ARTICLES

Oxygen Radical Absorbing Capacity of Anthocyanins

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Anthocyanins are natural colorants belonging to the flavonoid family. They are widely distributed among flowers, fruits, and vegetables. Using the automated oxygen radical absorbance capacity (ORAC) assay developed in our laboratory, we determined the antioxidant capacity of 14 anthocyanins including the aglycons delphinidin, cyanindin, pelargonidin, malvidin, peonidin, and their derivatives with different sugar linkages. Among these anthocyanins, kuromanin (cyanidin-3-glucoside) had the highest ORAC activity, which was 3.5 times stronger than Trolox (vitamin E analogue), while pelargonin had the lowest antioxidant activity but was still as potent as Trolox. Different patterns of hydroxylation and glycosylation in anthocyanins appear to modulate their antioxidant properties. Therefore, in addition to their colorful characteristics, anthocyanins possess potent antioxidant properties.

Keywords: *Anthocyanins; flavonoids; colorants; ORAC; antioxidant; peroxyl radical*

INTRODUCTION

Anthocyanins are natural colorants belonging to the flavonoid family. They are widely distributed among flowers, fruits (particularly in berries), and vegetables and are responsible for the bright colors such as orange, red, and blue (Tables 1 and 2). They play a definite role in attracting animals in pollination and seed dispersal. They may also have a role in the mechanism of plant resistance to insect attack (Strack and Wray, 1993). The anthocyanins are glycosides and acylglycosides of anthocyanidins. Some common anthocyanidins with different hydroxyl or methoxyl substitutions in their basic structure, flavylium (2-phenylbenzopyrilium), are shown in Figure 1. There are over 250 naturally occurring anthocyanins (Strack and Wray, 1993), and all are *O*-glycosylated with different sugar substitutes (Francis, 1989). The most prevalent sugars substituted on the aglycon (anthocyanidins) in order of occurrence in nature are glucose, rhamnose, xylose, galactose, arabinose, and fructose. The common anthocyanins are either 3- or 3,5-glycosylated. When the number of sugar residues is higher than three, they may be attached to the basic molecule with alternating sugar and acyl linkages (Francis, 1989).

The daily intake of anthocyanins in humans has been estimated to be as much as 180−215 mg/day in the U.S. (Kühnau, 1976) due to their widespread distribution and occurrence in fruits and vegetables (Table 2). Despite the relatively high potential intake in humans, the physiological impact of the anthocyanins is not well studied. Nevertheless, anthocyanins have been shown to have some positive therapeutic effects including in the treatment of diabetic retinopathy (Scharrer and Ober, 1981), in fibrocystic disease of the breast in human (Leonardi, 1993), and on vision (Politzer, 1977; Timberlake and Henry, 1988). A commercial extract of *Vaccinium myrtillus* (bilberry), called *V. myrtillus* anthocyanin (VMA), containing largely glycosides of delphinidin and cyanidin (Baj et al., 1983) has been used to treat various microcirculation diseases resulting from capillary fragility (Mian et al., 1977; Timberlake and Henry, 1988) and has been used to maintain normal vascular permeability (Robert et al., 1977; Miskulin et al., 1980; Detre et al., 1986). VMA also prevents cholesterol-induced atherosclerosis in the rabbit (Kadar et al., 1979). Anthocyanins may also have other potential physiologic effects as antineoplastic agents (Kamei et al., 1995), radiation-protective agents (Minkova et al., 1990; Akhmadieva et al., 1993), vasotonic agents (Colantuoni et al., 1991), vasoprotective and antiinflammatory agents (Lietti et al., 1976), chemoprotective agents against platinum toxicity in anticancer therapy (Karaivanova et al., 1990), and hepatoprotective agents against carbon tetrachloride damage (Mitcheva et al., 1993), and possibly other effects due to their diverse actions on various enzymes and metabolic process (Carpenter et al., 1967; Wheeler et al., 1967; Ferrell et al., 1979; Gibb

Table 1. Color and Distribution of Major Anthocyanidins in Some Common Fruits and Vegetables

compd	color[a]	fruits and vegetables[a]
delphinidin	bluish red	Concord grape, blueberry, bilberry, black currant
cyanidin	orange red	strawberry, blackberry, rhubarb, black currant, cherry, red cabbage, bilberry, cranberry, elderberry, Concord grape, corn, plum, raspberry, red onion
pelargonidin	orange	strawberry, corn
malvidin	bluish red	grape, blueberry, bilberry
peonidin	red	cherry, cranberry, sweet potato, plum

[a] Sources: Francis (1989); Timberlake and Harry (1988); Strack and Wray (1993); Terahara et al. (1994).

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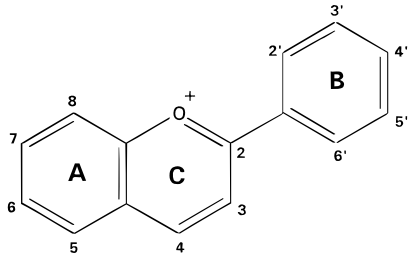
Table 2. Total Anthocyanin Content in Some Common Fruits and Vegetables[a,b]

fruits and vegetables	total anthocyanins
blackberry	83−326
blueberry	25−495
raspberry	
black	214−428
red	20−60
sweet cherry	350−450
cranberry	78
juice	18−87
strawberry[c]	7−30
juice[d]	21−333
red grapes	30−750
red wine[e]	100−1000
currant	
black	250
red	12−19
apple (Scugog)	10
red cabbage[f]	25
red onion	9−21

[a] All values expressed on fresh weight basis (mg/100 g) except values for juice and wine, which were expressed in mg/L. [b] Data from Mazza and Miniati (1993) unless noted differently. [c] From Kikoku et al. (1994). [d] From Bakker et al. (1994). [e] From Glories (1988). [f] From Timberlake and Henry (1988).



Substitution Patterns

Aglycone	3	5	7	3'	4'	5'
Delphinidin	OH	OH	OH	OH	OH	OH
Cyanidin	OH	OH	OH	OH	OH	H
Pelargonidin	OH	OH	OH	H	OH	H
Malvidin	OH	OH	OH	OCH_3	OH	OCH_3
Peonidin	OH	OH	OH	OCH_3	OH	H

Figure 1. General structure of anthocyanidins and substitution patterns of some common compounds.

et al., 1987; Saija et al., 1990; Costantino et al., 1995). No adverse effects were observed in animals fed a grape color extract containing principally anthocyanins (Becci et al., 1983a,b). Also there appears not to be any adverse effects in humans of grape skin extract as this extract was approved by the Food and Drug Administration to be used as a food colorant (Timberlake and Henry, 1988).

These above-observed biological activities of anthocyanins may contribute significantly to the beneficial effects of consumption of fruits and vegetables. The consumption of fruits and vegetables has been showed to protect against cancer (Doll 1990; Dragsted et al, 1993; Ames et al, 1993; Willett, 1994), cardiovascular disease (Armstrong et al., 1975; Verlangieri et al., 1985), and cerebrovascular disease (Acheson and Williams, 1983; Gillman et al., 1995). Since this protection was attributed to the various antioxidants contained in the fruits and vegetables (Ames, 1983; Gey, 1990; Steinberg et al., 1989; Steinberg et al., 1991) and the reported biological activities of anthocyanins could also be due to their antioxidant properties, the objective of this study was to assess the antioxidant capacity of some common anthocyanins by using an automated oxygen radical absorbance capacity (ORAC) assay (Cao et al., 1995) with a peroxyl radical (ROO•) generator.

MATERIALS AND METHODS

Chemicals. β-Phycoerythrin (β-PE) from *Porphydium cruentum* and pelargonidin were purchased from Sigma (St. Louis, MO). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). All other anthocyanins and aglycons (13 compounds) except pelargonidin were purchased from Indofine Chemical Co., Inc. (Somerville, NJ).

Automated ORAC assay. The procedure was based on a previous report of Cao et al. (1993), as modified for the COBAS FARA II (Cao et al., 1995). The automated ORAC assay was carried out on the COBAS FARA II centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ) with a fluorescence detector. The fluorescent filters were set to pass the light with an excitation wavelength of 540 nm and an emission wavelength of 565 nm. Since anthocyanins did not dissolve well in water and acetone, all compounds were dissolved in dimethyl sulfoxide (DMSO) first and then diluted with phosphate buffer (75 mM, pH 7.0). The final assay mixture contained 0.2% (v/v) DMSO with anthocyanins present in concentration ranges from 0.25 to 1 μM. DMSO at 0.2% (v/v) had little effect on the ORAC assay (data not shown). The same amount DMSO was also added to the blank and standard. Briefly, in the final assay mixture (0.4 mL total volume), β-PE (1.67×10^{-8} M) was used as a target of free radical damage, AAPH (4 mM) as a peroxyl radical generator, and Trolox as a control antioxidant standard. The analyzer was programmed to record the fluorescence every 2 min after AAPH addition. Final results [oxygen radical absorbance capacity against peroxyl radicals ($ORAC_{ROO•}$)] were calculated using the differences of areas under the quenching curves of β-PE between a blank and a sample and expressed as μmole of Trolox equivalent per μmole of compound.

Data Analysis. Linear regression analyses of ORAC activity (Y) versus anthocyanin concentrations (X) with four data points of each compound were computed using the regression procedure in Microsoft Excel for Windows. A linear fit ($Y = a_0 + a_1X$) adequately described the data as assessed by the correlation coefficient. The standard errors of the y-intercept (a_0) and the standard errors of estimate of the regression coefficient (a_1, slope) were obtained from the regression procedure. The a_1 coefficient represented the ORAC activity relative to Trolox as a standard.

RESULTS AND DISCUSSION

We tested five groups of available anthocyanins with a total of 14 compounds which are common colorants in fruits and vegetables. Least-squares regression lines were computed between anthocyanin concentration (μM) (X) and ORAC (μM Trolox equivalents) (Y). The best fit as assessed by the correlation coefficient was a linear line. The regression and correlation coefficients for the anthocyanins that were tested are presented in Table 3. All of the regression correlation coefficients (r) are greater than 0.98, which reflects the linearity between the concentration of the compounds and the ORAC (Trolox equivalent) value. Compounds with slopes greater than 1.0 will have a stronger antioxidant activity against peroxyl radicals than Trolox. The largest slope among the compounds tested was kuromanin (3.491) while the lowest was pelargonin (1.067), which was as potent as Trolox against peroxyl radicals. Other studies, using different antioxidant assays, have also shown that anthocyanins are antioxidants (Tamura and Yamagami, 1994; Yoshiki et al., 1995; Rice-Evans et al., 1995, 1996) and they prevent lipid peroxidation

Table 3. Regression Coefficients of Anthocyanins Concentration (μM) (X) and ORAC Activity (Y) (Trolox Equivalents, μM)

compd	common name	coefficients[a]		
		a_0[b] (intercepts)	a_1[c] (slopes)	r[d]
delphinidin		−0.024 ± 0.042	1.809 ± 0.068	0.997
cyanidin		0.019 ± 0.018	2.239 ± 0.029	0.999
cyanidin-3-glucoside	kuromanin	−0.004 ± 0.006	3.491 ± 0.011	0.999
cyanidin-3-rhamnoglucoside	keracyanin	0.045 ± 0.057	2.992 ± 0.093	0.998
cyanidin-3-galatoside	ideain	0.019 ± 0.028	2.027 ± 0.025	0.999
cyanidin-3,5-diglucoside	cyanin	−0.051 ± 0.059	1.689 ± 0.052	0.999
pelargonidin		−0.008 ± 0.020	1.540 ± 0.033	0.999
pelargonidin-3-glucoside	callistephin	0.190 ± 0.166	1.560 ± 0.145	0.991
pelargonidin-3,5-diglucoside	pelargonin	0.051 ± 0.050	1.067 ± 0.043	0.998
malvidin		0.100 ± 0.102	2.009 ± 0.167	0.989
malvidin-3-glucoside	oenin	0.026 ± 0.059	1.404 ± 0.052	0.998
malvidin-3,5-diglucoside	malvin	0.055 ± 0.071	1.550 ± 0.062	0.998
peonidin		0.034 ± 0.041	1.693 ± 0.035	0.999
peonidin-3-glucoside		−0.013 ± 0.017	1.805 ± 0.014	0.999

[a] Regression coefficients: Y (ORAC, μM Trolox equivalent) = $a_0 + a_1X$ (concentration, μM). [b] All a_0 coefficients are not significantly different from zero ($p > 0.05$). [c] All a_1 coefficients significantly greater than zero ($p < 0.05$). [d] Multiple correlation coefficient. [e] Since all a_0 coefficients are not significantly different from zero, the slope (a_1) directly reflects the antioxidant potency (ORAC) against peroxyl radicals. A slope of 1.0 would have the same potency as Trolox, a water-soluble α-tocopherol analogue.

(Tamura and Yamagami, 1994) and act as scavengers of superoxide anion (Sichel et al., 1991) and nitric oxide radical (van Acker et al., 1995).

Our data also enabled us to assess some structure−activity relationships in these compounds. By comparing the ORAC value of five aglycons (Table 3) and their substitution patterns (Figure 1), one can see the effect of different hydroxyl (OH) substitutions and methylations on antioxidant properties of anthocyanins against peroxyl radicals. Among the aglycons of the same hydroxylation pattern in the A and C rings, compounds with only one OH group in the B ring (4′-OH) including pelargonidin, malvidin, and peonidin had lower ORAC activities compared to a compound with 3′,4′-di-OH substitution (cyanidin). This is in agreement with results by Bors et al. (1990), indicating that the 3′- and 4′-OH in the B ring (catechol) structure are important determinants for the radical scavenging potential in flavonoids with a saturated 2,3-bond (in flavan-3-ols, flavanones, cyanidin chloride). However, delphinidin was an exception to this principle in this study since it also has 3′,4′-di-OH substitution but had a low ORAC activity. The importance of the hydroxyl groups on both the 3′ and 4′ positions of the B ring in contributing to the high antioxidant capacity has also been shown in flavones (Cao et al., 1997; Rice-Evans et al., 1996).

In other flavonoids, increasing the number of hydroxyl groups increases the antioxidant capacity (Cao et al., 1997). However, this does not hold true for the anthocyanidins where pelargonidin, cyanidin, and delphinidin have 4, 5, and 6 hydroxyl groups, respectively (Figure 1), but the ORAC values were 1.54, 2.24, and 1.81. The 5′-hydroxylation appeared to decrease the ORAC activity in the presence of 3′,4′-OH (delphinidin vs cyanidin). This extra hydroxylation in delphinidin does not change the Trolox equivalent antioxidant capacity (TEAC) compared to that of cyanidin in the TEAC assay (Rice-Evans et al., 1996) but does increase the chemiluminescence intensity in the chemiluminescence assay (Yoshiki et al., 1995).

It is interesting that none of the anthocyanidins we tested had antioxidant activities as high as flavones with the same hydroxylation patterns as tested with the ORAC assay [delphinidin (1.81) vs myrecetin (4.32), cyanidin (2.24) vs quercetin (3.29), pelargonidin (1.54) vs kaempherol (2.67)] (Cao et al., 1996). This reflects the importance of the 2,3-double bond in conjunction with a 4-oxo group in the C ring in contributing potent antioxidant capacities in flavones. This also supports findings by Bors et al. (1990) that this 2,3-double bond in conjugation with a 4-oxo group is another important determinant for antioxidative potential and is responsible for electron delocalization from the B ring. Although this criterion has been supported by Rice-Evans et al. (1996) in explaining the antioxidant potency in flavones, their data do not show much difference in antioxidant potency between flavones and anthocyanins.

There were two other interesting findings from our study presented in Table 3: (1) Glycosylation of the anthocyanidins may modulate the antioxidant capacity depending on the aglycons. Glycosylation either increased (kuromanin vs cyanidin), decreased (oenin vs malvidin), or did not have a significant effect (pelargonidin vs callistephin) on the ORAC activity of the aglycons in this study. Effects of glycosylation on the antioxidant potency of anthocyanins have also been found by other investigators (Yoshiki et al., 1995; Rice-Evans et al., 1996). Rice-Evans and co-workers (1996) have shown a general trend of decreasing TEAC by glycosylation, while Yoshiki et al. (1995) have shown an enhancing effect in the chemiluminescence intensity in malvidin. (2) Different sugars may have different effects on the antioxidant activity of an anthocyanin. Using cyanidin as an example, 3-glycosylation in the C ring increased ORAC activity for glucose and rhamnoglucose but decreased activity for galactose. The order of antioxidant potency in the ORAC assay was as follows: kuromanin (cyanidin-3-glucoside) > keracyanin (cyanidin-3-rhamnoglucoside) > cyanidin > ideain (cyanidin-3-galactoside) (Table 3). The data from Rice-Evans et al. (1996) have also shown a differential effect on antioxidant potency of different sugars on the same aglycon (keracyanin > ideain). The effects are particularly intriguing when comparing kuromanin and ideain since the only difference between glucose and galatose is the orientation of one hydroxyl group at their pyran ring. Obviously, even the orientation of this hydroxyl group affected their antioxidant potency against peroxyl radical. Therefore, it is conceivable that different sugar molecules had differential effects on the antioxidant potency. Since the radical scavenging efficiency of an antioxidant depends on its ability to form a stable radical itself (Bors et al., 1990; Kandaswami and Middleton, 1994), different sugar molecules may provide

different molecular structures in which they may either enhance or diminish the stability and affect the potency.

It is not surprising that a different order of antioxidant activity of anthocyanins was obtained by different investigators (Sichel et al., 1991; Yoshiki et al., 1995; Rice-Evans et al., 1996). Antioxidant activity of a compound depends upon which free radical or oxidant is used in the assay (Halliwell and Gutteridge, 1995), and chemically different methods for measuring antioxidant activity will produce different hierarchies of antioxidants (Halliwell and Gutteridge, 1990). Different assays with different free radical generators, end-points, and quantification systems were used by other investigators (Sichel et al., 1991; Yoshiki et al., 1995; Rice-Evans et al., 1996). However, the ORAC assay is superior to other similar methods because it uses an area-under-curve (AUC) technique and thus combines both inhibition time and inhibition degree of free radical action by an antioxidant into a single quantity (Cao et al., 1995). The peroxyl radical ($ROO^{\bullet}$) used in this study is a common free radical found in the body (Halliwell and Gutteridge, 1990) and has been used in other antioxidant activity assays (Wayner et al., 1985; Glazer, 1990; Ghiselli et al., 1994). It is slightly less reactive than $OH^{\bullet}$ and thus possesses an "extended" half-life of seconds instead of nanoseconds (Grisham, 1992). The ORAC assay, which was also used for determining the total antioxidant activity of fruits (Wang et al., 1996), measures the antioxidant activities of all *traditional* antioxidants including ascorbic acid, α-tocopherol, β-carotene, glutathione, bilirubin, uric acid, melatonin (Cao et al., 1993; Pieri et al., 1994), and flavonoids (Cao et al., 1997).

The antioxidant activities of anthocyanins measured using the ORAC assay may contribute significant antioxidant properties to fruits (Wang et al., 1996), particularly in those with bright colors. Among the fruits and vegetables commonly consumed, grapes and their associated products including wine, juice, and raisins may be the most important source of our dietary anthocyanins considering they contain significant amount of anthocyanins (Table 2). Grapes are the world's largest fruit crop (Mazza, 1995), and the annual utilization of grapes in the United States in 1993 is 7 lb per capita (U.S. Census Bureau, 1995). Large amounts of anthocyanins from red grape skin are present (malvidin-3-glucoside is the principal colorant) in red wine in contrast to white wine (Pierpoint, 1986; Glories, 1988; Mazza, 1995; Frankel et al., 1995). The anthocyanins in red wine have been shown to contribute to the strong protection of the red wine against low-density lipoprotein oxidation (Frankel et al., 1995). Ingestion of red wine has also been shown to increase the antioxidant capacity in serum (Whithead et al., 1995). These findings support the notion of red wine consumption contributing to the so-called "French Paradox", in that there are low incidences of and mortality rates from ischemic heart disease in France despite the fact that saturated fat intakes, serum cholesterol, blood pressure, and prevalence of smoking are no lower there than elsewhere (Renaud and Lorgeril, 1992; Burr, 1995).

In summary, anthocyanins are natural colorants with potent antioxidant properties. Different hydroxylations and glycosylation may modulate their antioxidative properties. Anthocyanins are potential candidates in disease prevention and treatment, but further research is needed to elucidate their impacts on human health.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; ORAC, oxygen radical absorbance capacity; β-PE, β-phycoerythrin; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; DMSO, dimethyl sulfoxide; VMA, *Vaccinium myrtillus* (bilberry) anthocyanins; TEAC, Trolox equivalent antioxidant capacity.

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LITERATURE CITED

Acheson, R. M.; Williams, D. R. R.. Does consumption of fruit and vegetables protect against stroke? *Lancet* **1983**, *1* (8335), 1191−1193.

Akhmadieva, A. Kh.; Zaichkina, S. I.; Ruzieva, R. Kh.; Ganassi, E. E. The protective action of a natural preparation of anthocyan (pelargonidin-3,5-diglucoside). *Radiobiologiia* **1993**, *33* (3), 433−435.

Ames, B. M. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science* **1983**, *221*, 1256−1263.

Ames, B. M.; Shigena, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.

Armstrong, B. K.; Mann, J. I.; Adelstein, A. M. Eskin, F. Commodity consumption and ischemic heart disease mortality, with special reference to dietary practices. *J. Chronic Dis.* **1975**, *28*, 455−469.

Baj, A.; Bombardelli, E.; Gabetta, B.; Martinelli, E. M. Qualitative and quantitative evaluation of Vaccinium myrtillus anthocyanins by high-resolution gas chromatography and high-performance liquid chromatography. *J. Chromatogr.* **1983**, *279*, 365−372.

Bakker, J.; Bridle, P.; Bellworthy, S. J. Strawberry juice color: a study of quantitative and qualitative pigment composition of juices from 39 genotypes. *J. Sci. Food Agric.* **1994**, *64*, 31−37.

Becci, P. J.; Hess, F. G.; Gallo, M. A.; Johnson, W. D.; Babish, J. G. Subchronic feeding study of color extract in beagle dogs. *Food Chem. Toxicol.* **1983a**, *21* (1), 75−77.

Becci, P. J.; Hess, F. G.; Babish, J. G.; Gallo, M. A.; Voss, K. A. Reproduction study of grape color extract in rats. *Food Chem. Toxicol.* **1983b**, *21* (1), 79−83.

Bors, W.; Heller, W.; Michel, C.; Saran, M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. *Methods Enzymol.* **1990**, *186*, 343−355.

Burr, M. L. Explaining the French paradox. *J. R. Soc. Health* **1995,** *115* (4), 217−219.

Cao, G.; Alessio, H. M.; Culter, R. G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **1993**, *14*, 303−311.

Cao, G.; Verdon, C. P.; Wu, A. H. B.; Wang, H.; Prior, R. L. Automated oxygen radical absorbance capacity assay using the COBAS FARA II. *Clin. Chem.* **1995**, *413*, 1738−1744.

Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: Structure−activity relationships. *Free Radical Biol. Med.* **1997**, *22* (5), 749−760.

Carpenter, J. A.; Wang, Y.; Powers, J. J. Effect of anthocyanin pigments on certain enzymes. *Proc. Soc. Exp. Biol. Med.* **1967**, *124* (3), 702−706.

Colantuoni, A.; Bertuglia, S.; Magistretti, M. J.; Donato, L. Effects of Vaccinium myrtillus anthocyanosides on arterial vasomotion. *Arzneim.-Forsch.* **1991**, *41* (9), 905−909.

Costantino, L.; Rastelli, G.; Albasini, A. Anthocyanidines as inhibitors of xanthine oxidase. *Pharmazie* **1995**, *50*, 573−574.

Detre, Z.; Jellinek, H.; Miskulin, M.; Robert, A. M. Studies on vascular permeability in hypertension: action of anthocyanosides. *Clin. Physiol. Biochem.* **1986**, *4* (2), 143−149.

Doll, R. An overview of the epidemiological evidence linking diet and cancer. *Proc. Nutr. Soc.* **1990**, *49*, 119−131.

Dragsted, L. O.; Strube, M.; Larsen, J. C. Cancer-protective factors in fruits and vegetables: biochemical and biological background. *Pharmacol. Toxicol.* **1993**, *72* (Suppl. 1), 116−135.

Ferrell, J. E., Jr; Chang Sing, P. D. G.; Loew, G.; King, R.; Mansour, J. M.; Mansour, T. E. Structure/activity studies if flavonoids as inhibitor of cyclic AMP phosphodiesterase and relationship to quantum chemical indices. *Mol. Pharmacol.* **1979**, *16*, 556−568.

Francis, F. J. Food colorants: Anthocyanins. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 273−314.

Frankel, E. F,; Waterhouse, A. L.; Teissedre, P. L. Principal Phenolic Phytochemicals in Selected California Wines and Their Antioxidant Activity in Inhibiting Oxidation of Human Low-Density Lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.

Gey, K. F. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. *Biochem. Soc. Trans.* **1990**, *18*, 1041−1045.

Ghiselli, A.; Serafini, M.; Maiani, G.; Assini, E.; Ferro-luzzi, A. A fluorescent-based method for measuring total antioxidant capability. *Free Radical Biol. Med.* **1994,** *18*, 29−36.

Gibb, C.; Glover, V.; Sandler, M. *In vitro* inhibition of phenol-sulphotransferase by food and drink constituents. *Biochem. Pharmacol.* **1987**, *36* (14), 2325−2330.

Gillman, M. W.; Cupples, L. A.; Gagnon, D.; Posner, B. M.; Ellison, R. C.; Castelli, W. P.; Wolf, P. D. Protective effect of fruits and vegetables on development of stroke in men. *JAMA, J. Am. Med. Assoc.* **1995**, *273*, 1113−1117.

Glazer, A. N. Phycoerythrin fluorescence-based assay for reactive oxygen species. *Methods Enzymol.* **1990**, *186*, 161−168.

Glories, Y. Anthocyanins and tannins from wine: organoleptic properties. *Prog. Clin. Biol. Res.* **1988**, *280*, 123−134.

Grisham, M. B. *Reactive metabolites of oxygen and nitrogen in biology and medicine*; R. G. Landes Co.: Austin, TX, 1992; p 10.

Halliwell, B.; Gutteridge, J. M. C. The antioxidants of human extracellular fluids. *Arch. Biochem. Biophys.* **1990**, *280* (1), 1−8.

Halliwell, B.; Gutteridge, J. M. C. The definition and measurement of antioxidants. *Free Radical Biol. Med.* **1995**, *18* (1), 125−126.

Kadar, A.; Robert, L.; Miskulin, M.; Tixier, J. M.; Brechemier, D.; Robert, A. M. Influence of anthocyanoside treatment on the cholesterol-induced atherosclerosis in the rabbit. *Paroi Arterielle* **1979**, *5* (4), 187−205.

Kamei, H.; Kojima, T.; Hasegawa, M,; Koide, T.; Umeda, T.; Yukawa, T.; Terabe, K. Suppression of tumor cell growth by anthocyanins in vitro. *Cancer Invest.* **1995**, *13* (6), 590−594.

Kandaswami, C.; Middleton, E. J. Free radical scavenging and antioxidant activity of plant flavonoids. *Adv. Exp. Med. Biol.* **1994**, *366*, 351−376.

Karaivanova, M.; Drenska, D.; Ovcharov, R. A modification of the toxic effects of platinum complexes with antocyans. *Eksp. Med. Morfol.* **1990**, *29* (2), 19−24.

Kikoku, Y.; Fukuhara, K.; Saito, I.; Oota, H. Identification and high performance chromatographic determination of strawberry anthocyanin pigments. *J. Jpn. Soc. Food Sci. Technol.* **1995**, *42* (2), 118−123.

Kühnau, J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet* **1976**, *24*, 117−191.

Leonardi, M. Treatment of fibrocystic disease of the breast with myrtillus anthocyanins. Our experience. *Minerva Ginecol.* **1993**, *45*, 617−621.

Lietti, A.; Cristoni, A.; Picci, M. Studies on Vaccinium myrtillus anthocyanosides. I. Vasoprotective and antiinflammatory activity. *Arzneim-Forsch.* **1976**, *26* (5), 829−832.

Mazza, G. Anthocyanins on grapes and grape products. *Crit. Rev. Food Sci. Nutr.* **1995**, *35* (4), 341−371.

Mazza, G.; Miniati, E. *Anthocyanins in fruits, vegetables, and grains*; CRC Press Inc.: Boca Raton, FL, 1993.

Mian, E.; Curri, S. B.; Lietti, A.; Bombardelli, E. Anthocyanosides and the walls of the microvessels: future aspects of the mechanism of action of their protective effect in syndromes due to abnormal capillary fragility. *Minerva Med.* **1977**, *68* (52), 3565−3581.

Minkova, M.; Drenska, D.; Pantev, T.; Ovcharov, R. Anti-radiation properties of alpha tocopherol, anthocyans, and pyracetam administered combined as a pretreatment course. *Acta Physiol. Pharmacol. Bulg.* **1990**, *16* (4), 31−36.

Miskulin, M.; Detre, Z.; Jellinek, H.; Robert, A. M. Variation in vascular permeability in the initial phase of experimental hypertension in rat. *Paroi Arterielle* **1980**, *6* (4), 213-216.

Mitcheva, M.; Astroug, H.; Drenska, D.; Popov, A.; Kassarova, M. Biochemical and morphological studies on anthocyans and vitamin E on carbon tetrachloride induced liver injury. *Cell. Mol. Biol.* **1993**, *39*, 443−448.

Pieri, C.; Marra, M.; Moroni, F.; Recchioni, R.; Marcheselli, F. Melatonin: a peroxyl radical scavenger more effective than vitamin E. *Life Sci.* **1994**, *55*, PL271−276.

Pierpoint, W. S. Flavonoids in human diet. *Prog. Clin. Biol. Res.* **1986**, *213*, 125−140.

Politzer, M. Experience in the medical treatment of progressive myopia. *Klin. Monatsbl. Augenheikd.* **1977**, *171* (4), 616−619.

Renaud, S.; de Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

Rice-Evans, C.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Res.* **1995**, *22*, 375−383.

Rice-Evans, C.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20* (7), 933−956.

Robert, A. M.; Godeau, G.; Moati, F.; Miskulin, M. Action of anthocyanosides of Vaccinium myrtillis on the permeability of the blood-brain barrier. *J. Med.* **1977**, *8* (5), 321−332.

Saija, A.; Princi, P.; D'Amico, N.; De Pasquale, R.; Costa, G. Effects of *Vaccinium myrtillus* anthocyanins on triiodothyronine transport in the rat. *Pharmacol. Res.* **1990**, *22* (Suppl. 3), 59−60.

Scharrer, A.; Ober, M. Anthocyanosides in the treatment of retinopathies. *Klin. Monatsbl. Augenheikd.* **1981**, *178* (5), 386-389.

Sichel, G.; Corsaro, C.; Scalla, M.; Di Bilio, A. J.; Bonomo, R. P. In vitro scavenger activity of some flavonoids and melanin against $O_2^{-\bullet}$. *Free Radical Biol. Med.* **1991**, *11*, 1−8.

Steinberg, D. Antioxidants and atherosclerosis: a current assessment. *Circulation* **1991**, *84*, 1420−1425.

Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Beyond cholesterol: Modification of low-density lipoprotein that increases its atherogenicity. *New Engl. J. Med.* **1989**, *320*, 915−924.

Strack, D.; Wray, V. The anthocyanins. *The Flavonoids: Advances in research since 1986*; Harborne, J. B., Ed.; Chapman & Hall: London, 1993; pp 1−22.

Tamura, H.; Yamagami, A. Antioxidative Activity of Monoacylated Anthocyanins Isolated from Muscat Bailey A Grape. *J. Agric. Food Chem.* **1994**, *42*, 1612−1615.

Terahara, N.; Yamaguchi, M.; Honda, T. Malonylated anthocyanins from bulds of red onion, Allium cepa L. *Biosci., Biotechnol., Biochem.* **1994**, *58* (7), 1324−1325.

Timberlake, C. F.; Henry, B. S. Anthocyanins as natural food colorants. *Prog. Clin. Biol. Res.* **1988**, *280*, 107−121.

U.S. Census Bureau. *Statistical abstract of the United States: 1995*, 115th ed.; Washington, DC, 1995.

van Acker, S. A. B. E.; Tromp, M. N. J. L.; Haenen, G. R. M. M.; van der Vijgh, W. J. F.; Bast, A. Flavonoids as scavengers of nitric oxide radical. *Biochem. Biophys. Res. Commun.* **1995**, *214* (3), 755−759.

Verlangieri, A. J.; Kapeghian, J. C.; el-Dean, S.; Bush, M. Fruit and vegetable consumption and cardiovascular mortality. *Med. Hypotheses* **1985**, *16*, 7−15.

Wang, H; Cao, G.; Prior, R. Total Antioxidant Capacity of Fruits. *J. Agric. Food Chem.* **1996,** *44* (3), 701−705.

Wayner, D. D. M.; Burton, G. W.; Ingold, K. U.; Locke, S. Quantitative measurement of the total, peroxyl radical-trapping antioxidant capacity of human plasma by controlled peroxidation. *FEBS Lett.* **1985**, *187*, 33−37.

Wheeler, O. R.; Carpenter, J. A.; Powers, J. J.; Hamdy, M. K. Inhibition of enzymes by the anthocyanin malvidin-3-monoglucoside. *Proc. Soc. Exp. Biol. Med.* **1967**, *125* (2), 651−657.

Whitehead, T. P.; Robinson, D.; Allaway, S.; Syms, J.; Hale, A. Effect of red wine ingestion on the antioxidant capacity of serum. *Clin. Chem.* **1995,** *41* (1), 32−35.

Willett, W. C. Diet and health: what should we eat? *Science* **1994**, *264*, 532−537.

Yoshiki, Y.; Okubo, K.; Igarashi, K. Chemiluminescence of anthocyanins in the presence of acetaldehyde and tert-butyl hydroperoxide. *J. Biolumin. Chemilumin.* **1995**, *10*, 335−338.

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