

# Anthocyanins

Subjects: [Food Science & Technology](#) | [Pharmacology & Pharmacy](#) | [Horticulture](#)

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Anthocyanins are biologically active water-soluble plant pigments that are responsible for blue, purple, and red colors in various plant parts—especially in fruits and blooms. Anthocyanins have attracted attention as natural food colorants to be used in yogurts, juices, marmalades, and bakery products. Numerous studies have also indicated the beneficial health effects of anthocyanins and their metabolites on humans, including free-radical scavenging and antioxidant activity.

Anthocyanin metabolites

antioxidants

cardioprotection

hepatoprotection

nephroprotection

neuroprotection

## 1. Introduction

Anthocyanins are pigments belonging to the flavonoid group, which is widely distributed in plants. They are responsible for blue, purple, and red colors in flowers, fruits, and vegetables and protect plants from environmental stresses such as high sunlight irradiance [1] or low nitrogen [2]. Chemically, anthocyanins are produced when anthocyanidins are glycosylated. The most abundant anthocyanidin in plants is cyanidin. Other anthocyanidins are less abundant, and their frequency decreases in this order: delphinidin, peonidin, pelargonidin, petunidin, and malvidin [3]. Anthocyanidins are flavylium ion derivatives that vary in terms of their substituent groups: –H, –OH, or –OCH<sub>3</sub>. Usually, anthocyanidins are glycosylated at the C3 or C3 and C5 sites, but the glycosylation of other sites has also been reported [4]. The biological activity of anthocyanins depends on their structure; however, all samples, including those with different compositions and amounts of anthocyanins, extracted from various berries and vegetables, are biologically active [5]. Azevedo et al. [6] established that the radical scavenging activity and reducing properties of anthocyanins strongly depend on the chemical structures of particular anthocyanins; this effect increases with the presence of catechol and pyrogallol groups in ring B of cyanidin-3-glucosides and the respective aglycones. Some studies have shown that delphinidin has the highest antioxidant activity compared with the other five anthocyanidins due to the three hydroxyl groups on the B-ring [5][7]. An increasing body of evidence shows that anthocyanin intake can have a protective effect on human and animal brains, hearts, livers, and kidneys, and many of the therapeutic effects may be purported to the antioxidant activities of anthocyanins and their metabolites [8][9][10][11][12]. The antioxidant activity of these compounds manifests through direct and indirect methods of action. Thus, anthocyanins can directly scavenge reactive oxygen species (ROS) [13][14], whereas the indirect pathways involve stimulation of the synthesis or activity of antioxidant enzymes (catalase, superoxide dismutase (SOD), glutathione peroxidase) [15]; inhibition of ROS-forming enzymes, such as nicotinamide adenine dinucleotide

phosphate (NADPH) oxidase and others [16][17]; or even mild uncoupling of mitochondrial respiration preventing ROS generation [9][18]. It can also be assumed that for effective therapeutic action of anthocyanins, both the ROS scavenging activity and the modulation of cellular antioxidant systems are required [14].

## 2. Natural Sources of Anthocyanins

Anthocyanins are natural, water-soluble plant pigments that are responsible for blue, red, or purple colors in plants. Plant genotypes, agro-climatic conditions, and fruit or vegetable maturity are significant factors in the composition and quantity of anthocyanins [19]. Therefore, the main sources of anthocyanins in human diet are fruits and vegetables, which accumulate anthocyanins in both the peel and flesh; however, their content varies greatly (Table 1).

Table 1. Maximum amount of anthocyanins (mg 100 g<sup>-1</sup> of fresh weight (FW)) in fruits and vegetables.

Source	Anthocyanin amount mg 100 g <sup>-1</sup> FW	Dominant Anthocyanins	References
Bilberry	772.4	Dp3gal, Dp3glc, Dp3ara, Mv3glc, Cy3gal, Cy3glc, Cy3ara	[20]
Blackcurrant	478.6	Dp3rut, Cy3rut, Dp3glc, Cy3glc	
Golden currant	615.5	Cy3rut, Cy3glc, Pn3rut	[21][22]
Redcurrant	66.7	Cy3glc, Cy3rut, Cy3sam	
Elderberry	580.0	Cy3sam, Cy3glc	[19]
Grapes	116.4	Mv3glc, Cy3glc, Dp3glc, Pt3glc, Pn3glc	[23]
Sour cherry	147.0	Cy3rut	[24]
Sweet cherry	244.0	Cy3rut, Pn3rut	[25]

Wild strawberry	10.0	Pg3glc, Cy3glc	[26]
Black carrot	126.4	Cy3xylglcgal, Cy3xylgal	[27]
Eggplant	8.7	Dp3glc, Dp3rut,	[28][29]
Red cabbage	23.4	Cy3glc, Cy3rut, Dp3glc, Dp3rut, Cy3diglc5glc	[30][31]
Red chicory	39.2	Cy3glc	[29]
Purple wheat	23.5	Cy3glc	[32]

Cy—cyanidin; Dp—delphinidin; Mv—malvidin; Pg—pelargonidin; Pn—peonidin; Pt—petunidin; ara—arabinoside; gal—galactoside; glc—glucoside; rut—rutinoside; sam—sambubioside; xyl—xyloside.

### 3. Stability of Anthocyanins in Foods and Beverage

Anthocyanins are natural plant compounds that are increasingly being used in the food and pharmaceutical industry due to their effects on human health. However, the low stability of anthocyanins is still an obstacle to their use [33]. The stability of anthocyanins depends on the pH, temperature, light, presence of solvents and oxygen, and other factors [34]. Anthocyanins are more stable under acidic conditions. At a pH of 1.0, flavylium cations are the predominant species and contribute to the development of purple and red pigments, while at pH 2.0–4.0, the blue quinoidal species predominates. When the pH value reaches 7.0, anthocyanins are usually degraded [34]. The storage temperature affects the concentration of anthocyanins in extracts; for instance, 11% of rosella anthocyanins were lost after storage for 60 days at 4 °C, while 99% of anthocyanins were degraded in the same extracts stored at 37 °C for the same period [35].

The stability of anthocyanins from various *Ribes* species was reported to depend on their composition and storage conditions [31]. Anthocyanins in redcurrant berry extract have been shown to be more stable at room temperature and in the presence of light than extracts from berries of golden currant and gooseberry. After the storage of anthocyanin extracts under dark and cold conditions (+4 °C) for 84 days, up to 90% of redcurrant, 80% of gooseberry and golden currant, and up to 50% of blackcurrant anthocyanins remained intact [31].

Thermal food processing negatively affects the nutritional value of anthocyanin-rich juices as it results in anthocyanin degradation [36]. Thermal processing is responsible for the loss of up to 35% of anthocyanins. Even short-term thermal treatment (5 s at 85 °C) resulted in a loss of 9% of anthocyanins from strawberry juice, while

pasteurization for 15 min resulted in a loss of 21% [37]. Boiling of red cabbage resulted in a loss of 41.2% of anthocyanins, while the anthocyanin concentration remained the same after steaming or stir-frying. Possibly, as highly water-soluble pigments, anthocyanins may be lost by leaching in the case of boiling [5].

Various anthocyanin stabilization methods are being developed. For instance, the improvement of anthocyanin thermal stability by yeast mannoproteins at pH 7.0 has been studied. The complexes were found to effectively protect anthocyanins from degradation during heating at 80 and 126 °C [38]. Mixing of clarified acerola juice with montmorillonite resulted in 50% more anthocyanins, regardless of time or pH changes [39]. Copigmentation is another natural tool that can be used to enhance anthocyanin stability. The most studied copigments are phenolic acids such as hydroxycinnamic and hydroxybenzoic acids [40]. Babaloo and Jamei [41] established that caffeic acid provides more stability for anthocyanins than benzoic, tannic, and coumaric acids. Encapsulation with polysaccharides, such as  $\beta$ -cyclodextrin, maltodextrin, or Arabic gum, is also important for the stabilization of anthocyanins. The protective effect of  $\beta$ -cyclodextrin was evident for all blackberry anthocyanins after thermal treatment at 90 °C for 2 h [42].

Novel techniques for phenolic compound isolation from natural products that avoid the degradation of these compounds have been developed. Block freeze concentration has been employed to extract anthocyanins from strawberries and enrich yogurt with the obtained concentrated strawberry juice. As a result, yogurt with a high anthocyanin content and greater antioxidant activity was produced; however, it had a short shelf life [43]. The foam mat drying technique is now considered to be an effective dehydration method to produce powder from juices or pulp. Only a small reduction in anthocyanins (7–9%) was observed after the storage of jambolana juice powder produced by foam mat drying for 150 days [44]. When foam-mat freeze-drying was used for powder production from blueberry juice, 80–100% of anthocyanins remained after processing; the most stable was Cy3glc [45].

## 4. Biological Effects Related to the Antioxidant Activity of Anthocyanins—In Vivo Studies in Humans

Epidemiological and clinical studies suggest that an anthocyanin-enriched diet may lower levels of certain oxidative stress biomarkers in humans, and this could be associated with reduced risk of cognitive decline and the development of neurodegenerative and cardiovascular diseases, as well as 358 having sustained hepatic function and kidney protecting activities [19][46][47][48][49][50][51][52][53][54][55][56][57].

A randomized clinical trial [58] evaluated the effects of a standardized maqui berry (*Aristotelia chilensis* (Mol.) Stuntz) extract (containing 162 mg of anthocyanins) on products of lipid peroxidation in healthy, overweight, and smoker adults. The results suggested that supplementation with the extract can be related to a limited term (max for 40 days) reduction in oxidized low-density lipoprotein (LDL) levels and a decrease in urinary F2-isoprostanes. Another study [59] concluded that the acute consumption of anthocyanin-rich red *Vitis labrusca* L. grape juices could be related to decreased levels of thiobarbituric acid reactive substances and lipid peroxides in the serum of

healthy subjects. It has also been demonstrated that regular (for 30 days) anthocyanin-rich sour cherry consumption could suppress the formation of ROS by circulating phagocytes and decrease the risk of systemic imbalance between oxidants and antioxidants [60]. It is worth noting that a portion (300 g) of blueberries, the dietary source of anthocyanins provided to young volunteers involved in a randomized cross-over study, significantly reduced  $H_2O_2$ -induced DNA damage in blood mononuclear cells [61]. In another human pilot intervention study, the consumption of anthocyanin-rich bilberry (*Vaccinium myrtillus L.*) pomace extract was found to modulate transcription factor E2-related factor 2 (Nrf2)-dependent gene expression in peripheral blood mononuclear cells [62].

A single-blind randomized placebo-controlled intervention trial, which lasted for 8 weeks and involved 72 unmedicated subjects, revealed that the administration of various berries (including bilberries, chokeberries, and blackcurrants) increased both the concentration of high-density lipoprotein (HDL) cholesterol and the plasma antioxidant capacity [63]. Higher dietary anthocyanin and flavan-3-ol intake was associated with anti-inflammatory effects in 2375 Framingham Heart Study Offspring Cohort participants [64]. Interestingly, the consumption of 300 mL of red wine (a total dose of anthocyanins was 304  $\mu M$ , which was the highest amount among detected compounds) with a meal was shown to prevent the postprandial increases in plasma lipid hydroperoxides and cholesterol oxidation products and therefore protect against a potential pro-atherosclerogenic effect [65]. Similar findings were obtained in a randomized cross-over trial, in which it was concluded that moderate consumption of red wine decreases erythrocyte SOD activity [66]. In another randomized double-blind trial, 150 subjects with hypercholesterolemia consumed a purified anthocyanin mixture derived from bilberries and blackcurrants (320 mg/d) for 24 weeks [67]. It was found that anthocyanin consumption significantly decreased the levels of inflammatory biomarkers (C-reactive protein, soluble vascular cell adhesion molecule-1 and plasma IL-1 $\beta$ ) and increased the HDL cholesterol level. Recently, it was shown that a daily intake of 150 g of anthocyanin-rich blueberries resulted in clinically relevant improvements in endothelial function and systemic arterial stiffness; probably due to the improved nitric oxide bioactivity and HDL status [68].

Nonalcoholic fatty liver disease (NAFLD), defined by excessive lipid accumulation in the liver, is the hepatic manifestation of insulin resistance and metabolic syndrome. NAFLD encompasses a wide spectrum of liver diseases ranging from simple uncomplicated steatosis, to steatohepatitis, cirrhosis, and hepatocellular carcinoma [48]. Zhang et al. [69] reported that anthocyanins extracted from bilberry and blackcurrant (320 mg/day) and administered for 12 weeks ameliorated liver injury in patients with NAFLD. It was observed that a so called “anthocyanin group” exhibited significant decreases in the plasma alanine aminotransferase, cytokeratin-18 M30 fragment, and myeloperoxidase levels. It was also found that consumption of *Myrica rubra* Sieb. and Zucc. juice (250 mL for 4 weeks) protected young adults (18–25 years old) against NAFLD by improving the plasma antioxidant status and inhibiting the inflammatory and apoptotic responses involved in this disease [70].

In another study, red fruit juice (40% red grape juice, 20% blackberry juice, 15% sour cherry juice, 15% blackcurrant juice, and 10% elderberry juice) with high polyphenol and anthocyanin contents was tested for its preventive potential in hemodialysis patients [71]. For this purpose, 21 subjects consumed 200 mL/day of juice according to the following protocols: 3-week run-in; 4-week juice 411 uptake; 3-week wash-out. The results revealed a significant decrease in DNA oxidation damage and protein and lipid peroxidation and an increase in the reduced glutathione level; the effects were attributed to the high anthocyanin and polyphenol contents of the juice [71]. Another study [72] demonstrated that regular consumption of concentrated red grape juice by hemodialysis patients could be associated with the reduced neutrophil NADPH-oxidase activity and plasma concentrations of oxidized LDL and inflammatory biomarkers.

## 5. Conclusion

In conclusion, anthocyanins are valuable biomolecules with a broad variety of biological effects on human health, and we suggest adding more anthocyanin rich fruits, vegetables, and their products to the daily diet. Numerous studies indicate that bilberry, blackcurrant, elderberry, and other berries have the highest total concentrations of anthocyanins; therefore, the consumption of fresh berries and their processed products may have greater beneficial effects on humans. Various anthocyanin stabilization methods have been developed e.g., copigmentation with other phenolic acids, encapsulation with polysaccharides, block freeze concentration, and powder production using the foam mat drying technique. All of them enable anthocyanins to be preserved during processing, thus increasing their bioavailability and delivery to target tissues in the human body. However, long-term studies on the impacts of anthocyanin-rich product consumption on human health are still rare. Further studies could focus on the identification and tracking of individual anthocyanin metabolites and on the determination of the exact dosage and delivery platforms sustaining the antioxidant properties of anthocyanins *in vivo*. In addition, as an alternative to natural sources, synthesis of the most bioactive anthocyanins in bioreactors should be considered.

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