Anthocyanins and heart health

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Summary. Anthocyanins are the largest group of water-soluble pigments in the plant kingdom and belong to the family of compounds known as flavonoids. Major sources of anthocyanins are blueberries, cherries, raspberries, strawberries, black currants, purple grapes and red wine. In recent years several studies have shown that anthocyanins display a wide range of biological activities including antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic activities. In addition they display a variety of effects on blood vessels, platelets and lipoproteins able to reduce the risk of coronary heart diseases. However, until the absorption and metabolic fate of anthocyanins *in vivo* is unravelled, it would be unwise to conclude that a high consumption of them will reduce the risk of chronic diseases. Long-term intervention trials must be properly designed and carried out to provide definite proof. In the meantime a more complete knowledge of the identity of anthocyanin metabolites and their tissue distribution should be reached.

Key words: anthocyanins, bioavailability, cardiovascular disease.

Riassunto *(Antociani e salute del cuore)*. Gli antociani sono il più ampio gruppo di pigmenti idrosolubili presenti nel regno vegetale ed appartengono alla famiglia dei flavonoidi. Le principali fonti di antociani sono mirtilli, ciliegie, lamponi, ribes, fragole, uva nera e vino rosso. Recentemente numerosi studi hanno dimostrato che gli antociani sono in grado di esercitare attività biologiche di vario tipo fra le quali quelle antiossidante, anti-infiammatoria, anti-microbica, anti-carcinogenica. Inoltre essi esercitano vari effetti su vasi sanguigni, piastrine e lipoproteine, capaci di ridurre il rischio di malattie cardiache. D'altra parte, finché non saranno chiariti sia l'entità del loro assorbimento che il loro destino metabolico *in vivo*, potrebbe essere imprudente concludere che un elevato consumo di antociani è in grado di ridurre il rischio di malattie croniche. Trials clinici a lungo termine, condotti seguendo protocolli appropriatamente disegnati e ben controllati, sono pertanto necessari per ottenere una prova definitiva della loro efficacia. Nello stesso tempo il raggiungimento della piena conoscenza dei metaboliti attivi e della loro distribuzione nei tessuti è imprescindibile al fine di comprenderne il reale meccanismo di azione *in vivo*.

Parole chiave: antociani, biodisponibilità, malattia cardiovascolare.

INTRODUCTION

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom [1]. Chemically, they are polyhydroxylated or polymethoxylated glycosides or acylglycosides of anthocyanidins which are oxygenated derivatives of 2-phenylbenzopyrylium or flavylium salts [1, 2]. They belong to the family of compounds known as flavonoids, and they are distinguished from other flavonoids as a separate class by virtue of their ability to form flavylium cations [1-3].

Anthocyanins are responsible for most of the red, blue, and purple colors of fruits, vegetables, flowers, and other plant tissues or products. They are particularly abundant in berries and other fruits with red, blue, or purple color, and in red wines [1]. Color differences between anthocyanins are largely determined by the substitution pattern of the B-ring of the aglycone, the pattern of glycosylation, and the degree and nature of esterification of the sugars with aliphatic or aromatic acids, and by the pH, temperature, type of solvent, and the presence of co-pigments [1-6]. Approximately 400 individual anthocyanins have been identified [1, 7-9]. The six anthocyanidins commonly found in plants are classified according to the number and position of hydroxyl and methoxyl groups on the flavan nucleus, and are named pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin *(Figure 1)*. The most commonly occurring anthocyanidin in nature is cyanidin.

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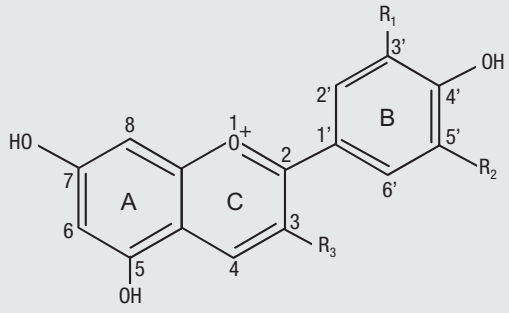

Substitution pattern

	R_1	R_2	R_3
Delphinidin	OH	OH	OH
Cyanidin	OH	H	OH
Petunidin	OCH_3	OH	OH
Peonidin	OCH_3	H	OH
Malvidin	OCH_3	OCH_3	OH
Pelargonidin	H	H	OH

Fig. 1 | *Structural classification of common anthocyanidin species.*

The average intake of dietary flavonoids is estimated at about 23 mg/day for Holland [10] and 650 mg/day for USA [11, 12]. The daily intake of anthocyanins in humans has been estimated at 180-215 mg/d in USA [11]. This value is considerably higher than the intake of other flavonoids such as flavones and flavonols in the Dutch diet (23 mg/d, measured as aglycones) [10]. Major sources of anthocyanins are blueberries, cherries, raspberries, strawberries, black currants, purple grapes and red wine. Servings of 100 g of berries can provide up to 500 mg of anthocyanins [1].

Anthocyanin biological activities

In recent years, numerous studies have shown that anthocyanins display a wide range of biological activities [13, 14] including antioxidant [15-18], anti-inflammatory [19, 20], antimicrobial [21] and anti-carcinogenic activities [22-24]; improvement of vision [25, 26]; induction of apoptosis [27]; and neuroprotective effects [28, 29].

In addition, anthocyanins display a variety of effects on blood vessels [30, 31] and platelets [32, 33] that may reduce the risk of coronary heart disease [34].

Anthocyanins are potent antioxidant superior to classical antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoulene (BHT), and α-tocopherol [15, 17]. Glycosylation of an anthocyanin decreases radical scavenger activity compared with the aglycone, as it reduces the ability of the anthocyanin radical to delocalize electrons. In accordance with this, Fukumoto and Mazza [17] reported increased antioxidant activity with increase in the hydroxyl groups and decreased antioxidant activity with glycosylation of anthocyanidins *(Table 1)*.

The antioxidant activity (scavenging free radicals, metal chelation, protein binding) of anthocyanins including the protection of LDL against oxidation, has been demonstrated in a number of different *in vitro* systems [35-37]. Recently, we found that pelargonidin, cyanidin, delphinidin, peonidin, malvidin, malvidin 3-glucoside, and malvidin 3,5-diglucosides have strong inhibitory effects on NO production in LPS/IFN-γ-activated RAW 264.7 macrophage. At the range of 16-500 μM, these compounds inhibited NO production by >50% without showing any cytotoxicity [20]. Their inhibitory effects were comparable to that of quercetin, which has been extensively studied and shown to exert anti-inflammatory and antioxidant effects. Anthocyanin-rich berry extracts also showed considerable inhibitory effects on NO production, and their inhibitory effects were significantly correlated with the content of total anthocyanins. Additionally, Cao *et al.* [38] reported increased serum antioxidant capacity measured as ORAC, TEAC and TRAP, after the consumption of strawberries or red wine; and Mazza *et al.* [18] found that the concentration of anthocyanins in the serum of male subjects that had consumed 1.2 g anthocyanins from freeze dried blueberries was positively correlated with the serum antioxidant capacity *(Figure 2)*.

Anthocianyns and heart diseases

The association between grape phenolics and coronary heart disease has been ascribed in part to the presence of anthocyanins in red wine [39, 40]. In addition, several epidemiological studies have shown that coronary heart disease mortality can be decreased by moderate consumption of red wine [41-43]. The primary mechanisms believed to be responsible for this reduced risk factor include reduced platelet coagulability [44, 45], and higher circulatory

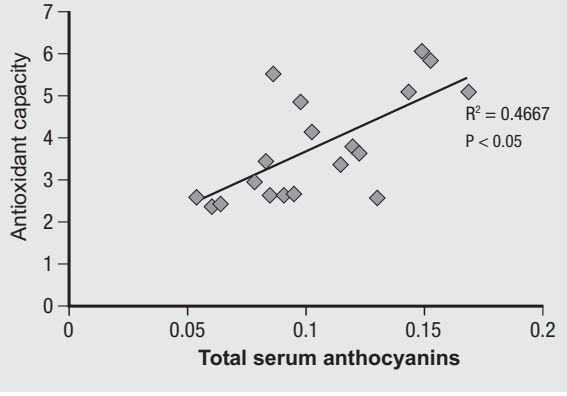

Fig. 2 | *Correlation between serum antioxidant capacity as measured by ORAC-total and concentration of total serum anthocyanins. Antioxidant value expressed as 1000 x μm Trolox equivalents/L, and anthocyanins expressed as cyanidin-3-glucoside chloride equivalents (Reprinted with kind permission from [18]).*

Table 1 | *Antioxidant and prooxidant activity of selected anthocyanins (adapted from: Fukumoto and Mazza [17])*

Compound	Current study				Selected results from literature	
	β-carotene method	DPPH method	HPLC method		DPPH assay[1]	ORAC assay[2]
	Initial slope[3] (x 10^{-6})	Antiradical activity[4]	Antioxidant activity[5] (μM of compound added)	Prooxidant activity[6]	Antiradical power[7] [antiradical activity]	ORAC slope[8]
Cyanidin	836 ± 69	-7.40 ± 0.15	200-300	+		2.239 ± 0.029
Cyanidin 3-glucoside	278 ± 32	-6.81 ± 0.30	300-400	+		3.491 ± 0.011
Cyanidin 3,5-diglucoside	220 ± 39	-3.32 ± 0.07	500-1000	+		1.689 ± 0.052
Delphinidin	897 ± 147	-8.86 ± 0.28	500-1000	+		1.809 ± 0.068
Malvidin	288 ± 34	-4.49 ± 0.28	1500-2000	+		2.009 ± 0.167
Malvidin 3-glucoside	448+40	-4.29 ± 0.42	500-1000	+		1.404 ± 0.052
Malvidin 3,5-diglucoside	266 ± 27	-2.56 ± 0.10	2000-2500	+		1.550 ± 0.062
Pelargonidin	nc	-4.63 ± 0.25	1500-2000	+		1.540 ± 0.033
Pelargonidin 3-glucoside	444 ± 94	-3.95 ± 0.22	2000-2500	+		1.560 ± 0.145
Pelargonidin 3,5-diglucoside	nc	-2.04 ± 0.10	2000-2500	+		1.067 ± 0.043
Peonidin	169 ± 22	-4.05 ± 0.17	1500-2000	+		1.693 ± 0.035
Peonidin 3-glucoside	251 ± 4	-3.38 ± 0.15	2500-3000	+		1.805 ± 0.014
Standards						
Ascorbic acid	nc	-1.83 ± 0.07	> 4000	+	3.7 [-1.85]	
α-Tocopherol	870 ± 21	-1.95 ± 0.07	2000-2500 (50%)	nd		
BHA	835 ± 50	-2.61 ± 0.01	1000-1500	nd	4.17 [-2.09]	
BHT	864 ± 76	-3.17 ± 0.07	200-300	nd	4.2 [-2.1]	

[1]*The DPPH assay used is described in Fukumoto and Mazza [17]*

[2]*The oxygen radical absorbing capacity (ORAC) assay measuring reaction with peroxyl radicals expressed as μM of Trolox equivalent per μM of compound. Results for anthocyanidins/anthocyanins were taken from Wang* et al. *[15].*

[3]*Values are means of slope coefficients calculated by linear regression ± standard deviations (n. = 3) in A450 nm after 90 minutes of incubation in the dark/μM of compound added.*

[4]*Values are means of slope coefficients calculated by linear regression ± standard deviations (n. = 3) in μM of DPPH/μM of compound.*

[5]*Antioxidant activity was defined by the concentration range of compound added needed to reach 0% malonaldehyde of the control.*

[6]*Prooxidant activity was positive (+) if the % malonaldehyde of the control was >100% in the concentration range tested.*

[7]*Antiradical power was defined as the reciprocal of the amount of antioxidant needed to decrease the initial DPPH concentration by 50%. The antiradical activity was equivalent to negative half of the antiradical power.*

[8]*Values are slope coefficients calculated by linear regression ± standard error.*

nc: not calculated since linear regression $r^2 < 0.800$.

nd: not detected.

high-density lipoprotein cholesterol (HDL) [42, 43]. Other mechanisms such as inhibition of lipoprotein oxidation, free-radical scavenging and modulation of eicosanoid metabolism [46-49] are also thought to play a role in the reduction of atherosclerosis.

A study of the relationships between vasodilation capacity, antioxidant activity and phenolic content of 16 red wines reported that total phenol content correlated well with vasodilation capacity and antioxidant activity of the wines, but only anthocyanins were correlated with vasodilation capacity [50]. Other recent studies support the hypothesis that vasodilation activity is connected to skin-derived compounds in the grapes. Andriambeloson *et al.* [51] found that only the anthocyanin and oligomeric condensed tannin-containing fractions of red wine showed vasorelaxant activity comparable to the original polyphenol fraction of the red wine. The phenolic acid derivatives, hydroxycinnamic acids and flavanol classes tested failed to induce this type of response.

Anthocyanin absorption and metabolism

Biological activities of anthocyanins are closely linked to their absorption and metabolism. Recent evidence from several laboratory indicates that anthocyanins are rapidly absorbed from both the stomach [52, 53] and small intestine [53, 54], and appear in blood circulation and urine as intact, methylated, glucuronide derivatives and/or sulfoconjugated forms [18, 55-58]. Recently, in rats fed an anthocyanin-rich diet for 15 days, anthocyanins have been found in several organs, including stomach, small intestine (jejunum), liver, kidney and brain. In the brain, total anthocyanin content (blackberry anthocyanins and peonidin 3-*O*-glucoside) reached 0.25 ± 0.05 nmol/g of tissue [59].

The metabolites persist in the urine for up to 24 h and may retain their basic anthocyanin structure [57, 58]. Pharmacokinetic evidence suggests that the concentration of the parent glycosides and their glucuronide derivatives are prominent in early blood samples (0-5 h), with increasing methylation occurring over time (6-24 h). This evidence suggests that anthocyanins bioactivity is likely altered over time as a result of metabolic transformation post consumption.

The absorption of anthocyanins from food is limited and the concentrations found in plasma are in the nM to low μM range [32, 52, 57-67]. In a very recent study conducted in our laboratory [58], the total cumulative concentration of anthocyanins (parent and metabolites) detected in the serum over a 7 h sampling regime was 172.96 ± 7.44 μg·h/mL, with a maximum concentration of 44.86 ± 2.82 μg/mL (Cmax) occurring within 2.8 h (tmax). Only 32.7% (52.54 μg·h/mL) of the total anthocyanins detected in the serum was the parent cyanidin 3-glycosides with an average of 67.3% identified as conjugated metabolites. Additionally, the total urinary excretion of metabolites and parent compounds over 24 h was 1071.54 ± 375.46 μg, reaching a maximal rate of excretion (Rmax) of 202.74 ± 85.06 μg/h at 3.72 ± 0.83 h (tmax) and having an elimination half-life (t1/2) of 4.12 ± 0.4 h. Correspondingly, only 32.5% (347.85 μg) of the anthocyanins excreted in the urine were the parent compounds with an average of 67.5% (723.69 μg) occurring as conjugated metabolites. Although, the absorption and elimination of parent anthocyanins appears relatively low compared to the initial dose (0.048%), glucuronidated and methylated anthocyanin metabolites were at levels more than twice that of the parent (intact) compounds [58].

Most other studies have also reported low relative urinary excretion, ranging from 0.004% to 0.1% of the intake [68], although Lapidot *et al.* [69] and Felgines *et al.* [56] measured higher levels of anthocyanin excretion (up to 5%) after red wine or strawberry consumption.

The seemingly low bioavailability of anthocyanin is not clearly apparent. It can be due to several reasons, including the likelihood that the concentration of some metabolites, such as protocatechuic acid, might have been below the detection limit of the analytical methods used. Also, with the predominance of the colourless carbinol (75-80%) and chalcone (15-20%) forms of anthocyanins present in blood and urine at neutral pHs, it is highly likely that these chemical forms may escape detection, and/or be chemically bound to other components in the plasma or urine, and therefore not included in the analyzed fraction. These shortcomings can be overcome by using labelled anthocyanins for identification of all metabolites generated from these flavonoids.

Conjugation likely affects the biological activity of anthocyanins and these metabolic products are probably responsible for the reported health benefits associated with the consumption of anthocyanin-rich products.

CONCLUSIONS

From the above, it is apparent that anthocyanins have diverse effects *in vitro* which suggest potential health benefits in general and reduction of coronary heart disease in particular. However, until the absorption and metabolic fate of anthocyanins *in vivo* is unravelled, it would be unwise to conclude that a high consumption of anthocyanis will reduce the risk of chronic disease, including heart disease.

Definite proof can only be obtained by large, long-term intervention trials. Such trials need to be seriously considered, and if initiated must be properly designed. In the meantime the evidence for the benefits connected with consumption of anthocyanin-rich products should include a more complete knowledge of the identity of anthocyanin metabolites and their tissue distribution using molecular, cell biology, animal and epidemiological studies. Future *in vitro* investigations on identifying the physiological effects of anthocyanins/flavonoids should be conducted with chemical structures that exist in the circulation (*i.e.* parent compound and metabolites) and at similar concentrations.

Acknowledgements

A shorter version of this paper is available from the electronic online food science journal, World of Food Science, accessible at www.worldfoodscience.org.

Submitted on invitation.
Accepted on 14 May 2007.

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