# Anthocyanins: Natural Colorants with Health-Promoting Properties

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## Key Words

oxidative stress, cardiovascular diseases, anti-inflammatory, anti-carcinogenic, absorption, metabolism

## Abstract

Anthocyanins are flavonoids in fruits and vegetables that render them vivid red to blue. To date, there have been more than 635 anthocyanins identified in nature, featuring six common aglycones and various types of glycosylations and acylations. Dietary consumption of anthocyanins is high compared to other flavonoids, owing to their wide distribution in plant materials. Based upon many cell-line studies, animal models, and human clinical trials, it has been suggested that anthocyanins possess anti-inflammatory and anti-carcinogenic activity, cardiovascular disease prevention, obesity control, and diabetes alleviation properties, all of which are more or less associated with their potent antioxidant property. Evidence suggests that absorption of anthocyanins occurs in the stomach and small intestine. Epithelial tissue uptake seems to be highly efficient, yet transportation into circulation, tissue distribution, and urine excretion are very limited. The bioactivity of bioavailable anthocyanins should be a focus of future research regarding their putative health-promoting effects.

## INTRODUCTION

**Cy:** cyanidin
**Pn:** peonidin
**Pg:** pelargonidin
**Mv:** malvidin
**Dp:** delphinidin
**Pt:** petunidin

Anthocyanins constitute the largest and probably the most important group of water-soluble natural pigments (Takeoka & Dao 2002). To date, there have been more than 635 anthocyanins identified in nature, and such a versatile group is responsible for the vivid blue, purple, and red color of many fruits, vegetables, and flowers (Andersen & Jordheim 2008). In fact, the word anthocyanin is derived from two Greek words, anthos and kyanos, meaning flower and dark blue, respectively (Delgado-Vargas & Paredes-Lopez 2003). Anthocyanins are believed to be important to plants as their color attracts animals, leading to seed dispersal and pollination. Owing to strong absorption of light, they may also be important in protecting plants from UV-induced damage (Mazza & Miniati 1993).

Anthocyanins are used as food colorants primarily in the beverage industry. As public concern about synthetic food dyes has increased recently, consumers and food manufacturers desire colorants from natural sources. Synthetic dyes commonly used in the food industry have been suspected to cause adverse behavioral and neurological effects (McCann et al. 2007). A recent trial involving 153 3-year-old and 144 8–9-year-old children concluded that when combined in the diet with sodium benzoate (E211), mixtures of artificial colorants including sunset yellow (E110), carmoisine (E122), tartrazine (E102), ponceau 4R (E124), quinoline yellow (E104), and allura red AC (E129) resulted in a statistically significant increase of hyperactivity in children (McCann et al. 2007). As promising alternatives to the most widely used synthetic food dye FD&C Red #40 (Allura red), anthocyanins are attracting great interest by the food industry and consumers.

## CHEMICAL STRUCTURE OF ANTHOCYANINS

### Chemical Structure of Flavonoids

Anthocyanins belong to a large group of polyphenolics named flavonoids, which are secondary metabolites synthesized by higher plants. Their aglycones share a C-6 (A ring)-C-3 (C ring)-C-6 (B ring) carbon skeleton (Harborne 1998). Based on the characteristics of the aglycones, flavonoids are divided into different subclasses (**Figure 1**). The presence or absence of double bonds and carbonyl groups on the C ring are the major differences among subclasses, whereas a shift of B ring substitution from C-2 to C-3 position separates isoflavones from others. Because quercetin, catechin, and isoflavones have similar structure to anthocyanins, their extensively studied bioactivities can provide the basis for the evaluation of anthocyanins.

### Anthocyanin Aglycones

Owing to the long chromophore of eight conjugated double bonds carrying a positive charge, anthocyanins are intensely colored under acidic conditions. The maximum absorption in the visible range is usually between 465 nm and 550 nm, whereas the other maximum absorption band falls in the UV range between 270 nm and 280 nm (Eder 2000). Differing in the patterns of hydroxylation and methylations on the different positions of the rings (**Figure 1**), there are close to 25 different aglycones that have been identified in nature (Andersen & Jordheim 2006). However, only six of them are commonly found in nature, and approximately 95% of all anthocyanins are derived from these six anthocyanidins (aglycones): cyanidin (Cy), peonidin (Pn), pelargonidin (Pg), malvidin (Mv), delphinidin (Dp), and petunidin (Pt) (Eder 2000, Kong et al. 2003). The color varies among aglycones (**Table 1**) with the B ring possessing more hydroxyl groups falling on the blue end of the spectrum and those possessing more methoxyl groups falling on the red end of the spectrum (Delgado-Vargas & Paredes-Lopez 2003, Heredia et al. 1998).

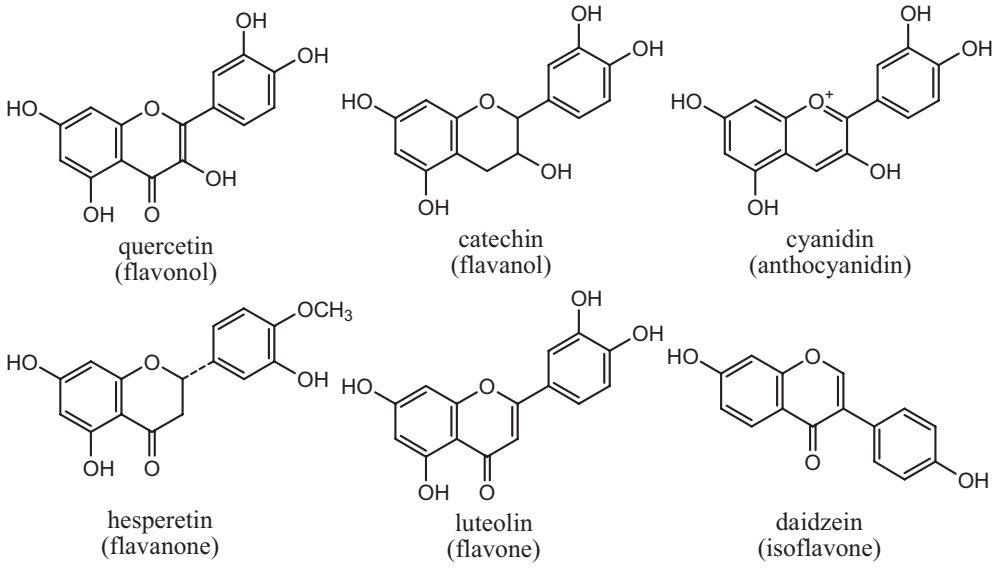


**Figure 1**

Representative aglycone structures of the common flavonoid subclasses.

## Glycosylation and Acylation

The hydroxyl groups on the aglycone may be substituted by sugar moieties, which may in turn be further linked to other sugars through glycosidic bonds or acylated with organic aromatic or aliphatic acids (cinnamic acid, malonic acid, and acetic acid, to name a few) through ester bonds (**Figure 2**). When the aglycone (anthocyanidin) is glycosylated, it is known as anthocyanin. Both glycosylation and acylation affect the physical and chemical properties of anthocyanins in that they modify the molecular size and polarity of the molecule. Glycosylation increases water solubility, whereas acylation decreases water solubility. The aglycone form of anthocyanins is rarely found in nature because of its poor stability. Glycosylation improves anthocyanin stability by forming an intramolecular H-bonding network within the anthocyanin molecule (Borkowski et al. 2005). Glucose (glu) and rhamnose (rha) are the more common sugar moieties attached to the aglycone, but galactose (gal), arabinose (ara), xylose (xyl), rutinose (rut), sambubiose (sam), and other sugars

**Table 1 Differences on chemical structure, color, and $\lambda_{max}$ of anthocyanidins most commonly found in nature**

| Name | Substitution | | Color | $\lambda_{max}$ (nm) in HCl acidified MeOH |
|---|---|---|---|---|
| | $R_1$ | $R_2$ | | |
| Cy | OH | H | Magenta | 535 |
| Pn | $OCH_3$ | H | Magenta | 532 |
| Pg | H | H | Red | 520 |
| Mv | $OCH_3$ | $OCH_3$ | Purple | 542 |
| Dp | OH | OH | Purple | 546 |
| Pt | $OCH_3$ | OH | Purple | 543 |

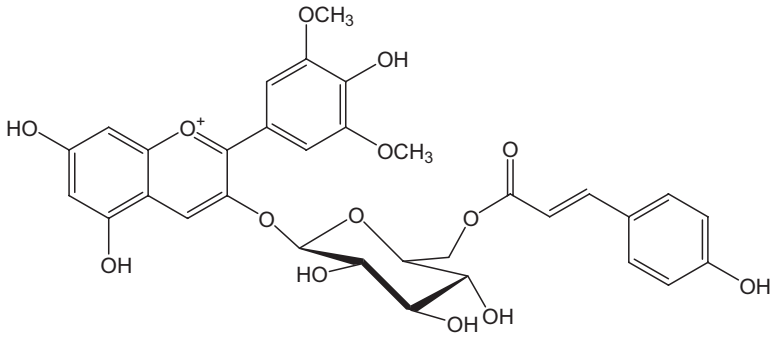


**Figure 2**

Chemical structure of an acylated anthocyanin (Mv-3-(*p*-coumaroyl)glu) found in grape skin.

are also frequently found. Acylated organic acids that can be found attached to the anthocyanin molecule comprise a broad range of compounds as well, which are normally classified into aliphatic acids and cinnamic acids. The various types of glycosidic and acyl substituents that can be found attached to the anthocyanidins molecule, as well as the different numbers of substitutions that can be attached to the molecule, are responsible for the wide variability of anthocyanin chemical structures reported in nature.

## The Influence of pH on Anthocyanin Chemical Structure

Anthocyanins are unique among flavonoids as their structures reversibly undergo pH-dependent transformation in aqueous solution (**Figure 3**). Four major anthocyanin forms exist in equilibria: the red flavylium cation, the blue quinonoidal base, the colorless carbinol pseudobase, and the colorless chalcone (Brouillard & Delaporte 1977). At a pH below 2, anthocyanins exist predominantly in the red flavylium cation form. Rapid hydration of the flavylium cation occurs at the C-2 position to generate the colorless carbinol pseudobase at pH values ranging from 3 to 6. As red color is bleached out in this transformation, the mechanism of reaction has been extensively investigated.

The fundamental work conducted by Brouillard & Dubois (1977) demonstrated that the hydration process is fairly rapid and, depending on the extent of pH change, can take between 30 and $\sim 10^3$ s to reach equilibrium. The $pK_h$ for the hydration reaction has been well studied with Mv-3-glu, a major anthocyanin in grapes and wines, using different methodologies (Asenstorfer et al. 2003, Brouillard & Delaporte 1977, Houbiers et al. 1998). The reported $pK_h$ was 2.60, 2.80, or 1.76 using UV/Vis spectroscopy, $^1$H NMR spectroscopy, or electrophoresis respectively. It is noteworthy that under the same conditions the 3,5-di-glu has less proportion in cation form than the corresponding 3-mono-glu, whereas acylation leads to noticeably increased cations especially at a pH above 4 (Dangles et al. 1993). For example, a larger number of acylated cinnamic acids attached to the anthocyanin results in higher $pK_h$, and thereby more red color is retained at low acidic conditions. This characteristic of acylated anthocyanins makes them preferable food colorants in moderately acidic foods such as yogurt. The reverse transition from carbinol pseudobase to flavylium cation is almost instant upon acidification (Brouillard & Delaporte 1977). The carbinol form can further equilibrate to an open ring form, the colorless chalcone pseudobase (**Figure 3**), at a slow rate. The reaction is favored by increased temperature. However, at any pH condition the chalcone form exists in a much smaller proportion as compared with the

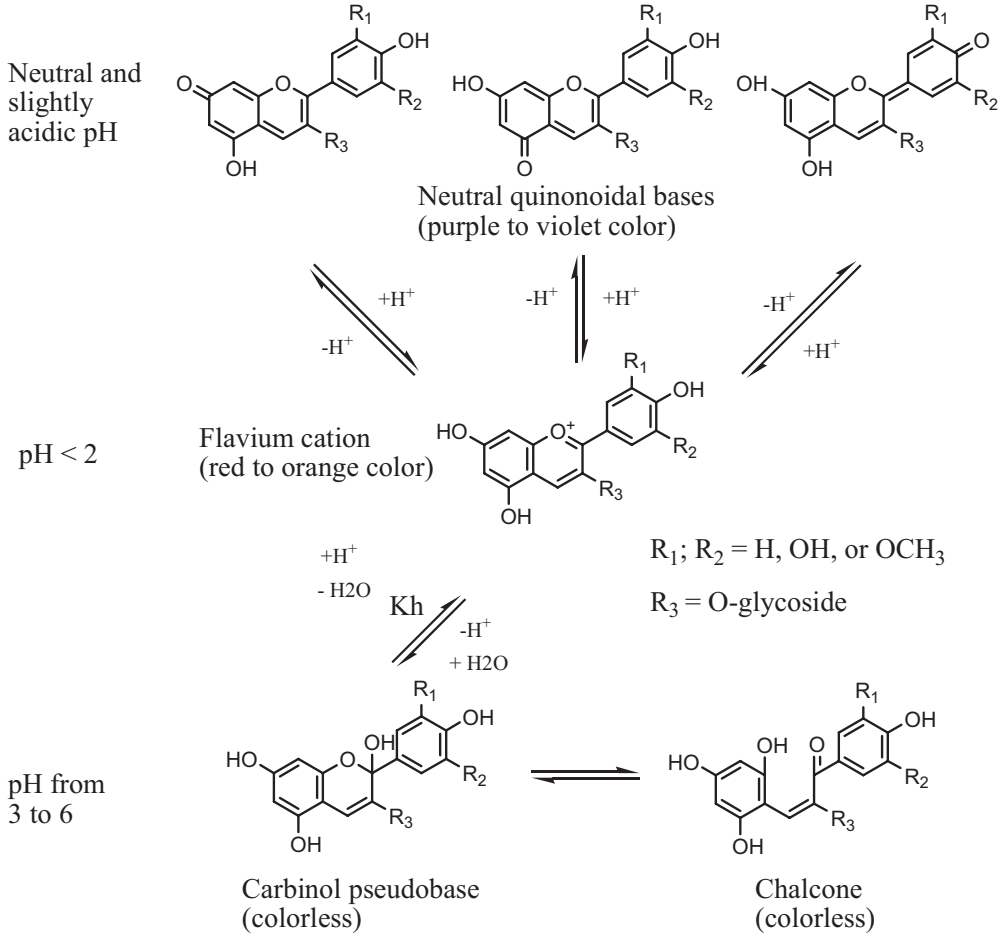


**Figure 3**

Scheme of the pH-dependent structural interconversion between dominant forms of mono-glycosylated anthocyanins in aqueous phase. (Source: Houbiers et al. 1998)

carbinol form. Reconversion of chalcone to flavylium cation is a very slow process taking hours to reach completion (Francis 1989).

Deprotonation of the flavylium cation to generate the quinonoidal base occurs at slightly acidic to neutral condition, and the reaction is extremely fast (Brouillard & Dubois 1977). At such a condition, kinetic competition between the deprotonation and hydration reactions predominantly favors deprotonation. As the pH increases above 8, the quinonoidal base can be ionized to carry one or two negative charges (Asenstorfer et al. 2003, Chen & Hrazdina 1982).

## ANTHOCYANINS IN THE HUMAN DIET

### Occurrence of Anthocyanins in Plant Materials

Anthocyanins are water-soluble vacuolar pigments found in many plant tissues (Shahidi & Naczk 2004). Edible anthocyanin sources in nature include colored fruits such as berries, cherries,

peaches, grapes, pomegranates, and plums as well as many dark-colored vegetables such as black currant, red onion, red radish, black bean, eggplant, purple corn, red cabbage, and purple sweet potato (Eder 2000, Wu et al. 2006a). Although most commonly accumulated in flowers and fruits, they are also present in leaves, stems, and storage organs (Delgado-Vargas & Paredes-Lopez 2003). Total anthocyanin content varies substantially across plant species and even cultivars (Wu et al. 2006a). Available data show a very wide range of anthocyanin content in plant material with berries usually providing the most anthocyanins per serving. Environmental factors such as light, temperature, and altitude also affect anthocyanin concentration considerably (Shahidi & Naczk 2004).

Abundance of the six common anthocyanidins in the edible parts of plants varies greatly. Some commodities, such as strawberry, contain a limited number of anthocyanin pigments, whereas others, such as low-bush blueberry, may contain a complex mixture. In a previous review, Kong et al. (2003) estimated the following abundance order: Cy (50%), Pg (12%), Pn (12%), Dp (12%), Pt (7%), and Mv (7%). In a later published summary including more anthocyanins (Andersen & Jordheim 2006), the abundance order was estimated to be Cy (30%), Dp (22%), Pg (18%), Pn (7.5%), Mv (7.5%), and Pt (5%). In both reports, the three nonmethylated anthocyanidins (Cy, Dp, and Pg) were shown to be more widespread than the three methylated anthocyanins (Pn, Mv, and Pt). Considering that more than 90% of anthocyanins contain glucose as a glycosylating sugar (Andersen & Jordheim 2006), it is not surprising that Cy-3-glu is the most widespread anthocyanin in nature (Kong et al. 2003).

## Anthocyanins in Foods and Beverages

Dietary anthocyanin sources include many colored fruits and vegetables as well as fruit-based processed foods and beverages such as jelly, juices, and red wine. The global anthocyanin consumption from black grapes alone is estimated to be 10,000 tons annually (Clifford 2000). With regard to mass consumed, anthocyanins constitute perhaps the most important subclass of flavonoids. Daily intake of anthocyanins had previously been estimated to be 180–215 mg per day per person (Kühnau 1976), but according to a recent report by the USDA evaluating more than 100 common foods, the estimation was 12.5 mg per day per person in the United States (Wu et al. 2006a). Still, this is a significant number compared with other phytochemicals with known or proposed health-promoting benefits. It has to be noted that dietary habits and choices have great impact on anthocyanin consumption. For example, one serving of blueberry increases anthocyanin consumption to greater than 500 mg. Likewise, one serving of Concord grape provides approximately 200 mg, and one serving of elderberry can supply 2000 mg anthocyanins. Regular red wine drinkers or juice drinkers can also benefit more from anthocyanins, as one bottle can readily provide more than 200 mg of anthocyanins (Clifford 2000). As the consumers become increasingly concerned about the adverse health effect of synthetic food dyes, more and more food manufacturers are attempting to use anthocyanins as substitutes for FD&C red #40 (allura red, E129), the most widely used synthetic colorant. For instance, application of anthocyanin-based colorants in fruit yogurt and many types of fruit-flavored dry mixes is becoming more popular. Indeed, synthetic dyes are not allowed in the rapidly growing natural foods market, where anthocyanins are becoming increasingly important. Acylated anthocyanins are usually used as food colorants because of superior stability over nonacylated anthocyanins (Giusti & Wrolstad 2003). However, certain commodities such as elderberry and barberry can provide high levels of nonacylated anthocyanins at relatively low cost, thus they also have potential use in the food industry (Jing & Giusti 2005, Wallace & Giusti 2008).

## Toxicity of Anthocyanins

Animals and humans have consumed anthocyanins since ancient times. No adverse impact on health has been reported with oral consumption of anthocyanins in foods (Brouillard 1982). The use of anthocyanins from natural sources as food colorants in foods and beverages is widely permitted within Europe (E163), Japan, the United States, and many other countries (Eder 2000). Based on early toxicological studies including mutagenicity, reproductive toxicity, teratogenicity, as well as acute and short-term toxicity evaluations, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that anthocyanin-containing extracts had a very low toxicity (WHO 1982). The no-observed-effect-level (NOEL) for young rats was determined to be approximately 225 mg $kg^{-1}$ body weight in a two-generation reproduction study. Based on the above result, the estimated acceptable daily intake (ADI) for human was estimated to be 2.5 mg $kg^{-1}$ body weight per day in 1982, using the equation of ADI = NOEL/100 (Clifford 2000).

**ROS:** reactive oxygen species

**ORAC:** oxygen radical absorbance capacity



# PUTATIVE HEALTH-PROMOTING EFFECTS OF ANTHOCYANINS

Interests in dietary polyphenols, including anthocyanins, drastically intensified after the recognition of their potential health benefits (Scalbert & Williamson 2000). Epidemiological studies have suggested a reverse association between high consumption of polyphenols and incidence of some chronic diseases. For example, drinking red wine regularly has been associated with the relatively low incidence of coronary heart disease in French people despite a high-fat diet, well known as the French Paradox (Renaud & de Lorgeril 1992). Since then, a vast number of studies have been carried out on the biological effects of polyphenols, using in vitro and in vivo models. Anthocyanins are among the most abundant polyphenols in fruits and vegetables and possess potent antioxidant activity. In vitro models have the merits of low cost and high efficiency, thus they have been widely employed. Animal and human clinical studies on health benefits of anthocyanins are still in the early stage. To date, suggested health benefits of anthocyanins have been in some way related to their antioxidant activity (Kong et al. 2003). It must be noted that not a single class of compounds can explain most of the health-promoting effects of consuming fruits and vegetables. Apparently, the phytochemicals contained in fruits and vegetables work collaboratively to benefit our body (Seeram et al. 2004, Zhang et al. 2008).

## Relief of Oxidative Stress

Reactive oxygen species (ROS), including free radicals, singlet oxygen, and peroxides, are generated in the body. They are important to the immune system, cell signaling, and many other normal body functions. However, if ROS are overly produced, they can elicit cellular damage, leading to degenerative diseases such as inflammation, cardiovascular disease, cancer, and aging (Allen & Tresini 2000).

Anthocyanins are potent antioxidants in vitro. They effectively quench free radicals and terminate the chain reaction that is responsible for the oxidative damage. Because pH in the human body is generally neutral except in the stomach, the antioxidant activity of anthocyanins at neutral pH is of particular importance. Using a widely accepted antioxidant assessment method, the oxygen radical absorbance capacity (ORAC) assay, antioxidant activity of 14 anthocyanins including Dp, Cy, Pg, Mv, Pn, and their glycosylated derivatives was determined in aqueous phase at neutral pH (Wang et al. 1997). Among these anthocyanins, Cy-3-glu had the highest ORAC value, 3.5 times as potent as Trolox, a water-soluble vitamin E analog. Pg had the lowest ORAC value among the tested anthocyanins, but was still as potent as Trolox. In linoleic acid

autoxidation, liposome, rabbit erythrocyte membrane, and rat liver microsomal systems Cy-3-glu and its aglycone Cy were shown to have similar antioxidant potency as vitamin E (α-tocopherol) (Tsuda et al. 1994). Such potent antioxidant activity from anthocyanins may have protective effects in the biological environment. An in vitro study using human erythrocytes treated with $H_2O_2$ as an oxidative model revealed that red wine fractions rich in anthocyanins significantly lowered ROS in human red blood cells (Tedesco et al. 2001).

**LDL:** low-density lipoprotein

The protective effect of anthocyanins on oxidative stress–induced damage is promising as shown using in vivo models. In a rat study utilizing hepatic ischemia-reperfusion as an oxidative stress model, Cy-3-glu efficiently attenuated changes of biomarkers in liver injury (Tsuda et al. 2000). In another rat study, the animals were fed vitamin E–deficient diets for 12 weeks followed by supplementation with purified anthocyanin-rich extracts. The anthocyanin diet significantly improved plasma antioxidant capacity and lowered the level of hydroperoxides and 8-Oxo-deoxyguanosine, indicating significant reductions of the vitamin E deficiency–induced lipid peroxidation and DNA damage, respectively (Ramirez-Tortosa et al. 2001).

## Prevention of Cardiovascular Diseases

Oxidation of low-density lipoprotein (LDL) triggers accumulation of macrophage white blood cells in the artery wall. Rupture of the plaque deposits oxidized cholesterol into the artery wall, leading to atherosclerosis and eventually cardiovascular diseases (Aviram 2000, Aviram et al. 2005). Dietary antioxidants, including anthocyanins, have the potential to increase serum antioxidant capacity and thereby protect against LDL oxidation and prevent cardiovascular diseases. Research initially focused on anthocyanin-rich red wine because of the famous French paradox (Renaud & de Lorgeril 1992). Using a chemiluminescent assay of serum antioxidant capacity (SAOC), the effects in normal human subjects ingesting 300 mL of red wine, white wine, or high dose (1000 mg) of vitamin C were studied. In subjects who ingested red wine, the mean SAOC was increased by 18% and 11% after 1 h and 2 h, both higher than that in the white wine group, although not as high as that in the vitamin C group (Whitehead et al. 1995).

Following the pioneering studies on red wines, more attention has been given to anthocyanins and other polyphenols present in red wines. A trial involving seven human subjects demonstrated that daily consumption of 125 mL of concentrated red grape juice markedly raised serum total antioxidant capacity (TAC) as compared with the baseline. The susceptibility of LDL to oxidation was also reduced. Therefore, the nonalcoholic red grape extract was suggested to have similar beneficial effects to red wine (Day et al. 1997). Other anthocyanin-rich foods have also been extensively studied. Monitoring of chemiluminescent emission intensity of human blood plasma for 8 h following oral administration of black currant anthocyanins demonstrated a rapid increase of plasma antioxidant capacity until 2 h (Matsumoto et al. 2002). Spray-dried elderberry juice containing high anthocyanin content was investigated with respect to the protective effect on human LDL in vitro (Abuja et al. 1998). A concentration-dependent prolongation of the lag phase was observed in copper-induced oxidation. Meanwhile, a similar prolongation effect was also observed in peroxyl-radical-driven LDL oxidation, together with a reduction of maximum oxidation rate. It is important to note that anthocyanins may not explain all of the protective effects observed in these foods, but likely contributed to some extent. In a UV light radiation–induced lipid peroxidation model, three purified anthocyanins (Pg-3-glu, Cy-3-glu, and Dp-3-glu), as well as their aglycones, all demonstrated strong inhibition of lipid peroxidation and acted as active oxygen radical scavenging agents (Tsuda et al. 1996).

## Anti-Inflammatory Activity

Inflammation is a complex biological response in response to tissue injury. Many cancers occur at sites of inflammation because inflammatory cells provide a microenvironment favorable for tumor development, and therefore anti-inflammatory therapy has the potential to prevent early neoplastic progression and malignant conversion (Coussens & Werb 2002). Because cyclooxygenases (COXs) convert arachidonic acid to prostaglandins that stimulate inflammation, inhibitory effect on COX enzymes is highly desirable (Seeram et al. 2001). Cy aglycone was reported to possess better anti-inflammatory activity than the positive control aspirin in the COX activities assays (Wang et al. 1999). Purified anthocyanin fractions from tart cherries, sweet cherries, bilberries, blackberries, blueberries, cranberries, elderberries, raspberries, and strawberries were evaluated using COX-inhibitory assays (Seeram et al. 2001). All the anthocyanin fractions demonstrated inhibitory effect on COX-1 and COX-2 enzymes, whereas strawberry, blackberry, and raspberry showed the highest activity, comparable to that of the positive controls ibuprofen and naproxen at 10 μM concentrations. In an in vivo study, the therapeutic efficacy of blackberry anthocyanins (Cy-3-glu accounted for 80%) was investigated in rats with carrageenan-induced lung inflammation (Rossi et al. 2003). All parameters of inflammation were effectively reduced in a dose-dependent manner by anthocyanins.

**COX:** cyclooxygenase



## Anticarcinogenic Activity

Anticancer activity of anthocyanins has been established largely based on in vitro evidence. Anthocyanins extracted from flower petals were found to be more potent than combined nonanthocyanin flavonoids regarding cell growth inhibition in a human malignant intestinal carcinoma-derived HCT-15 cell line (Kamei et al. 1995). The dose required for 50% inhibition ranged from 0.5 to 5 $\mu g\ mL^{-1}$ for representative individual anthocyanins and anthocyanidins, whereas higher concentrations of other flavonoids were required to exhibit the same effect. Similarly, the anthocyanin fraction isolated from red wine was also discovered to be significantly more effective than nonanthocyanin flavonoids in red wine or white wine using HCT-15 cell line and AGS cell line, which was derived from human gastric cancer (Kamei et al. 1998). The antiproliferative effect of anthocyanin fraction from four cultivars of muscadine grapes was evaluated using two human colon cancer-derived cell lines, HT-29 and Caco-2 (Yi et al. 2005a). In all cultivars and both cell lines, greater inhibitory activity was observed from the anthocyanin fraction than from the phenolic acids fraction or the crude extract. Zhao et al. (2004) demonstrated that anthocyanin fractions from commercially available bilberry, chokeberry, and grape extracts all exerted antiproliferative effects in the HT-29 cell line. Similar results were found with other anthocyanin-rich extracts from other sources, including purple corn, purple carrot, and red radishes (Jing et al. 2008). A dose of 25 $\mu g\ mL^{-1}$ chokeberry anthocyanins provided 50% inhibition of the carcinoma cell line, notably not affecting the growth of normal colonic NCM460 cells. More in-depth investigation revealed that the chokeberry anthocyanins arrested the cell cycle of HT-29 cells by blocking at the G1/G0 and G2/M phases (Malik et al. 2003). Highly purified anthocyanins have also been evaluated. Four anthocyanins isolated from strawberry by means of medium-pressure liquid chromatography (MPLC) were all shown to reduce cell viability of human oral (CAL-27, KB), colon (HT29, HCT-116), and prostate (LNCaP, DU145) cancer cells at 100 $\mu g\ mL^{-1}$ dose level, although different sensitivity was recorded for each individual compound (Zhang et al. 2008). Additionally, the results from different studies show that the antiproliferative effects of the different anthocyanins on the colon cancer cells are highly dependant on the chemical structure of the pigments, including type of aglycone, glycosylation pattern as well as acylation (Jing et al. 2008).

Anthocyanins are shown to be promising phytochemicals responsible for at least part of the anticancer property of many fruits and vegetables, but it is more than likely that anthocyanins work collaboratively with other phytochemicals to help the body defense. Seeram et al. (2004) evaluated the antiproliferative effects of total cranberry extract versus its flavonol glycosides (gly), anthocyanins, proanthocyanidins, and organic acids fractions using human oral (KB, CAL27), colon (HT-29, HCT116, SW480, SW620), and prostate (RWPE-1, RWPE-2, 22Rv1) cancer cell lines. Both the anthocyanin fraction and the proanthocyanidin fraction exhibited substantial inhibitory effect on all but the SW480 cell lines. However, the combination of these two fractions was the most active against all cell lines. Studies by Jing et al. (2008) suggest that the combined effects of anthocyanins and other phenolics from a number of anthocyanin-rich fruits and vegetables are mainly additive rather than synergistic or antagonistic.

In animal studies, the growing body of data has demonstrated chemopreventive effect of anthocyanins in multiple types of cancers. Nevertheless, the observed preventive effects were primarily related to the gastrointestinal tract (GIT)-related organs including the oral cavity, the esophagus (Reen et al. 2006, Stoner et al. 1999), and the colon (Hagiwara et al. 2001, 2002; Harris et al. 2001; Lala et al. 2006). In the GIT lumen, anthocyanins are largely available and can contact directly with the epithelial layer (He et al. 2005). In contrast, availability of anthocyanins to non-GIT organs requires blood delivery. This is probably one of the reasons why strawberry anthocyanins failed to inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-and benzo[a]pyrene-induced lung cancer in a mice model (Carlton et al. 2000).

Anticancer activity of anthocyanins may be attributed to the additive effect of multiple mechanisms (Duthie 2007, Hou 2003, Lala et al. 2006). Possible mechanisms that have been suggested include antimutagenic activity (Gasiorowski et al. 1997, Ohara et al. 2004, Yoshimoto et al. 2001), inhibition of oxidative DNA damage (Singletary et al. 2007), inhibition of carcinogen activation and induction of phase II enzymes for detoxification (Shih et al. 2007, Srivastava et al. 2007), cell cycle arrest (Renis et al. 2008), inhibition of COX-2 enzymes, induction of apoptosis (Yi et al. 2005a,b), and antiangiogenesis (Bagchi et al. 2007). In the particular case of GIT-related cancer, the influence of anthocyanins on the GIT luminal condition is of great importance too. Bruce et al. (2000) suggested two mechanisms that initiate colon cancer development, one involving a local irritation that produces a local inflammatory response and the other relating to an electrolyte imbalance. Both mechanisms result from a defect in the epithelial barrier, and both lead to elevated ROS and COX-2 levels in epithelial cells. Therefore, agents that can improve colon luminal condition, hence reduce epithelial barrier damage, can inhibit expression of COX-2 and inflammation, or can quench ROS in local cells have the potential to prevent colon cancer. Anthocyanins have been shown to be powerful antioxidants and COX-2 inhibitors, as discussed previously in this section. However, Lala et al. (2006) suggested that the inhibitory effect of dietary anthocyanins in a colon carcinogen azoxymethane (AOM)-induced rat colon cancer model was primarily attributed to the direct effect on improving colon luminal condition. The patterns of inhibition on colonic cell proliferation and large aberrant crypt foci (ACF) multiplicity (**Figure 4**) were not correlated with the total antioxidant capacity in the diet, with anthocyanin absorption, or with the colonic mucosa COX-2 mRNA levels. The highest correlation was between colon cancer growth and the total anthocyanin content in the colonic lumen as represented by fecal anthocyanin concentration. Luminal anthocyanins appeared to promote fecal moisture content and fecal excretion of bile acids, a group of endogenous tumor-promoting compounds (**Figure 4**). In addition, luminal anthocyanins may benefit colon health by protecting the epithelial cells against oxidative damage and microbial infection.

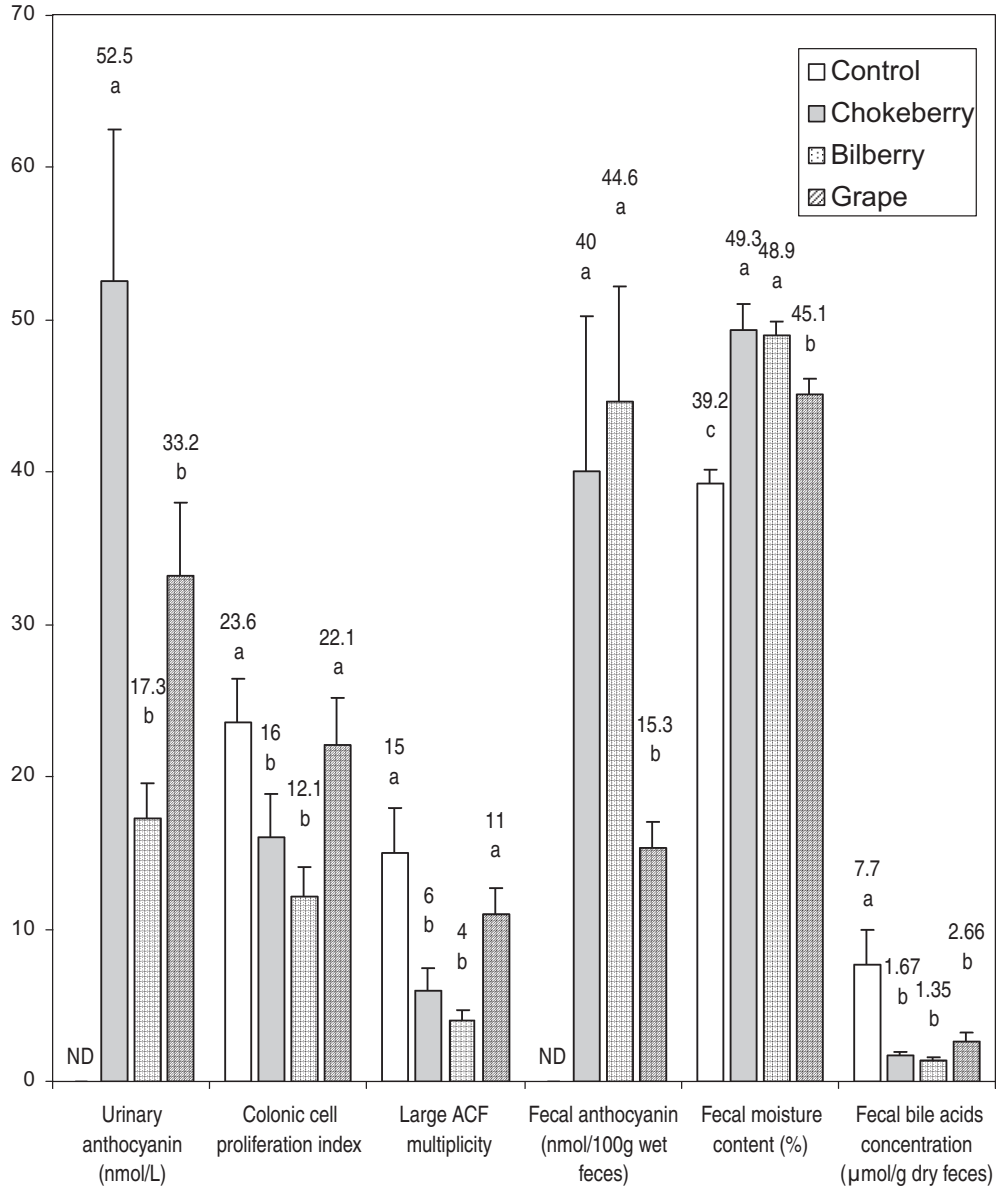


**Figure 4**

Effect of feeding anthocyanin-rich diets on total anthocyanin concentration in rat urine, colonic cell proliferation index, large aberrant crypt foci (ACF) multiplicity, total anthocyanin concentration in feces, fecal moisture content, and fecal bile acids concentration (Source: Lala et al. 2006). Data are means ± SE. Values with a different letter differ significantly ($P < 0.05$) within a same category.

## Prevention of Obesity

Obesity is the result of accumulated excessive adipose tissue caused by the imbalance of energy intake and expenditure. It is usually associated with various metabolic disorders. Consumption of anthocyanins can possibly ameliorate the function of adipocytes, and thus may prevent metabolic

syndrome and obesity (Tsuda 2008). In a fundamental study conducted by Tsuda et al. (2003), 24 male mice were fed control, purple corn extract, high-fat, or high-fat plus purple corn extract diet for 12 weeks. Supplementation with purple corn color suppressed the high-fat diet–induced gain of body weight and white/brown adipose tissue weights. Downregulation of the mRNA levels of enzymes involved in fatty acid and triacylglycerol synthesis was suggested to contribute to this antiobesity effect. Two additional in vivo studies supported anthocyanin's antiobesity effect on high-fat diets. In one of the studies, black soybean anthocyanins were found to effectively reverse the weight gain of high-fat diet–group rats to the same as that in the control group (Kwon et al. 2007). Serum lipid composition was also improved by the addition of black soybean anthocyanins into the high-fat diet. Serum triglyceride and cholesterol levels were significantly reduced, whereas the high-density lipoprotein (HDL)-cholesterol concentration markedly increased. In the second study, male mice were fed a high-fat diet for 8 weeks with or without supplementation of blueberry anthocyanins in drinking water (Prior et al. 2008). Both the whole blueberry and the purified blueberry anthocyanins were evaluated. The purified anthocyanins resulted in lower body weight gains and body fat than the controls, whereas the whole blueberry with the same level of anthocyanins actually increased obesity, probably owing to added calorie intake from sugar. A further study of anthocyanin's effect on gene expression of adipocytes employed an in vitro model using isolated rat adipocytes (Tsuda et al. 2005). The total RNA isolated from the adipocytes was analyzed using GeneChip microarray. After the treatment of adipocytes with 100 μM of Cy-3-glu or Cy, 633 and 427 genes, respectively, were upregulated by greater than five-fold. Based on the gene expression profile, the upregulation of hormone-sensitive lipase and enhancement of the lipolytic activity were suggested to be the result of anthocyanin treatment on adipocytes.

## Control of Diabetes

Type 2 diabetes is a metabolic disorder associated in part with insulin resistance. Insulin secreted from the β-cells of the pancreas is responsible for stimulation of blood glucose transport into skeletal muscle and adipose tissue as well as suppression of hepatic glucose production (Ghosh & Konishi 2007). Obesity and excessive intake of high-fat or high glycemic–index foods are possible reasons for the relative inadequacy of insulin in late stages of type 2 diabetes. Anthocyanins have the potential to control obesity and consequently may contribute to the prevention of type 2 diabetes. Furthermore, the antioxidant activity of anthocyanins may protect β-cells from glucose-induced oxidative stress (Al-Awwadi et al. 2005). Sugimoto et al. (2003) examined the protective effects of boysenberry anthocyanins against oxidative stress in streptozotocin-induced diabetic rats. Increased plasma and liver biomarker oxidation was observed in diabetic rats as compared with control rats. Administration of a diet with boysenberry anthocyanins restored or tended to restore the biomarkers to the level of the control rats. The results indicated that anthocyanins are effective in preventing the development of in vivo oxidation that may lead to diabetes. More details about the role of anthocyanins in diabetes prevention can be found in the comprehensive review by Ghosh & Konishi (2007).

## Improvement of Eye Vision

Anecdotal evidence suggests that consumption of anthocyanins can improve eye vision (Kramer 2004). In a double-blind, placebo-controlled crossover study with healthy human subjects, feeding black currant anthocyanin concentrate at 12.5, 20, and 50 mg per subject resulted in dose-dependent lowering of the dark adaptation threshold (Nakaishi et al. 2000). The effect with the

highest dose (50 mg per subject) had a statistically significant effect ($P = 0.011$). However, a systematic review of placebo-controlled trials revealed conflicting evidence in the use of anthocyanins to improve night vision (Canter & Ernst 2004). The negative outcomes reported may be associated with low doses tested in some trials, the different methodologies used for evaluation, the variation of subjects, and the source of anthocyanins (Ghosh & Konishi 2007). Recently, a study on blueberry anthocyanin distribution in pig tissues confirmed that anthocyanins accumulated in pig eyes after feeding a blueberry diet for 4 weeks (Kalt et al. 2008). Although the detected concentrations in eye tissue were extremely low (pmol $g^{-1}$), the concentrations were comparable to that in other evaluated tissues including liver.

### Antimicrobial Activity

Plant phenolics are well known to play an important role in the defense against pathogens. Thus, their effects on human intestinal bacteria, both beneficial and pathogenic, have been extensively investigated (Nohynek et al. 2006). In a study of the phenolic compounds in eight common Finnish berries, the berry extracts as well as the representative individual phenolic compounds contained in the berries were evaluated against human intestinal bacteria (Puupponen-Pimiä et al. 2001). All four anthocyanins tested including Pg chloride, Cy chloride, Dp chloride, and Cy-3-glu were found to be effective inhibitors of Gram-negative *Escherichia coli* strain CM 871, a DNA repair-deficient strain, but did not inhibit regular *E. coli* and the beneficial Gram-positive probiotic bacteria. Therefore, the antimicrobial activity of anthocyanins was speculated to involve reactions related to DNA. In another study evaluating berry phenolics against severe human pathogens, anthocyanin fraction was the most potent phenolic fraction in berries for reducing viability of *Salmonella enterica* serovar Typhimurium (Nohynek et al. 2006). Such an effect was attributed to the ability of anthocyanins to induce the release of lipopolysaccharide molecules from the outer membrane of the Gram-negative bacteria.

## BIOAVAILABILITY AND METABOLISM OF ANTHOCYANINS

To validate the prominent health-promoting effects revealed in many in vitro models, it is important to consider the anthocyanin bioavailability in vivo. Anthocyanin levels detected in the plasma and urine after ingestion of anthocyanin-rich materials are in general very low. The doses reported in some in vitro studies might have little relevance to in vivo conditions given that the level of intact anthocyanins exposed to tissues (except GIT luminal side tissues) could be very limited owing to the observed low concentration in blood (Kroon et al. 2004). Another important issue is the form of metabolites that are present in the tissues. Some metabolites of flavonoids have comparable or even more potent bioactivity than the precursors (Setchell et al. 2002). Therefore, to truly evaluate the bioactivity of anthocyanins, it is critical to understand their bioavailability and metabolism.

Regarding absorption and metabolic pathways, anthocyanins have been thought to differ from the common flavonoids given that only intact anthocyanin glycosides were detected in urine and plasma (Felgines et al. 2002). However, improved analytical techniques have revealed that anthocyanins are also methylated, sufated, and glucuronidated (Felgines et al. 2003, 2005, 2007; Kay et al. 2005; Wu et al. 2002). It is now believed that the absorption, metabolism, and excretion of anthocyanins share some similarities with structurally related flavonoids. In this section, both anthocyanins and several well-documented flavonoids are discussed together as a whole.

## Overview of Flavonoid Absorption and Metabolism

After consumption of flavonoid-containing foods, the flavonoids are released from the food matrix by chewing. Absorption could start in the stomach. Flavonoids absorption by the stomach would appear in the blood extremely rapidly after ingestion (Piskula et al. 1999). The small intestine is the major site for flavonoid absorption. Endogenic β-glucosidases are involved at this stage to release aglycones from primarily flavonoid-glu and to a lesser extent -gal, -xyl, and -ara. Free aglycones are more hydrophobic and have smaller size than the glycosides, thus are more likely to penetrate the epithelial layer passively. In contrast, intact glycosides are also absorbed by the small intestine, either by inefficient passive diffusion or by the sodium-dependent glucose transporter (SGLT1). Acylated flavonoids are generally recognized as nonabsorbable in the small intestine owing to their larger molecular size and lack of a free sugar moiety for transporter binding. However, recent evidence suggests that acylated anthocyanins are slightly bioavailable in the intact form (Harada et al. 2004, He et al. 2005), although, likely owing to their increased molecular size, acylated anthocyanins are much less efficiently absorbed than their counterparts without the acylation (He et al. 2006, Mazza et al. 2002).

Unabsorbed flavonoids travel down to the colon, where a substantial amount of microorganisms ($\sim 10^{12}$ $cm^{-3}$) reside to provide catalytic and hydrolytic potential (Scheline 1973). Glycosidic and ester bonds are thereby cleaved by colonic microflora (Bokkenheuser et al. 1987). Aglycones then undergo spontaneous ring fission to some extent to generate simple compounds such as phenolic acids. The released aglycones and generated phenolic acids could be absorbed by the colon, yet only marginal absorption is expected because the colon is much less efficient than the small intestine with respect to absorption. For this reason, it is anticipated that the sugar moiety of flavonoids' glycosides governs the absorption and bioavailability of the aglycones of many flavonoids. So far, little is known about the effect of enzymatic deglycosylation on anthocyanin absorption.

Flavonoids, including anthocyanins, taken up from GIT lumen are subsequently metabolized by phase II drug-metabolizing enzymes to glucuronides, sulfates, and methylates in the intestine epithelium, liver, and kidney (Felgines et al. 2003, Kroon et al. 2004). The conjugated metabolites may be excreted into the jejunum via bile and later recycled in the intestine/colon by the process referred to as the enterohepatic circulation pathway.

## Gastric Absorption

Two research groups used similar approaches to demonstrate that anthocyanidin glycosides were efficiently absorbed in the stomach (Passamonti et al. 2003, Talavéra et al. 2003). Passamonti et al. (2003) injected grape anthocyanins into the stomach of 19 Wistar male rats that had surgically blocked cardias and collected blood from both the portal vein and the heart at 6 min intervals. Quantification of the anthocyanins by high-performance liquid chromatograph mass spectrometer (HPLC-MS) using single ion monitoring revealed that Mv-3-glu was present in both portal and systemic plasma ($0.789 \pm 0.491$ μM and $0.098 \pm 0.078$ μM, respectively; $n = 19$). Importantly, Mv 3-glu appeared in the plasma within 6 min, presenting evidence of stomach absorption. Pn-3-glu, Pt-3-glu, and Mv-3-glu-acetyl derivatives were inconsistently detected, perhaps owing to animal variability. Neither anthocyanin aglycones nor conjugated derivatives were detected in the plasma.

Talavéra et al. (2003) infused anthocyanin standards as well as bilberry and blackberry extract into the stomach of pylorus- and sphincter-ligated rats. Gastric contents and blood were collected from the gastric vein and abdominal aorta 30 min after the administration. HPLC analysis revealed that a high proportion (~25%) of anthocyanin monoglycosides, including glucoside and galactoside, was absorbed from the stomach, whereas the rutinoside was poorly absorbed. It was suggested

that gastric absorption of anthocyanins involves bilitranslocase (TC 2.A.65.1.1), an organic anion membrane carrier in the gastric mucosa (Passamonti et al. 2002).

## Direct Absorption in the Small Intestine

The small intestine is generally regarded as the most important site for absorption of nutrients. Absorption of anthocyanins in rat small intestine has been evaluated using an in situ perfusion method (Talavéra et al. 2004). Intestinal perfusion of anthocyanin supplemented in physiological buffers was conducted on anesthetized rats. The amount of anthocyanin remaining in the effluent was used to estimate the rate of anthocyanin absorption in the small intestine. Depending on their structures, the absorption rate of supplemented anthocyanins ranged from 22.4 ± 2.0% (Cy-3-glu) to 10.7 ± 1.1% (Mv-3-glu). Such high absorption rates seemed to contradict the very low levels of anthocyanins observed in the blood (Prior 2004). However, it has to be noted that these absorption rates were calculated based on the disappeared amount in the effluent, thus they could indicate the portion of anthocyanins being taken up into the small intestine tissue but not necessarily transferred into the blood. Recently, our research group also demonstrated that as high as 7.5% of the administered black raspberry anthocyanins could be taken up by rat small intestinal tissue, yet a very limited amount can be detected in urine (He et al. 2009).

## Deconjugation of Carbohydrate Moieties

Glycosylated flavonoids are more hydrophilic than the corresponding aglycones. For instance, quercetin has a partition coefficient (log value of concentrations in octanol/water) of 1.2 ± 0.1, whereas quercetin-3-rut has only 0.37 ± 0.06 (Scalbert & Williamson 2000). With smaller molecular size and better lipid solubility, aglycones are anticipated to penetrate the lipid bilayer of cell membranes, possibly leading to passive diffusion across the small intestine brush border. This pathway necessitates deglycosylation of ingested anthocyanins. Nonenzymatic deglycosylation (acid hydrolysis) is unlikely to play an important role, despite the strong acidic condition in the stomach. Deglycosylation of quercetin glycosides (Gee et al. 1998) or anthocyanidins glycosides (Pérez-Vicente et al. 2002) was not noted after pepsin-HCl digestion at pH 2.0 and 37°C for 2 h. Therefore, the question is left to the possibility of enzymatic deglycosylation in vivo.

Recent advances in the study of small intestinal β-glucosidases support the hypothesis that they deglycosylate some flavonoids, and play an important role in the digestion of dietary flavonoids. Day et al. (1998) were the first to investigate the effect of human β-glucosidases on flavonoids. Most of the monoglucosides tested were successfully deglycosylated by both human small intestine and liver β-glucosidases, regardless of the type of aglycone (quercetin, kaempferol, naringenin, apigenin, genistein, and daidzein). In contrast, rutinosides remained intact. The results agreed with a number of previous in situ or in vivo studies specifying the absorption site difference between mono-glu and rut (Gee et al. 1998, Hollman & Katan 1997, Hollman et al. 1997). Using in situ and ex vivo rat jejunum perfusion models, two research groups independently demonstrated that lactase-phlorizin hydrolase (LPH) was capable of hydrolyzing quercetin-glu efficiently and influencing the transport of quercetin across the epithelial membrane (Day et al. 2003, Sesink et al. 2003). Notably, the $K_m$ values of human- and animal-originated enzymes were different but comparable (Day et al. 2000, Lambert et al. 1999) (**Table 2**). These studies suggested the possibility of using animal models for future research.

Evidence of enzymatic deglycosylation of anthocyanins is still very limited. Examination of pig and rat GIT content indicated selective degradation of anthocyanin glucoside in the small intestine (He et al. 2005, 2009; Wu et al. 2005, 2006b), but further characterization of anthocyanin

**Table 2** $K_m$ of quercetin 4′-glu and genistein 7-glu by β-glucosidase from human and animal intestine and liver (Day et al. 1998, 2000; Lambert et al. 1999)

| | $K_m$ (μM) | | | |
|---|---|---|---|---|
| | Human | | Pig | Lamb |
| Substrate | Liver | Small intestine | Liver | Small intestine |
| Quercetin 4′-glu | 27 ± 13 | 37 ± 12 | 65 | 44 ± 7 |
| Genistein 7-glu | 13 ± 1 | 14 ± 5 | 35 | 85 ± 11 |

deglycosylation patterns under the effect of isolated small intestinal β-glucosidases is needed. Interestingly, even in the above-mentioned rat small intestine in situ perfusion model, the disappearance of Cy-3-glu was significantly higher than other glycosides of Cy (Talavéra et al. 2004). Limited information available suggests that anthocyanin-xyl and ara are better retained in the cecal content and feces as opposed to anthocyanin-gal and -glu (He et al. 2005). Further research is needed to elucidate the fate of such glycosides.

## The Influence of Colonic Microflora

The enzymes present in the small intestine, including β-glucosidase, cannot account for hydrolysis of all glycosidic bonds, and hence flavonoid-rha, -rut, and others can survive through the small intestine and reach the colon (Scalbert & Williamson 2000). There are no endogenous esterases in humans to release phenolic acids either. Thus, the esterase activity of colonic microflora is required for the metabolism of acylated flavonoids (Plumb et al. 1999). Using an in vitro anaerobic fecal fermentation model, Aura et al. (2002) demonstrated that human fecal flora readily deconjugates quercetin-rut, -glu, and glucuronide (glc). The deglycosylated quercetin undergoes ring fission to generate simple phenolics such as 3,4-dihydroxyphenylacetic acid and its derivatives. One of the microorganisms responsible for the degradation of flavonoids may be *Eubacterium ramulus*, as addressed by Schneider & Blaut (2000). Anaerobic incubation with a broad range of flavonoids was performed after inoculating the media with an exponentially growing culture of *Eubacterium ramulus* that had been previously isolated. The fermentation end products included hydroxyphenylacetic acids and hydroxyphenylpropionic acids. These degradation products, as well as the deglycosylated aglycones, may be absorbed by the colon, and consequently contribute to the bioactivity of ingested flavonoids.

Fermentation of Cy-3-rut and Cy-3-glu in the presence of human fecal slurry revealed that anthocyanins could also be converted by gut microflora (Aura et al. 2005). Hydrolysis of Cy-3-glu was almost complete after 2 h of incubation, and less than 1/3 of the Cy-3-rut remained. Protocatechuic acid (PC), a ring fission product of Cy aglycone, was the major metabolite. In another study, Cy-3,5-di-glu was incubated with human fecal suspension (Fleschhut et al. 2006). More than 90% of the Cy-3,5-glu was degraded after 2 h, and partial hydrolysis generated Cy-mono-glu as a degradation intermediate, which also underwent degradation in the meantime. Corresponding generation and accumulation of PC was again observed. Further examination of di-acylated anthocyanins from red radish revealed that the acyl group could be cleaved by fecal microflora and that the released acids were relatively stable (Fleschhut et al. 2006). Deacylated anthocyanins would then follow the same pathway of degradation as discussed above.

## Metabolism in Intestinal Mucosa and Tissues

Several phase II drug detoxification enzymes involved in xenobiotic conjugation appear to be the key enzymes for flavonoid metabolism after absorption. Catechol-O-methyltransferase

(COMT; EC 2.1.1.6), which occurs in various tissues, may transfer a methyl group to the flavonoid aglycone (Ichiyanagi et al. 2005, Kuhnle et al. 2000). Uridine diphosphoglucose glucuronosyl transferase (UDPGT; EC 2.4.1.17) and uridine diphosphoglucose glucose dehydrogenase (UDPGD; EC 1.1.11.22), both abundant in liver and intestine, were proposed to catalyze the glucuronidation of flavonoid aglycones (Yang et al. 1998). Cytosolic enzymes phenol sulfotransferases (SULT; EC 2.8.2.1) are widely distributed throughout the body. They are likely to sulfate flavonoids (Scalbert & Williamson 2000).

Some of the metabolites contribute to the bioactivity of flavonoids. For instance, methylated Cy-3-glu is converted to Pn-3-glu (Wu et al. 2002). Benzoic acid generated by the metabolism of quercetin-3-rut may provide antioxidant activity or even anticancer effects (Olthof et al. 2003). Equol as a colonic metabolite of daidzein is more estrogenic than daidzein and the other metabolites of isoflavones (Setchell et al. 2002). Similarly, it is possible that some of the degradation products of anthocyanins may possess enhanced activity as compared with the parent compounds.



## Tissue Distribution

The protective effects of flavonoids have been associated with diseases occurring in various tissues, but such claims are mainly based on in vitro evidence using different types of cell lines. Knowledge about their availability to target tissues is quite limited. Quercetin is one of the well-investigated flavonoids regarding distribution in tissues. For example, two groups of rats fed either 0.1% or 1% quercetin diet for 11 weeks demonstrated the same pattern of tissue distribution (de Boer et al. 2005). The combined concentration of quercetin and its metabolites was high in lung, testes, and kidney; moderate in thymus, heart, and liver; low in brown fat, muscle, and bone; and extremely low in white fat, brain, and spleen. The highest tissue concentrations were 3.98 nmol $g^{-1}$ and 15.3 nmol $g^{-1}$ in the lung for diets with 0.1 and 1% quercetin, respectively. The authors also reported that the liver (5.87 nmol $g^{-1}$ tissue) and kidneys (2.51 nmol $g^{-1}$ tissue) contained high concentrations of quercetin in pigs fed 500 mg quercetin $kg^{-1}$ body wt diet for 3 days, whereas brain, heart, and spleen had much lower concentrations.

Anthocyanin distribution in tissues has recently been evaluated in rat and pig models. Male Wistar rats were fed blackberry extract (370 nmol anthocyanin/d) for 15 d and killed at 3 h after the beginning of the last meal. Total anthocyanins averaged 605 nmol $g^{-1}$ in jejunum, 68.6 nmol $g^{-1}$ in stomach, 3.27 nmol $g^{-1}$ in kidney, 0.38 nmol $g^{-1}$ in liver, and 0.25 nmol $g^{-1}$ in brain (Talavéra et al. 2005). In pigs fed diets supplemented with 0, 1, 2, or 4% w/w blueberries for 4 weeks and fasted for 18–21 h before euthanasia, 1.30 pmol $g^{-1}$ of anthocyanins were identified in the liver, 1.58 pmol $g^{-1}$ in eyes, 0.878 pmol $g^{-1}$ in cortex, and 0.664 pmol $g^{-1}$ in cerebellum (Kalt et al. 2008). The results suggested that anthocyanins may potentially provide protection for brain and eye tissues after crossing the blood-brain barrier and the blood-retinal barrier, evidence also supported by another independent study using aged blueberry-fed rats (Andres-Lacueva et al. 2005).

## Excretion

Unabsorbed flavonoids are excreted through feces (Griffiths & Barrow 1972, He et al. 2005, Wiseman et al. 2004). The absorbed intact anthocyanins and flavonoid aglycones are largely excreted in urine (Felgines et al. 2002, McGhie et al. 2003). Conjugated flavonoid metabolites are likely excreted in urine as well (Wu et al. 2002), but alternatively a portion of them may reenter the jejunum with the bile, and later either are absorbed by the colon entering the enterohepatic circulation again (Ichiyanagi et al. 2005, 2006), or are excreted with feces. The lung has been reported as a major excretion site for many phytochemicals including quercetin (Walle et al.

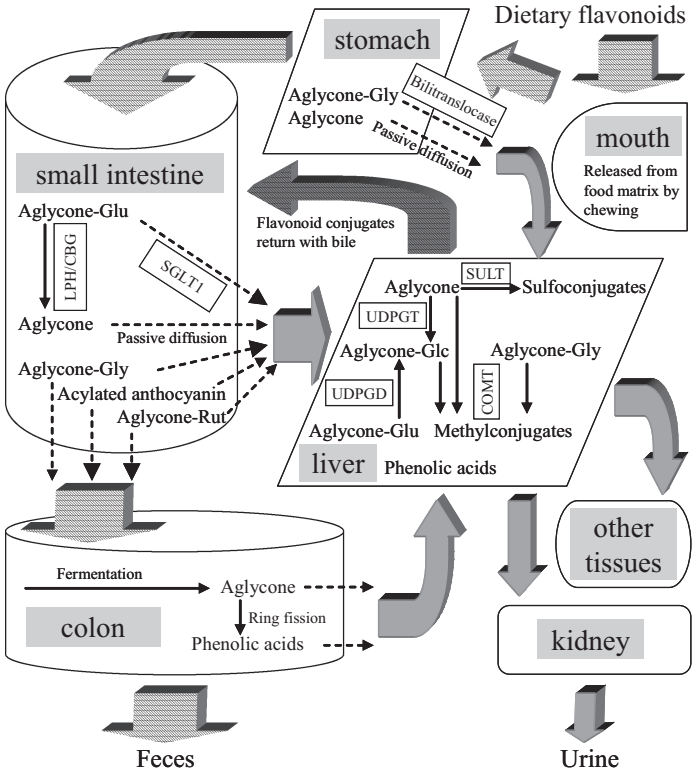


**Figure 5**

Intergrated putative pathways of dietary flavonoids absorption, metabolism, distribution, and excretion.

2001). More than 50% of the orally ingested $^{14}$C-labeled quercetin was found exhaled as $^{14}CO_2$ in humans. However, there are no data regarding the respiratory excretion of anthocyanins.

Understanding the bioavailability and metabolism pathway is important to the health benefits evaluation of anthocyanins. Such knowledge is also necessary for the screening of suitable anthocyanins from numerous sources to facilitate development of functional foods/supplements that promote human health. In the past decade our knowledge of the bioavailability and metabolism of anthocyanins has steadily increased. The pathways reviewed in this section are summarized in **Figure 5**.

## FUTURE RESEARCH

Interest in anthocyanins has increased substantially over the past decades, and it is expected to continue to increase. There is a combination of driving forces for this increase, including interest from consumers, the food industry, and the scientific community.

From the standpoint of consumers, there is an increased awareness and interest about the potential impact of foods on health and, with this, an increasing demand for natural ingredients in contrast to the use of synthetic and/or artificial ingredients in foods. Consumers are willing to pay more for products that are perceived more natural, healthier, and with potential disease prevention benefits in addition to their nutritional value. This, in turn, is stimulating the food industry toward the incorporation of more natural ingredients into foods, including the use of

anthocyanin-based colorants as an alternative to the use of synthetic dyes. Use of anthocyanin-based colorants presents a number of challenges, including stability for processing and storage, compatibility with the matrix, their ability to produce the desired color as well as the fact that they may contribute aromas and flavors that may not be desirable for the final product. Good progress has been made over the past few decades. However, owing to the complexity of the different food matrices and constant development of new food products, combined with the wide variability of anthocyanin chemical structures, this is an area that will need continued attention for years to come. More stable anthocyanins will be investigated including acylated anthocyanins, deoxyanthocyanins, and pyrano-anthocyanins, among other less common chemical structures. Stabilization of anthocyanins through copigmentation with other phenolics or other food components also needs to be investigated further.

Many researchers also are fascinated with this class of compounds, long ignored from the point of view of health impacts, owing to their low absorption into the plasma. Over the past few decades, it has become evident that anthocyanins are compounds that deserve close attention. Their abundance in the gastrointestinal tract makes them likely to impact the health of that local microenvironment. Large bodies of in vitro and animal tests suggest they do. Clinical trials are underway to confirm those observations in humans. In addition, the low concentrations of anthocyanins found in the plasma seem to be enough for these compounds to impact a number of different processes, including inflammation, obesity, and diabetes, among others. This is intriguing, and it is clear that more research is needed to understand the mechanisms and effectiveness in vivo. Future studies are needed to better understand the transformations that these compounds undergo in vivo, from the oral cavity, through the GIT, and after absorption and metabolism. There is evidence that a large portion of the dietary intake of anthocyanins will remain in the GIT. However, there is still a portion of the dietary intake that remains unaccounted for. Some possibilities are degradation products, and others may involve binding to membranes or proteins, based on evidence from different laboratories around the world.

The search for the perfect anthocyanin-based colorant will not have universal application but may present itself in the form of a specific function for a particular application. And anthocyanin-based colorants will be more desirable because of their dual value of providing color and enhancing health, making foods more appealing and rewarding.

## SUMMARY POINTS

1. Anthocyanins belong to a subgroup of flavonoids. The combination of various aglycones, glycosylations, and acylations results in more than 635 anthocyanins in nature. Their aglycone structures undergo reversible transformation at different pHs.
2. The stability of anthocyanins is determined intrinsically by the types of glycosylation and acylation, and it is affected externally by the pH environment, temperature, light intensity, enzyme, and the presence of other compounds interacting with anthocyanin molecules.
3. Human consumption of anthocyanins is among the highest of all flavonoids, and the toxicity of dietary anthocyanins is extremely low.
4. Anthocyanins have been suggested to possess anti-inflammatory activity, anticarcinogenic activity, as well as preventive effects on cardiovascular diseases, obesity, and diabetes. All the putative health-promoting effects are more or less associated with their potent antioxidant property.

5. Accumulating evidences suggest that anthocyanin absorption occurs in the stomach and small intestine. Uptake into the epithelial tissues seems to be quite efficient, yet transportation into circulation, tissue distribution, and urine excretion are very limited.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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**Errata**

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