, 1931, 1935; Robertson and Robinson, 1926), following the publication of the classic paper by Willstätter and Everest (1913). During his five years (1923–1928) as professor of organic chemistry in Manchester he completed the synthesis of most of the anthocyanidins and made headway with synthesis of anthocyanins (Cocker, 1987). Lady Robinson found her greatest interest in the study of plant pigments because of the pleasure she found in her beautiful Oxford garden (Simonsen, 1954). The review of Blank (1947) covered the two decades since the appearance of the second edition of Onslow's monograph (1925).

The purification and identification of anthocyanins was greatly aided by the application of paper chromatographic methods, notably by Harborne and his coworkers (Harborne, 1958). The technique was devised by Gordon, Martin, and Synge (1943) and Consden, Gordon, and Martin (1944) and introduced in the anthocyanin field by Bate-Smith (1948). Martin and Synge were awarded Nobel Prizes in 1952 for their invention of partition chromatography (Tiselius, 1952). Paper chromatography revolutionized and enormously simplified investigations in plant sciences (Thompson et al., 1959). In the United States, Geissman used paper chromatography (Scott-Moncrieff, 1981) for his study of gene action in *Antirrhinum* and for the study of a large number of chalcones, aurones, and anthocyanins in order to find clues as to their biosynthesis. Research of the distribution of anthocyanins in plants was resumed with the work of Harborne (1967).

Jeffrey B. Harborne spent two years (1953–55) as a post-doctoral fellow with Professor T. S. Geissman at the University of California, working on phenolic plant pigments (Hanson, 2002). He was a pioneer in the separation and structural identification of anthocyanins (Cooper-Driver, 2001), using both spectral (1957, 1959) and chromatographic methods (1959), and developing suitable solvents for separating pigments with varying glycosylation patterns, working also in microscale (1962–1965). At that time paper chromatography opened up a whole new world, as before it had been almost impossible to separate mixtures of water-soluble plant phenolics (Grayer and Williams, 2007). Jeffrey Harborne formed with Bate-Smith and Swain an eminent triumvirate in the plant polyphenol area (Bueno, submitted; Quideau et al., 2011).

Research into anthocyanin pigments has been responsible for several important breakthroughs in molecular biology, including the isolation and identification of a plant transcription factor gene for the first time, the isolation of one of the first cDNAs for a plant cytochrome P450 enzyme, the first demonstration of antisense RNA technology in a transgenic plant, and the first description of transgene co-suppression (Mlodzinska, 2009; Davies, 2004).

ANTHOCYANIN ANALYSIS: A BRIEF SURVEY

Many difficulties are associated with anthocyanin analysis because suitable, simple, and pure standards for anthocyanin compounds are not easily available and even if they were, they may not be very similar to the complex type of pigment that might be present in the extract. The analysis of anthocyanins is also complex (Ignat et al., 2011) as a result of their ability to undergo structural transformation and complexation reactions. In addition, they are difficult to measure in a way independent of other flavonoids, as they have similar structural and reactivity characteristics and they also react with the usual reagents used for phenolic analysis such as Folin-Ciocalteu and vanillin (Biesaga and Pyszynska, 2009; Welch et al., 2008; Rivas-Gonzalo, 2003; Tura and Robards, 2002; Antolovich et al., 2000). The large number of chemical groups that may bind to the flavylium molecule has contributed to a large variation in structure, also making the analysis of anthocyanins difficult (Corradini et al., 2011; Jackman et al., 1987a).

Although spectrophotometric methods based on color are possible, such as differential methods (relying on differences in color produced by pH change, requiring conversion of all chemical forms to the red flavylium cation by adding normal acid), the best method requires prior separation and then measuring the individual compounds by chromatography (Timberlake, 1980). Isolation of anthocyanins from the sample matrix is generally a prerequisite to any comprehensive analysis scheme, although enhanced selectivity in the subsequent quantitation step may reduce the need for sample manipulation (Truong et al., 2010; Liu et al., 2008).

The maximum of absorption at 520 nm in the visible region is the most common wavelength used in the spectrophotometric

TABLE 3
Early anthocyanin history

Year	Achievement	Author
1664	“Experiments and Consideration Touching Colours.” Indicator character of syrup of violets (tincture of the flowers)	Boyle
1682	Indicator character of anthocyanins in “The Anatomy of Plants”	Nehemiah Grew
1835	Introduction of the term <i>anthocynin</i> . Examination of the color changes that take place when extracts from flowers are heated with acids and alkalis	Marquart
1836	Two types of colored chromogens in flowers: erythrogen, which turns red by the addition of acid, and xanthogen, giving rise to yellow pigments with alkalis	Hope
1837	Attempt to isolate certain of the pigments in some berries and in autumn leaves by precipitation of lead salts and regeneration with hydrogen sulfide; process used successfully by Grafe (1906) and Willstätter (1916) in some cases. More than one anthocyanin pigment existed	Berzelius
1849–1850	Attempt to prepare cornflower coloring matter by repeated precipitation of its aqueous solution with alcohol (the first stage in the method later developed by Willstätter and Everest, which isolated the pure cornflower pigment)	Morot
1854	Cyanin, the 7-O-glycoside of cyanidin (present in blue cornflower), isolated in crude form. Belief that the red and purple flower pigments were all merely different forms of the same blue anthocyanin, i.e., cyanin	Fremy and Cloez
1854	Examination of the decoloration of anthocyanins in solution; by adding acid the color reappears, concluding that no reaction of reduction as suggested by Fremy and Cloez occurred	Filhol
1858	Attempt to separate pigments from various sources (red wine, bilberry) by the Berzelius method or a slight modification thereof (use of ethereal hydrogen chloride to decompose the lead salt)	Glenard Heise and Glan (1888–1894)
1895	Classification of the anthocyan pigments, hereby completely dispelling the one pigment idea that had so often been brought forward.	Weigert
1899	Conclusion that a considerable number of different anthocyan pigments existed	Overton
1903	Preparation for the first time of an anthocyan in a crystalline condition, from the scarlet pelargonium (geranium) and verbena flowers (but his record of their composition and properties was quite erroneous)	Griffiths
1905	Description of crystalline anthocyanin in situ and readily followed instructions for the preparation of substances outside the plant, although on a small scale. Beautiful, clear, and descriptive publication that spurred research in the area. Decided in favor of their being glucosides	Molish
1906–1911	Repetition of Molish experiments on a large scale. Results of some careful analysis of the pigment of the hollyhock (<i>Althea rosea</i>) in 1906, followed by further analysis of the pigment of scarlet <i>Pelargonium</i> flowers in 1911 (no less than 10 g of crystals in this case was obtained)	Grafe
1913	Publication of the classic paper. Establishment of the main lines in subsequent investigation. Parent name anthocyanin for the glycosides and anthocyanidin for the aglycone. First structural enquiry; it was shown to be a glucoside of a pentahydroxy-2-phenyl-benzopyrilium salt	Willstätter and Everest
1914	Publication of “The Vegetable Chameleon” (because of the well-known reversible discoloration of the pigments in acid and alkalis), with a detailed account of the extraction and properties of colored substances in the flowers, fruits, and leaves of plants	Tswett
1914	Pelargonidine, the first anthocyanidine prepared, reported at a meeting of the Prussian Academy of Sciences on July 30	Willstätter
1920	Cyanidine, second anthocyanidin prepared, reported to the Baeyer Academy of Sciences and the Chemical Society at Munich	Willstätter

(Continued on next page)

TABLE 3
Early anthocyanin history (*Continued*)

Year	Achievement	Author
1920	Article on the anthocyanins of the young leaves of wine grape, coining the term leucoanthocyanin, but failed to mention the previous work of Tswett, published six years before	Rosenhein
1920	New synthetic methods condensing o-hydroxybenzaldehydes with ω -hydroxyacetophenone were explored	Robinson and Robinson
1925	Publication of the second edition of the monograph <i>The anthocyanin pigments of plants</i>	Wheldale Onslow
1926–1939	Collaboration, with the encouragement of Haldane, with geneticists (John Innes Horticultural Institute) and chemists (Sir Robert Robinson and Gerturde Robinson Oxford team) makes major headway in illuminating the chemical genetics of flower pigmentation	Scott-Moncrieft
1927	Research concerning the structure of some anthocyanidins and anthocyanins	Karrer et al.
1931–1932	Copigmentation, the bluing of anthocyanins by flavones and related substances is suggested.	Robinson, Lawrence
1932	Complete synthesis of several anthocyanins, including cyanin chloride, is achieved	Robinson and Robinson
1936	Chromatography of anthocyanins using aqueous solutions on solid adsorbents, alumina and gypsum (in 1934 the Robinsons used cellulose to study the behavior of plant pigments in water-amyl alcohol)	Karrer and Strong; Karrer and Weber
1939	Elegant explanation of how the resonating flavylium structure accounts for the pigment depth and intensity in color	Pauling
1947	Publication of a review on anthocyanin pigments of plants	Blank
1948	Paper chromatography of anthocyanins and related substances in plant extracts	Bate-Smith
1950s	The availability of radioisotope tracer molecules fueled major new progress toward elucidating the flavonoid biosynthetic pathway	Melvin Calvin
1958	Review on paper chromatography on anthocyanins, showing in this way later that flavonoids and anthocyanins were present as complex mixtures of glycosides	Harborne
1960	The term leucoanthocyanin was an unfortunate one, as the name was originally given by Rosenheim on an erroneous assumption that the compounds were glucosides of a pseudo base	Swain
1967	Research on the distribution of anthocyanins in plants is resumed with the work of Harborne	Harborne
1968–1971	Series of papers dealing with quantitative methods for anthocyanins	Fuleki and Francis
1970s	It was established firmly that both anthocyanins and synthetic flavylium salts families of compounds undergo multiple structural transformations in aqueous solution, following the same basic mechanism	Brouillard and Dubois McClelland

measurement of total anthocyanins, a rapid and cost-effective determination carried out on raw materials with no sample hydrolysis (Wroldstad et al., 2002). This method is often adopted in the food industry to perform a rapid analysis of the total anthocyanin content in foods and beverages. Nevertheless, the spectrophotometric method does not provide any specificity as far as the molecular intensity of the anthocyanin present in the material is concerned. Spectrophotometric methods provide very useful qualitative and quantitative information; actually, spectroscopy is the main technique used for the quantitation of different classes of polyphenols due to its simplicity and low cost. Giusti and Wroldstad (2001, 2003, 2005) published excel-

lent reviews of the main methods used in the characterization and quantification of anthocyanins by UV-visible spectroscopy.

Anthocyanins have been analyzed by a number of separation techniques including paper, thin-layer, and column chromatographic methods, and capillary electrophoresis (Corradini et al., 2011; Ignat et al., 2011; Coté et al., 2010; Unger, 2009; Giusti and Jing, 2008; Welch et al., 2008; Mazza et al., 2004; Kong et al., 2003). Nowadays high-performance liquid chromatography (HPLC) is the method of choice because it is fast, sensitive, and quantitative, and almost all of the available HPLC methods for the determination of anthocyanins and anthocyanidins are based on reverse-phase columns. In combination with photodiode

array and mass spectrometric techniques, structural characterization of the pigments is readily achievable (Steimer and Sjöber, 2011; Wallace, 2010; Barnes et al., 2009; Heier et al., 2002).

Prior to about 2000, however, there were very few published reports on the use of liquid chromatography-mass spectrometry (LC-MS) for analysis and characterization of anthocyanins (Pascual-Teresa et al., 2002). MS techniques for anthocyanin analysis such as fast atom bombardment (FAB), matrix-assisted laser desorption ionization (MALDI), and time-of-flight (TOF) have proven to be very useful for structural elucidation, but they are not used routinely (Scotter, 2011b; Patel et al., 2010). The use of nuclear magnetic resonance (NMR) is also vital for the structural elucidation of anthocyanins (Andersen and Fossen, 2005; Andersen and Francis, 2004). The application of pulse technique and its associated Fourier transform have made NMR the most important tool for complete structure elucidation (Andersen and Fossen, 2003, 2005). Recent techniques in mass spectrometry and two-dimensional nuclear magnetic resonance spectroscopy (2-D NMR) have become vital for the determination of many anthocyanin linkage positions and aliphatic acyl groups (Andersen and Jordheim, 2010; Andersen, 2008; Jordheim, 2007). While other more sophisticated techniques such as MS (Flamini and Traldi, 2010; Hayasaka et al., 2005) and NMR spectroscopy are becoming more popular as research tools, their relatively high capital costs are still an impediment to their routine use in enforcement laboratories, a fact that must be taken into consideration (Scotter, 2010, 2011b).

It is necessary to point out that the analysis of plant and food phenolics comprises not only rather simple compounds that are readily extracted and analyzed by HPLC, but also polymers (Quideau et al., 2011), including reaction (e.g., oxidation) products, that can prove difficult to extract, to elute from the chromatographic stationary phases, and/or to characterize (Fanali et al., 2011; Ivanova et al., 2011; Kusznierevicz et al., 2011; Andersen and Jordheim, 2010; Tarascou et al., 2010). A variety of chemical and physical parameters, such as oxygen, high temperature, and most pH values, exert an influence over anthocyanin stability (Cavalcanti et al., 2011). The various anthocyanins have similar structures and occur often in complex mixtures, which make them rather difficult to isolate (Andersen and Jordheim, 2010). Improvements in the methods and instrumentation used for separation and structure elucidation of anthocyanins have made it easier to use smaller quantities of material for analysis, and to achieve results at increasing levels of precision (Mullen et al., 2010; Truong et al., 2010; Castañeda-Ovando et al., 2009; Rijke, 2005; Andersen, 2008).

Recent research on the extraction and analysis of anthocyanins will be a further subject of the present review.

ANTHOCYANIN PROFILES AS FINGERPRINTS

Total anthocyanin content among different plants or even cultivars in the same plant varies considerably, being affected by genes, light, temperature, and agronomic factors, i.e., growing conditions and maturity (Jing and Giusti, 2011; Amarowicz

et al., 2009; Cermak et al., 2009; Stein et al., 2002). In general, the level of anthocyanins in fruits is much higher than in vegetables (Horbowicz et al., 2008). Each different plant and plant part has a unique profile of anthocyanins (Hartmanova et al., 2010; Nakajima et al., 2004). For this reason the anthocyanin composition has been used as a botanical tool for taxonomical classification (Jing and Giusti, 2011).

Since the anthocyanin composition profile of many fruits and vegetables is distinctive, it can be used to distinguish (a fingerprint to classify) among species and/or varieties, to establish the geographic origin of, for example, a wine, to identify the variety of grape used in its manufacture, or in the control of the quality and authenticity of related food (Bordonaba and Terry, 2008; Giusti and Jing, 2008; Giusti et al., 1999), i.e., to detect the adulteration of fruit juices (Tian et al., 2005; Durst and Wrolstad, 2001). Considerable attention is paid to the application of anthocyanin analysis (often together with advanced statistical methods) in classification of wine (Hartmanova et al., 2010). Anthocyanins, which confer the characteristic color to red wine, can be used as markers to classify wines according to the grape variety (Vergara et al., 2010; Birse, 2007; Zimman et al., 2004), although this requires a complex separation with very high chromatographic efficiency, especially when dealing with aged red wines, because of the formation of pyranoanthocyanins (Rentzsch et al., 2007; Casassa and Catania, 2006). This family of compounds is formed through the reaction of anthocyanins with small molecules.

Pyroanthocyanins have been known for only two decades, i.e., that pyroanthocyanins or Visitin A type pigments are derived from reactions of anthocyanins at the C-4 position with pyruvic acid and other compounds to form cyclo addition products (Oliveira et al., 2009; Wrolstad et al., 2005), followed by aromatization through oxidation. The presence of this four ring increases stability against the nucleophilic attack water of bisulfite (Jordheim, 2007; Leopoldini et al., 2010). Pyranoanthocyanins are thought to be responsible for the orange hues (hypsochromic shift) observed in red wine during maturation (Oliveira et al., 2011; Birse, 2007; Rein, 2005).

CHEMICAL STRUCTURE OF ANTHOCYANINS

Anthocyanins are generally found in the form of glycosides. The aglycones are rarely found in plants other than as artifacts; the 3-deoxy forms the main exceptions appearing in, for example, red-skinned bananas, sorghum, and black tea. Glucose (the most common), galactose, rhamnose, arabinose, xylose, and glucuronic acid are the sugars most commonly encountered, usually as 3-glycosides or 3,5-diglycosides (Pereira et al., 2009; Freitas and Mateus, 2006); 3-diglycosides and 3-diglycoside-5-monoglycosides are less common. The four main biosides encountered are rutinose, sambubiose, lathyrose, and sophorose. In terms of glycoside distribution 3-glycosides occur approximately two and half times as often as 3,5-diglycosides, the most ubiquitous anthocyanin being cyaniding-3-glucoside (Kong et al., 2003). The de-glycosylated

or aglycone forms of anthocyanins are known as anthocyanidins, both parent names being introduced by Willstätter and Everest (1913) in their classic paper on the field (Livingstone, 1987; Robinson, 1953).

Differences among the anthocyanins lie in the number and position of hydroxyl and methoxyl groups, the nature and number of sugars attached to the molecule, the position of this attachment, and the degree and nature of esterification with aliphatic or aromatic acids attached to sugars in the molecule (McGhie and Walton, 2007; Andersen and Jordheim, 2006; Wu et al., 2006; Kong et al., 2003). Structures of the main anthocyanidins are shown in Figure 1 and Table 2, with cyanidin being the most common. The differences in chemical structures of these six common anthocyanidins occur at the 3' and 5' positions of the B ring. Most new anthocyanins discovered in the past 15 years have been based on the six common anthocyanidins encountered in higher plants. With regard to structural characterization, information may be obtained (Liu et al., 2008) on: (i) the structure of aglycone; (ii) the type of carbohydrates or other substituents present; (iii) the sequence of the glycan part; (iv) interglycosidic linkages; and (v) attachment points of the substituents to the aglycone. Since each anthocyanidin may be glycosylated and acylated by different sugars and acids, at different positions, the number of anthocyanins is 15–20 times greater than the number of anthocyanidins (Mazza and Brouillard, 1987). All anthocyanins and anthocyanidins appearing in nature show an unclosed hydroxyl group in the 4' position.

The sugar moieties of anthocyanins are normally connected to the anthocyanins through O-linkages (Figure 2). Glycosylation position effects in many cases are tied to the effect of subsequent O-acylation. O-acylation, a prevalent modification, is the addition of acid (acyl) group to the sugar residues of the anthocyanin, and anthocyanins may have single or multiple acyl groups (Gao and Mazza, 1994). It is particularly an important modification because it plays a significant role in the formation of pigment-stabilizing tertiary structures. More than 60% of the reported anthocyanins contain one or more acyl moieties, and the colors and functions of these pigments in plants are highly affected by the nature, number, and linkage positions of the acyl groups (Andersen and Jordheim, 2010; Andersen, 2008).

Anthocyanins are all amphoteric forming salts with either acids or bases. In addition, anthocyanins occur in plants as salts (indicated by the positive charges on the heterocyclic ring), and their color in plant cells depends mainly upon their mode of combination. The conjugated bonds in their structures (light conjugated double bonds carrying a positive charge), which absorb light at about 500 nm, are the basis for the bright red, blue, and purple color of fruits and vegetables, as well as the autumn foliage of deciduous trees (Wang and Stoner, 2008). Every color except green has been observed (either natural or synthetic), depending on aspects such as kind of substituents present in the B-ring, the local pH, the state of aggregation of the anthocyanins, complexation by organic molecules, or, as in the case of blue color, complexation by metal cations

(Quina et al., 2009). Note that chlorophylls are virtually the only green pigments found in plant food. Degradation products of chlorophyll can also contribute green-brown or brown color (Palmer, 1984).

The most widespread anthocyanins in fruits are also glycosylates in the 3-OH position (3-*O*-monoglycosides), providing thermal stability, and less extensively, in both 3-OH and 5-OH (3,5-*O*-diglycosides) positions, and possible acylation of sugar residues with organic acids. Although not common in berries, some anthocyanins may be acylated. Acylated anthocyanins have been reported in boysenberries and marionberries (Jing and Giusti, 2011). Acylation involving hydroxycinnamic acids has been reported to be an advanced character because it occurs mainly in highly evolved families (Harborne, 1986). Natural anthocyanins in which the 7' hydroxy group (a less common site of glycosylation) is glycosylated or replaced by a methoxy group are quite rare (Scotter, 2011a; Quina et al., 2009). In rare cases glycosylation has also been reported on the B ring.

The most frequent acylating agents are caffeic, ferulic, sinapic, and *p*-coumaric acids and 3,5-dihydroxycinnamic acids (hydroxycinnamic acids), *p*-hydroxybenzoic acid, and gallic acids (hydroxybenzoic acids), although aliphatic acids such as acetic, malic, malonic, oxalic, succinic, and tartaric acids may also occur. Common cinnamic and aliphatic acids acylated with sugar moieties on anthocyanins are depicted in Figures 3 and 4, respectively (He, 2004). Malonic acid, which is identified in 25% of the anthocyanins, is the most frequently acyl moiety of anthocyanins. Up to three acylating acids may be present simultaneously (Scotter, 2011a; Galvano, n.d.). These anthocyanins have some compounds designated as polyglycosides. In some cases very complex anthocyanins may be formed, with multiple glycosyl and acyl groups.

The nature of the individual sugars has little general effect, but their positions in the molecule can have a profound influence on its reactivity (Jordheim, 2007). The possible number of individual pigments became apparent when the possibilities above were considered (Andersen, 2008; Castañeda-Ovando et al., 2009). Other flavonoid glycosides may be attached to anthocyanins. These structures are formed through the bonding via ester linkages of an anthocyanin and a glycoside of a different flavonoid type (e.g., flavones) to the carboxyl functions of an organic diacid (e.g., malonic, succinic). Such structures have been found in flowers and orchids (Gould and Lister, 2006; Davies, 2004).

More than 30 monomeric anthocyanidins have been properly identified. Most of them are based on cyanidin (31%), delphinidin (22%), or pelargonidin (18%) (Tsao, 2010; Andersen and Jordheim, 2006). The other common anthocyanidins (peonidin, malvidin, and petunidin), which contains methoxy group(s) on their B-ring (Figure 1), represent together 21% of the isolated anthocyanins. One new methylated anthocyanidin, 7-*O*-methylcyanidin, five new desoxyanthocyanidins, and a novel type of anthocyanidin called pyroanthocyanidin have been reported (Pereira et al., 2009; Andersen, 2008) in the past

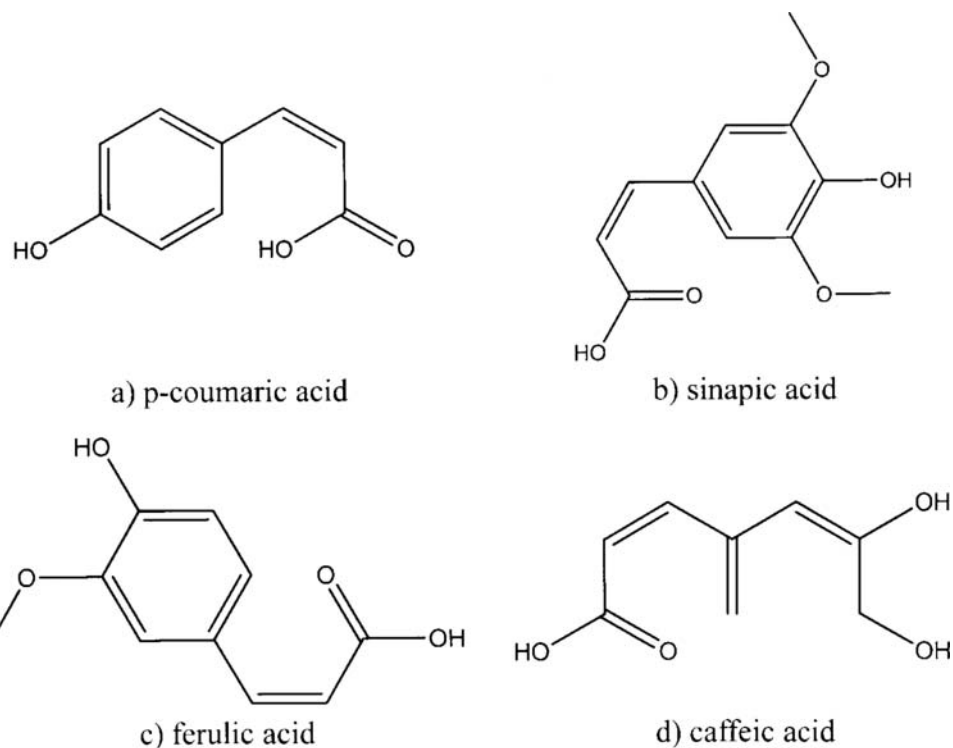


FIG. 3. Common cinnamic acids acylated with sugar moieties on anthocyanins (He, 2004).

decade. In spite of the structural diversity of anthocyanins, the three non-methylated anthocyanidins, cyanidin (Cy), delphinidin (Dp), and pelargonidin (Pg), are the most widespread in nature, being present in 80% of pigmented leaves, 69% of fruits, and 50% of flowers (Ghosh and Konishi, 2007; Kong et al., 2003).

The chemical structure of the anthocyanin influences its chemical properties and determines the stability, color, aqueous equilibrium, copigmentation effect, antioxidant reactivity, and potential biological activity (Kong et al., 2003; Prior and Wu, 2006). Many factors, such as pH, temperature, light, presence of other phenolic compounds, enzymes, metal ions, sugars,

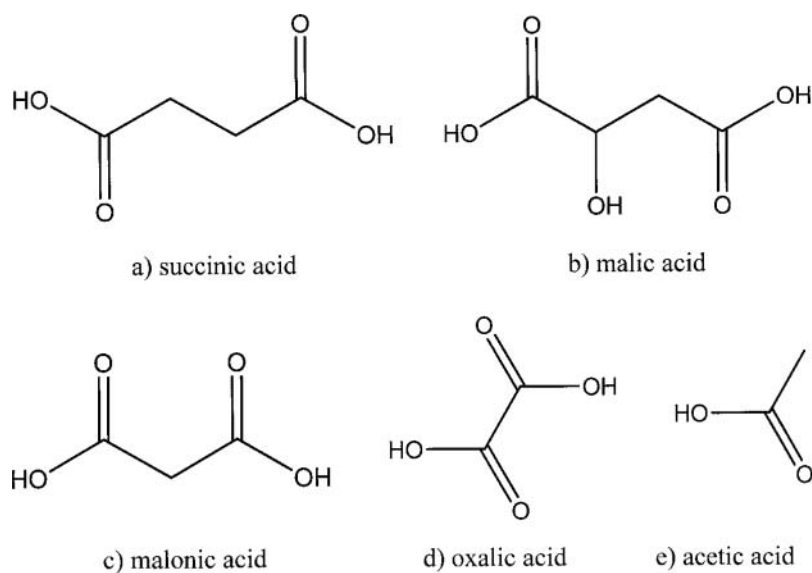


FIG. 4. Common aliphatic acids acylated with sugar moieties on anthocyanins (He, 2004).

ascorbic acid, and oxygen have impact on the stability of anthocyanins (Cavalcanti et al., 2011; Horbowicz et al., 2008). It is not surprising that the chemical structure of anthocyanins will affect their biological properties.

The number of hydroxyl groups and type of sugar moieties, as well as the acylated groups, obviously can influence the polarity, size, and spatial conformations of individual compounds (Bjoroy et al., 2009; Guzman et al., 2009; Estevez and Mosquera, 2008; Sakata et al., 2006) and consequently have a certain impact on bioavailability. Acidity constants in general (Asuero and Michalowski, 2011; Jabbari and Gharib, 2011; Asuero, 1989, 2007; Asuero et al. 1986a, 1986b; Herrador et al., 1986) are of vital importance in the analysis of bioactive compounds as well as in the interpretation of their mechanism of action; for example, drug transport potentials through biological membranes (Mielczarek, 2005) or antiradical properties (Musialik et al., 2009) are dependent on the acid-base properties shown by the flavonoids in question, i.e., anthocyanidins (Estevez et al., 2010; Guzman et al., 2009; Estevez and Mosquera, 2008). However, anthocyanin structure/function relationships are not well understood. In addition, impact of anthocyanin structures on their biological activities is dependent on experimental models, which complicate the enigma of the structure-bioactivity relationship (Jing and Giusti, 2011).

ACID-BASE EQUILIBRIA: A PRIMER

As indicated by Melo et al. (2007): "It is worth noting that the crucial and fundamental paper by Willstätter and Mallinson

(1915a,b) on the causes of color in flowers and fruits tends to be forgotten, and is seldom cited as such." Willstätter made the striking observation that the same pigment can give rise to different colors (Goto and Kondo, 1991) and attributed the variety of flower colors to different pH values of solution. A point of note is that anthocyanins are readily distinguished from other flavonoids as they undergo rearrangement in response to pH (Del Rio et al., 2010), and they have ability for electron delocalization and forming resonating structures following changes in pH, features that do not occur in other popular antioxidants (Zafra-Stone et al., 2007; Prior and Wu, 2006). Anthocyanins, like flavylium salts analogs (Gomes, 2009; Gomes et al., 2009), are involved in a complex network of chemical reactions, in which the different forms can be reversibly interconverted by changing the pH (Figure 5). This intrinsic complexity is even increased in microheterogeneous media such as micelles, which have been shown to significantly modify the position and dynamics of the equilibria involved in anthocyanin chemistry (Lima et al., 2002).

Anthocyanins exist in solution as various structural forms in equilibrium through hydration, proton transfer, and tautomerization reactions, and, depending on the particular structure, pH and temperature (Freitas and Mateus, 2006; Mazza et al., 2000); the relative amounts of each equilibrium form vary (Freitas et al., 2011; Es-Safi et al., 2008; Asenstorfer et al., 2006). The color of an anthocyanin solution is determined by the proportions of the different anthocyanin forms, namely, red flavylium cation (AH^+), violet quinoidal base (**A**), pseudobase or carbinol (colorless water adduct) (**B**), and yellow chalcones (**C**) (Ibrahim et al., 2011; Dougall and Baker, 2008). Interconversion between

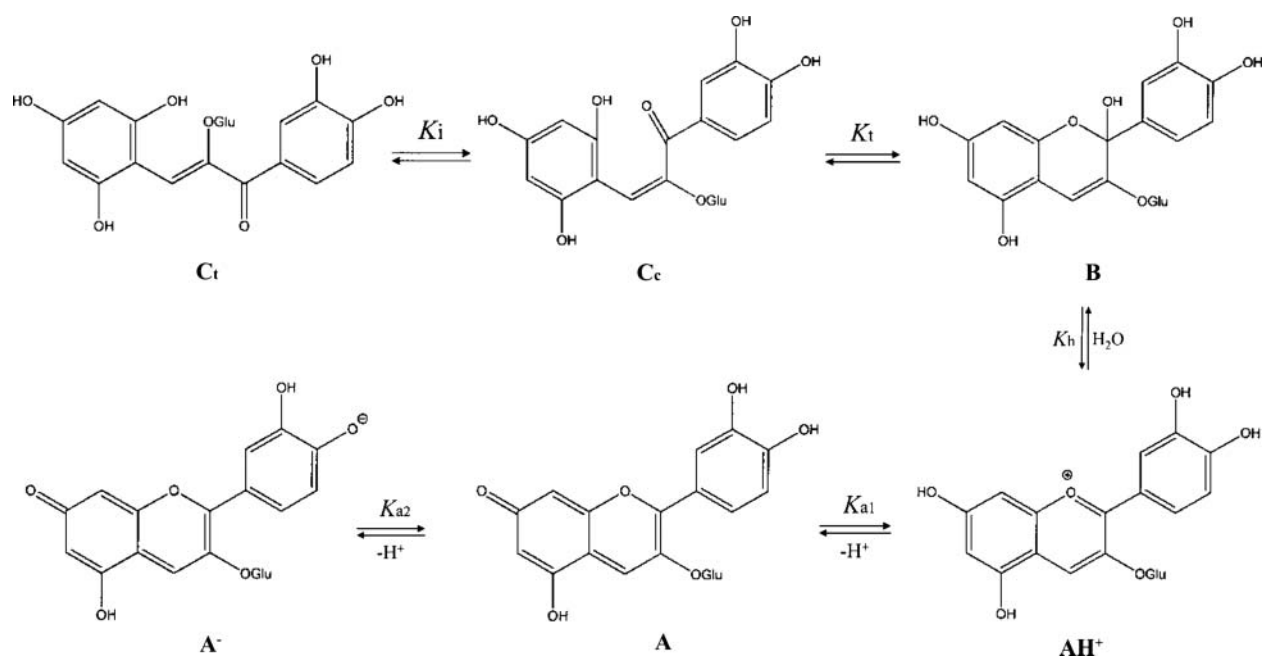
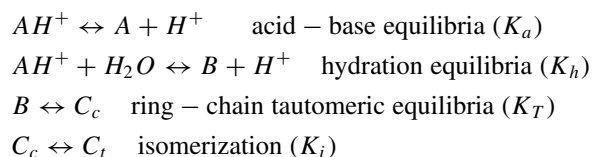


FIG. 5. Structural transformations of cyanidin-3-O-glucoside in strongly acid to alkaline solutions.

these four structures takes place according to:



Previous work carried out by Brouillard (1983) is the basis of our current understanding of the pH-induced changes of anthocyanins, in particular, a crucial point of the kinetic process: at moderately acidic pH values **B** is formed from hydration of **AH**⁺ and not from **A**. At pH 1–3 the flavylium cation is red colored, at pH 5 the resultant (nucleophilic attack of water) carbinol pseudobase (**B**) is colorless, and at pH 7–8 the blue purple quinoidal base (**A**) is formed. The ring-opening of the hemiketal B ring leads to cis-chalcone **Cc**. Finally, the trans-chalcone **Ct** is formed from its cis isomer. In an alkaline medium the anionic quinoidal base (**A**[−]) is formed. Vacuolar pH is generally regulated by vacuolar ATPase and pyrophosphate. Nature has developed strategies for stabilizing the red color of anthocyanins at pH values around 5, i.e., the pH of plant cell vacuoles in which anthocyanins are located in vivo (Mazza et al., 2000).

Various forms—including the colorless hydroxyl-adducts known as carbinol bases, pseudobases, or hemiketals—have been examined using pH-jump methods, UV-visible and fluorescence spectroscopy, and NMR spectroscopy. Using traditional methods obviously makes it very difficult to estimate simultaneously the number, concentration, and pure spectra profile of so many species present in such a complex mixture system without spectral resolution (Marco et al., 2011). Multivariate curve resolution (de Joan and Tauler, 2006) allows to investigation of the kinetic (photo) degradation of anthocyanins at different pH values, with and without radiation exposure (up to nine different species were detected and resolved by this method).

While the structural transformations of anthocyanins are well known, few results have been reported on the physicochemical properties of anthocyanin-derived pigments (Quijada-Morin et al., 2010). The intrinsic pH-dependent set of chemical reactions involving synthetic flavylium compounds has been confirmed to be basically identical to those of natural anthocyanins in acidic and neutral mediums. Substituent effects on the position of hydration, tautomerization, and chalcone isomerization equilibria of flavylium salts can be correlated by linear free energy relationships (LRERs) employing Hammett parameters (Freitas et al., 2011). Based on these relationships, it is possible to predict values of the apparent pK_a of flavylium ions that were not included in the original data set, as well as those of several naturally occurring anthocyanins.

Systematic investigations of photochemical and thermal reactions carried out with flavylium compounds reveal that these examples of multistate/multifunctional chemical systems may be used for information processing at the molecular level (Pina and Branco, 2011; Pina et al., 2011) according to principles similar to those that govern information transfer in living organ-

isms, i.e., they can behave as optical memories and logic gate systems: a write-read-erase molecular switch (Pina et al., 2011; Gomes et al., 2010; Pina et al., 2003). By using natural dyes as wide band-gap semiconductors in dye-sensitized solar cells, a successful conversion of visible light into electricity is possible. Anthocyanin plant extracts may be used as TiO₂ dye sensitizers (Polo et al., 2006).

COPIGMENTATION

Willstätter and Zollinger (1916) observed long ago the intensification of color on the addition of tannin to acidic oenin (malvidin-3-glucoside) solutions, the major pigment that they had isolated from Alicante grapes, resulting in a much bluer color. They also observed that the effect was not found in cyanin (cyanidin 3,5-diglucoside) solutions. Similar observations on the resultant additive complexes were made by others (i.e., Lawrence, 1932; Shibata et al., 1919), but the physicochemical feature of such phenomenon was initially described and investigated in detail in the pioneering works of Robinson and Robinson and was designated copigmentation (Robinson and Robinson, 1931; Robinson, 1933; Geissman, 1941), i.e., intermolecular stacking (formation of complexes and stabilization) with other flavonoids or by self-association, ranking almost 30 different cofactors by their ability to provide a blue shade to acidic oenin solutions (Boulton, 2001). By suitable combination of anthocyanins and copigments at appropriate concentrations and pH levels, Asen et al. (1972) were able to reproduce the spectra of flower petals.

Copigmentation is almost always a variation towards blueness and is induced by the presence of substances that are themselves colorless or only slightly colored (Lambert et al., 2011; Yawadio and Morita, 2007). Intramolecular copigmentation is due to the acylation of molecules, being more effective than intermolecular copigmentation. It has been suggested that in acylated anthocyanins the acyl groups interact with the basic anthocyanin structure, thus avoiding the formation of the hydrated species. The basic role of copigments is to protect the colored flavylium cation from the nucleophilic attack of the water molecule (Quina et al., 2009). The copigmentation complexes are easily disrupted by dilution returning anthocyanins to the dependent pH equilibria between their structural forms. This is the basis for characterizing the color due to copigmentation (González-Manzano et al., 2008). Copigmentation of flavonoids other than anthocyanins is also possible, but it is either a rare or an understudied phenomenon (Davies, 2004).

Cyanidin and delphinidin with this 3'-4'-dihydroxy group readily chelates metal ions with bathochromic shifts in the visible spectra (Smyk et al., 2008). As a result, anthocyanins with the ortho-dihydroxyl groups have the potential to scavenge hydroxyl radicals through the inhibition of ·OH generation by chelating iron, and to prevent iron-induced lipid peroxidation. The ortho-dihydroxyl group also helps to form anthocyanin-metal-copigment complexes at physiological pH ranges with various organic compounds such as ascorbic and partially through this

mechanism to spare Vitamin C (He, 2004). The methoxy group ortho in relation to the position 4' on the B ring in malvidin lowers the ability to form complexes with metal ions. Metal ions such as Al^{3+} and Fe^{3+} play a critical role in the generation of blue flowers in hydrangeas and tulips, respectively (Tanaka et al., 2010; Schreiber et al., 2010; Tanaka et al., 2008). The most interesting molecular complexes of anthocyanins are the metal-anthocyanins (Yoshida et al., 2009; Goto and Kondo, 1991).

In certain products, such as matured red wine and ports, chemical and enzymatic transformations occur, and an increasing number of anthocyanin-derived polyphenols are being found (Crozier et al., 2009; Birse, 2007; Zimman et al., 2004). Two groups of flavonoids, the anthocyanins and flavonols, are particularly important to the quality of red wines (Cheynier et al., 2006). Flavonols exist as catechin monomers and as oligomers and polymers, also called condensed tannins or proanthocyanidins (Bueno, submitted).

The most important red wine pigments are those derived from the parent anthocyanins, malvidin-3-glucoside being the most abundant in *Vitis vinifera*. These anthocyanin-like wine pigments include a variety of pyranoanthocyanin pigments, visitin A and B, vinyl-like pigments, and a large but transient group of ethyl-linked anthocyanin-flavanol pigments. All of these pigments share similar characteristics of the parent anthocyanin in that they participate in pH-dependent acid-base (protonation-deprotonation) equilibria and are subject to attack (to different extents) by water to form colorless hydrated species (Oliveira et al., 2011).

COLOR OF ANTHOCYANINS

Flavonoids and their colored class, anthocyanins, contribute to a wide range of colors: pale yellow, orange, red, magenta, violet, and blue (Andersen and Jordheim, 2009). Carotenoids are ubiquitously distributed in plants as essential components of photosynthesis and confer a yellow or red color on flowers when they are present (Tanaka and Ohmiya, 2008; Młodzinska, 2009). Betalains also result in yellow and red color, but only the families of Carophyllales (except for Carophyllaceae and Molluginaceae) produce betalains. To date, no plants producing both anthocyanins and betalains have been discovered (Tanaka et al., 2010; Stintzing and Carle, 2004). Anthocyanins, carotenoids, and other pigments contribute to the UV patterns that are visible to insects and serve to signal flowers that are attractive to pollinators (Ellestad, 2006; Mortensen, 2006).

Edible berries and selected fruits are rich sources of both anthocyanins and flavonol glycosides, which are responsible for the red, violet, purple, and blue color of the fruits (Ellestad, 2006). Berries are popularly consumed in North America in both fresh or frozen forms and in a variety of processed foods as yogurts, beverages, jams, canned fruits, and jellies (Seeram, 2008). Berry extracts are being commercialized as nutraceuticals and as dietary supplements to meet consumer demands (Jing and Giusti, 2011). Anthocyanins extracted from grape skins during wine making are also the main compounds responsible for

the color of young red wines (Oberholster, 2008; Birse, 2007; Zimman et al., 2004; Lapidot et al., 1999; Mazza and Francis, 1995). Food content of anthocyanins is generally proportional to color intensity and reaches values up to 2–4 g/kg fresh weight in blackberries. These values increase as the fruit ripens (Pandey and Rizvi, 2009).

Color differences between anthocyanins are largely determined by the structure, pH, temperature, type of solvent, and the presence of copigments. Their color greatly depends on the number of hydroxyl groups on the B ring; the larger the number of groups, the bluer the color (Tanaka et al., 2008). Aromatic acylation causes a blue shift and stabilizes anthocyanins. Aliphatic acylation does not change the color but increases the stability and solubility (Bakowska-Barczak, 2005; Giusti and Wrolstad, 2003). Details regarding the acyl groups of anthocyanins including some chemotaxonomic considerations are treated by Honda and Saito (2002).

Substitution of the third ring of aglycone with $-OH$ and $-OMe$ groups at the 3'- or 5'- positions produces a range of changes in the electron distribution over the molecules that result in a shift (Curtright et al., 1994) from the orange color of pelargonidin (strawberries) to the deep blue color of malvidin (blueberries) (Table 1). In 1939 Linus Pauling gave an elegant explanation of how the resonating flavylium structure accounts for the pigments' depth and intensity in color (MacKinney, 1940; Pauling, 1939). Pigments of pelargonidin and cyanidin derivatives usually produce red and purple, respectively, whereas those of delphinidin show purple or blue color. Blue flower color is restricted to the more highly evolved angiosperm family (Harborne and Williams, 2000). Blue flower color development is one of the most interesting topics in anthocyanin research and has been the subject of a recent detailed review (Yoshida et al., 2009). Classically bred roses, carnations, chrysanthemums, and lilies lack delphinidin-based anthocyanins, and this is the primary reason there are no blue/violet varieties in these species (Yoshida et al., 2009). However, most delphinidin glycosides are mauve in color, and the shift to blue region usually requires the presence of a flavone copigment and occasionally one or more cations (Shiono et al., 2005).

Anthocyanin copigmentation is a way to stabilize natural colors (Cavalcanti et al., 2011; Gallan et al., 2007; Mazza and Brouillard, 1987; Markakis and Jurd, 1974). Good early reviews of anthocyanins as food colorants are the ones by Bridle and Timberlake (1997), Francis and Markakis (1989), Timberlake and Henri (1988), Jackman et al. (1987b), and Mazza and Brouillard (1987). A number of good reviews have recently published on this topic (Aberoumand, 2011; Scotter, 2011a, 2011b; Melo et al., 2009; Motohasi and Sakagami, 2009; Garzon, 2008; Mercadante and Bobbio, 2008; Bakowska-Barczak, 2005; Wrolstad et al., 2005; Wrolstad, 2004) covering the various aspects of the field.

In summary, the major factors that cause the wide range of plant color due to the presence of anthocyanin pigments include: (i) the nature and concentration of the anthocyanidin; (ii) type of

anthocyanin glycosidation and acylation; (iii) metal complexes; (iv) anthocyanidin secondary structures (equilibrium forms); and (v) the nature of copigmentation (Andersen and Jordheim, 2010). Very small differences in chemical structures of the anthocyanins were found to have a very marked effect upon a flower color (Scott-Moncrieff, 1981). Anthocyanins with different aglycones and sugar moieties may have quite different responses in terms of their bioavailability and potential health effects (Wu et al., 2006). Several *in vivo* studies suggest that the food matrix has a significant effect on the absorption and metabolism of anthocyanins (Wallace, 2011; Yang et al., 2011).

The desire to understand and identify the source of natural colors has fascinated practicing chemists and their predecessors since before the Renaissance (Andersen, 2008). In spite of this, it is only in the past two decades that there has been an increasing interest in anthocyanins, mainly due to their potential health-promoting properties and their use as natural food colorants on one hand and to their appearance in cultivars and plant mutants with new colors and shapes on the other hand. As a result, the demand for natural plant colorants is increasing as they provide an alternative to synthetic dyes such as FD&C red no. 40 (Allura red) and the banned FD&C red no. 2 (amaranth). Note that in the United States, FD&C numbers are given by the Food and Drug Administration (FDA) for synthetic food dyes that do not exist in nature that are approved for use in foods, drugs, and cosmetics (Code of Federal Regulations, 2010), while in the European Union, E numbers are used for all additives, both synthetic and natural, that are approved in food applications.

Color appearance of food products is one of the major concerns of the food industry because color is an important factor for consumers when choosing a food product (Estupiñan et al., 2011; Downham and Collins, 2000). In a certain way, color may act as a “fingerprint” of a food product, being related to its flavor and at the same time an estimate of its overall quality (Mateus and Freitas, 2009; Chattopadhyay et al., 2008; Griffiths, 2005). Color is also the first attribute to be perceived in foods and beverages and is usually positively correlated with standards of quality by the consumer. Note that there is not legal definition of a natural color; hence, several disparities in categorization arise (Scotter, 2010, 2011b). Natural colorants can be extracted from plants, animals, fungi, and microorganisms and classified by their origins or chemical structures (Aberoumand, 2011). Food colorants may be organized in three different types, depending on their source (Mateus and Freitas, 2009; Henry, 1996):

- (a) Natural colors: organic colorants derived from natural edible sources such as anthocyanins.
- (b) Nature-identical colors: manufactured by chemical synthesis so as to be identical to colorants found in nature (e.g., β -carotene and riboflavin).
- (c) Synthetic colors: colors that do not occur in nature, produced by chemical synthesis (e.g., tartrazine and carmoisine).

The European Union permits natural food color additives in foods and beverages, and anthocyanins (E 163) are one of the nine designated natural color classes. Commission Directive 2008/128/EC defines anthocyanins as colorants obtained by extraction with sulfited water, acidified water, carbon dioxide, methanol, or ethanol from natural strains of vegetables and edible fruits. Anthocyanins contain common components of the source material, namely, anthocyanin, organic acids, tannins, sugars, minerals, etc., but not necessarily in the same proportions as found in the source material (European Union, 2008). Nevertheless, in spite of it being possible to extract color from any of the above raw materials (Table 2), the most common source, for economic reasons, is grape skins, a by-product of the wine industry. However, EU legislation permits the use of sources that include the concentrated juice of black currants, strawberries, cranberries, elderberries, cherries, and red cabbage. Other less familiar sources include radishes and black carrots.

With the current trend away from synthetic colors it is to be expected that the production and use of anthocyanins as natural colorants will increase in the future. Recently, interest in using some natural colorants in foods as “multifunctional food additives” is rising due to their additional nutritional and therapeutic effects. The anthocyanin-based natural colorants, with a variety of colors, ranging from orange to blue, are water soluble and nontoxic. They are also well known as multifunctional food additives with a quenching effect against ROS, which contributes to the prevention of coronary diseases (Wada et al., 2007).

Anthocyanins are gradually being incorporated into food and beverage products as a food colorant and as a functional food or dietary supplement. Increased development of anthocyanins with enhanced stability and prolonged shelf life will increase food applications and overall consumption and thereby increase its positive role in human health (Shipp and Abdel-Aal, 2010). Anthocyanins in blue and purple corn have been used for the production of naturally colored blue tortillas (Sugiyama Chemical Institute, 1977) for two decades. Raddish and potato extracts have color characteristics very similar to those of Allura red (a red dye used in food applications) and therefore have the potential to be incorporated as food colorants (Giusti and Wrolstad, 2003). Anthocyanins are widely used as natural food colorants, for example, the cyanidin acylglucosides from red cabbage (*Brassica oleracea* var. *capitata*) and *Perilla frutescens* (Tanaka et al., 2008). The anthocyanin colorant obtained from purple corn is approved in Japan and listed in the “Existent Food Additive List” as purple corn color; it is used for coloring beverages, jellies, candies, and so on in that country (Aoki et al., 2002). Extracts of several anthocyanin-containing fruits, especially grapes, have long been used as food colorants for food or beverages having a pH of 4 or below; above this pH value anthocyanins are rapidly decolorized (Mazza and Francis, 1995); blue-grey color changes occur at higher pH values. Acidic foods include soft drinks, fruit preserves (usually fresh/frozen rather than sulfited/canned), sugar confectioneries (particularly high

boiling/pectin jellies), dairy products (acidic products such as yogurt), frozen products (water ice at about pH 3), dry mixes (acidic dessert mixes and drink powders), and alcoholic drinks (Scotter, 2011b).

Generally, natural colorants have higher coloring capacities than synthetic ones, as seen from their relative absorptivities. Natural anthocyanins have powerful coloring properties, as only small doses of anthocyanins are required to display the color desired in several food matrixes (e.g., 30–40 ppm for soft drinks and 20–60 ppm for fruit preserves). Anthocyanins are almost exclusively required to provide a red color for foodstuffs, but in some cases betalains are preferred. In recent years, several approaches have been made aiming to apply different anthocyanin extracts to food matrixes (Sidani and Makris, 2011; Yang et al., 2011; Fang and Bhandar, 2010; Mateus and Freitas, 2009).

To date no plants producing both anthocyanins and betalains have been discovered (Tanaka et al., 2010), with one exception, *Mammillaria vivipara* var. *vivipara* (Chapman et al., 2006). A molecular explanation for the observed mutual exclusion of the anthocyanins and betalains is an unresolved issue (Grotewold, 2006a). Anthocyanins and betalains that are mutually exclusive in nature were also mixed to investigate their potential for food coloring purposes (Stintzing et al., 2006). Anthocyanins have extensively investigated by horticulturists and food scientists (Wrolstad, 2004) due to their importance to the color quality of fresh and processed fruit and vegetables. However, there is still much to be learned about the effects of processing and food matrix on anthocyan stability (Cavalcanti et al., 2011; Sidani and Makris, 2011; Yang et al., 2011).

ANTHOCYANIN INTAKE

Anthocyanins were incorporated into the human diet many centuries ago (Kuskoski et al., 2002). They were components of traditional herbal medicines used by North American Indians, Europeans, and the Chinese and were habitually derived from dried leaves, fruits, berries, storage roots, and seeds (Krenn et al., 2007; Konczak and Zhang, 2004). Anthocyanins are widely ingested by humans, mainly due to consumption of red fruits (like berries and red grapes), vegetables such as red cabbage, red wines, cereals, and purple corn (Galvano et al., 2004). Accurate estimation of anthocyanin content in foods and daily intake is critical in food science, nutrition, and other related research fields.

It has been calculated that consumption of anthocyanins could exceed 200 mg/day (Robards et al., 1999). Depending on nutritional habits, the daily intake of anthocyanins for individuals has been estimated to range from several milligrams to hundreds of milligrams per person. Anthocyanins in foods are typically ingested as components of complex mixtures of flavonoid components (Raghvendra et al., 2011), their intake being increased by consuming flavonoid supplements, i.e., grape seed extract, ginkgo biloba, or pycnogenol. In the United States, average daily intake of anthocyanins has been estimated at 215 mg during the summer and 180 mg during the winter

(Clifford, 2000). These values are thought to be higher for regular red wine consumers. Indeed, despite not being the best source of anthocyanins compared to fruit berries, anthocyanins from red wine may complex with other wine components; thereby their consumption levels should be significantly higher than initially expected (Mateus and de Freitas, 2009).

However, Wu et al. (2006) estimated the mean daily intake of anthocyanins to be 12.5 mg/person in the United States; such a huge difference of total anthocyanin daily intake estimation must result from different food intake data. The influence of methodological differences in the assessment, as well as nutritional, social, and cultural differences of the investigated populations, may also explain the wide range of anthocyanin consumption estimated by different authors. Intake estimations have almost exclusively been conducted in adult populations. However, anthocyanin intake and its trends in German infants and toddlers have been studied (Drossard et al., 2011). In a survey with Italian subjects, anthocyanin daily intake was in the range 25 to 215 mg/person, depending on gender and age (and this intake is large enough to induce pharmacological effects) (Delgado-Vargas et al., 2000). The daily estimated intake of anthocyanins is high compared with the intake of other dietary flavonoids such as genistein and quercetin (estimated as 20–25 mg/d) (Wallace, 2011).

In processed foods to which foods containing anthocyanins were added as raw materials, such as canned food, bread, cereals, and baby foods, anthocyanins generally could not be detected. Anthocyanins were barely detected in baby foods prepared from fruits high in anthocyanins such as blueberries. However, Chun et al. (2007) estimated the mean daily total flavonoid intake to be 189.7 mg/person, of which about 1.6% was anthocyanidins. It has been suggested that a combination of different redox-active components (i.e., antioxidants or reductants) may be needed for proper protection against oxidative damage, cooperating in an integrated manner as in plant cells (Halvorsen et al., 2006). A glass of red wine provides around 115 mg of polyphenols, contributing towards a total intake of phenolic compounds of 1171 mg/person/day (Saura-Calixto and Goñi, 2006; Scalbert et al., 2005). The scientific aspects of the Mediterranean diet have been the subject of study (Martínez-González et al., 2009; Giugliano and Esposito, 2008; Serra-Majem et al., 2006; Covas et al., 2002).

Anthocyanins are found in many foods because they are responsible for the cyan and red colors of several fruits such as red berries (grapes, elderberries, cranberries, raspberries, black currants, mulberries, etc.), cherries, pomegranates, and plums, and also of eggplants, beetroots, and pink potatoes (Valls et al., 2009; Giusti and Jing, 2008; Andersen and Markham, 2006). All these fruits are regularly consumed in diets, and consequently they have also been used for a long time by the food industry to produce juices, soft drinks, alcoholic beverages, and pies, among other products (Krenn et al., 2007). This traditional use has exploited the organoleptic characteristics of anthocyanins to create colorful and attractive products (Valls et al., 2009; Bechtold and

TABLE 4
Some areas of study in the field of anthocyanins with some selected examples of applications

Area of study	Reference
Development of analytical techniques for their purification and separation	Scotter, 2010, 2011b; Valls et al., 2009; Welch et al., 2008; Krenn et al., 2007; Mazza et al., 2004
Applications in foods	Yang et al., 2011; Day et al., 2009, Meulenberg, 2009; Motohashi and Sakagami, 2009
Identification and distribution in plants	Corradini et al., 2011; Truong et al., 2010; Horbowicz et al., 2008; Tian et al., 2005
Tracking color and pigment changes	Aberoumand, 2011; Cavalcanti et al., 2011; Raghvendra et al., 2011; He and Giusti, 2010
Biosynthesis	Hichri et al., 2011; He et al., 2010; Tanaka et al., 2008, 2010; Vogt, 2010
Quantitative analysis using chromatographic and electrophoretic techniques	Corradini et al., 2011; Fanali et al., 2011; Coté et al., 2010; Hartmanova et al., 2010; Ignat et al., 2011; Ivanova et al., 2011; Kusznierevicz et al., 2011; Wallace, 2010; Barnes et al., 2009; Liu et al., 2008
Effects in the plant stress	Mlodzinska, 2009
Chemotaxonomic investigations and wine adulteration	Bordonaba and Terry, 2008; von Baer et al., 2008; Honda and Saito, 2002
Health beneficial properties	Bueno et al., 2011; Cisowska et al., 2011; Jing and Giusti, 2011; Siva et al., 2011; McCune et al., 2011; Visioli et al., 2011; Wallace, 2011; Yang et al., 2011; Pascual-Teresa et al., 2010; Pascual-Teresa and Sánchez Ballesta, 2009; Thomasset et al., 2009; Motohashi and Sakagami, 2008; Seeram, 2008; Wang and Stoner, 2008; Wu and Prior, 2008; Ghosh and Konishi, 2007; McGhie and Walton, 2007; Zafra-Stone et al., 2007

Mussak, 2009). Intake of anthocyanin is increasing because extracts and juices with high anthocyanin contents from fruits and vegetables are now much more commercially available than earlier (He, 2004). Due to antioxidant and other potential beneficial properties, grapes, various berries, red cabbage, and other anthocyanin-rich foods are becoming more popular (McCune et al., 2011; Rao and Snyder, 2010). Berry extracts are also being commercialized as nutraceuticals and dietary supplements to meet consumer demands (Jing and Giusti, 2011).

CONCLUDING REMARKS

Anthocyanins are one of the flavonoid groups that have been most widely studied in recent decades (Valls et al., 2009). As a matter of fact, the heaviest concentration of chemical, biochemical, and biological research on phenolic compounds over the past century has focused on the anthocyanins (Whiting, 2001). A number of topics, i.e., anthocyanidin equilibrium forms, biosynthesis and molecular biology, and anthocyanin localization in plant cells, have seen important progress recently (Andersen, 2008). The main areas of study are compiled in Table 4. The most prolific area of flavonoid and anthocyanin research is connected with their possible applications to human health benefits.

Anthocyanins usually contain a single glucoside unit but many anthocyanins contain two, three, or more sugars attached at multiple positions, or occurring as oligosaccharide side chains. Anthocyanidins are rarely found in nature because of their poor stability. Glycosylation confers increased structural

stability and water solubility to the parent aglycone. Intensity and type of the color of anthocyanins are affected by the number of hydroxyl and methoxyl groups: if there are more hydroxyl groups, the color goes toward a more bluish shade; if there are more methoxyl groups, then redness is increased (Giusti and Jing, 2008; Heredia et al., 1998). A free hydroxyl group at one of the 5, 7, or 4' positions is essential for generating the in situ colors responsible for plant pigmentation, which arises mainly from the loss of an acidic hydroxyl hydrogen from the flavylium structure (Scotter, 2011b). Position 3 is always occupied by a sugar (whether acylated or not), and glucose can occur additionally in position 5, 7, 3', and possibly 4'. The possible number of individual pigments becomes apparent when the possibilities indicated above are considered (Andersen, 2008; Castañeda-Ovando et al., 2009).

The glycosylation, acylation, and methylation of anthocyanidins are the primary sources of structural diversity (Tanaka et al., 2010). The nature and accumulation of anthocyanins are principally regulated by genetic factors, but also by external factors, such as light and temperature. In spite of the increasingly large number of structures derived from only about 30 different anthocyanidins, almost 94% of the new anthocyanins discovered are in fact based on only the 6 common anthocyanidins: pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin. Anthocyanins show a λ_{\max} between 465 and 550 nm, as well as a significant absorption in the UV range between 270 and 280 nm (Wallace, 2011; He and Giusti, 2010; Stintzing and Carle, 2004).

Depending on the pH, anthocyanins in aqueous solutions exist in the form of different species in equilibria, hydration, proton-transfer, tautomerization, and isomerization equilibria being involved in their rich chemistry. Color variation and stabilization of anthocyanins in aqueous solution may have causes such as self-association, copigmentation, and intermolecular or intramolecular hydrophobic interactions (sandwich-type stacking); with, for example, flavones and aromatic acids, hydrogen bonds and charge transfer interactions may also be involved. Copigmentation protects the anthocyanidin molecule from the hydration reaction and also controls somewhat its color in response to its lightness (Gallan et al., 2007; Sakata et al., 2006). Copigmentation occurs with all six common anthocyanidins, in contrast to metal chelation, which occurs only with anthocyanins that have an ortho-dihydroxyl system linked to the B ring (Asen et al., 1972); therefore, cyanidin and delphinidin can form complexes while malvidin cannot.

The innumerable shades of blue, purple, violet, mauve, and magenta, and nearly all the reds that appear in flowers, fruits, leaves, and stems are due to anthocyanin pigments. Plants adopt many sophisticated mechanisms to exhibit and stabilize the flower color they need, especially blue colors (Yosida et al., 2009). Apart from their physiological roles in plants, anthocyanins are regarded as important components in human nutrition (Ross and Kasum, 2002). Anthocyanins present in various fruits and vegetables, especially in edible berries, provide natural pigmentation and exhibit a wide range of antioxidant protection and therapeutic benefits, including the integrity of genomic DNA; potent cardioprotective, neuroprotective, anti-inflammatory, and anticarcinogenic properties; and improvement in visual acuity and cognitive behavior (Wallace, 2011; Jaganath and Crozier, 2010; Yadav et al., 2009; Jaldappagari et al., 2008; Pascual-Teresa and Sánchez-Ballesta, 2008; Zafra-Stone et al., 2007).

Dietary choice can make a substantial impact on the amount of anthocyanin consumed (McGhie and Walton, 2007). Wine contains 200–300 mg of anthocyanins/L, and these anthocyanins are transformed into various complex structures as the wine ages (Pandey and Rizvi, 2009). Beverages with higher amounts of anthocyanins, functional drinks enriched with extracts, and many others are all part of the functional food revolution (Valls et al., 2009). The food industry first used anthocyanins as natural food colorants, but has now started to focus on possible health applications as nutritional supplements, functional food formulations, medicines, and so on (Zhao, 2007). However, the benefits of anthocyanins as dietary supplements are still only potential and many aspects of their antioxidant action must be clarified (Thomasset et al., 2009; Walle, 2004).

Because of their importance to the color quality of fresh and processed fruit and vegetables anthocyanins have been extensively investigated by horticulturists and food scientists (Barrett et al., 2010; Wrolstad, 2004). Both natural and synthetic colorants are added to food for various reasons, i.e., restoration of initial appearance and compensation for varying quality.

However, in spite of the advantages of synthetic pigments with respect to heat, light, pH, stability, and purity in comparison with natural colorants, i.e., carotenoids, anthocyanins, and betalains, they are increasingly being rejected by consumers owing to health concerns.

Although widespread use of anthocyanins as food dyes has long been envisaged, problems of chemical and color stability in processing, formulation, and storage conditions still represent a significant barrier to this application at a commercial scale. There is still much to be learned about the effects of processing and food matrix on anthocyanin stability (Cavalcanti et al., 2011), as shown above. Anthocyanin chemical stabilization (Sidani and Makris, 2011; Yang et al., 2011; Fang and Bhandar, 2010; Falcao et al., 2009) is the main focus of recent studies due to their abundant potential applications, their beneficial effects, and their use as alternatives to artificial colorants. Since anthocyanins are highly reactive compounds that readily degrade or react with other constituents in mixtures to form colorless or brown compounds, it is necessary to increase their stability in order to retain their color and functionality (Shipp and Abdel-Aal, 2010).

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