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Cyanidins: metabolism and biological properties

Fabio Galvano[a,*], Luca La Fauci[b], Giuseppe Lazzarino[c], Vincenzo Fogliano[d], Alberto Ritieni[d], Salvatore Ciappellano[e], Nino C. Battistini[f], Barbara Tavazzi[g], Giacomo Galvano[b]

[a]*Department of Agro-forestry and Environmental Science, University of Reggio Calabria, P.zza S. Francesco 7, 89061 Gallina di Reggio Calabria, Italy*
[b]*Department of Agronomical, Agrochemical and Animal Production Science, University of Catania, Catania, Italy*
[c]*Department of Chemical Science, Laboratory of Biochemistry, University of Catania, Italy*
[d]*Department of Food Science, University Federico II, Napoli, Italy*
[e]*Department of Food Science and Microbiology Science and Technologies, University of Milano, Milano, Italy*
[f]*Department of Hygiene Science, University of Modena, Modena, Italy*
[g]*Institute of Biochemistry and Clinical Biochemistry, Catholic University of Rome "Sacro Cuore", Rome, Italy*

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Abstract

Cyanidin and its glycosides belong to the anthocyanins, a widespread class of water-soluble plant compounds that are responsible for the brilliant color (red, orange, blue) of fruits and flowers. They are widely ingested by humans as it has been estimated a daily intake around 180 mg, mainly deriving from fruits and red wines. This paper reviews the literature on the biological activities, absorption and metabolism of cyanidins, with emphasis to the antioxidant, antimutagenic and other protective activities ascribed to these compounds. Their role in contrasting development of cancer and other pathologies is also reviewed. It is concluded that a great deal of work is still necessary to i) definitively clarify the metabolism of cyanidins in human beings; ii) assess the dietary burden and variations within and between populations; iii) evaluate the relationship between cyanidin glycosides-rich food consumption and incidence of given pathologies. The amount of work to be performed is even more significant when considering a possible therapeutic use of cyanidin glycosides-based drugs. With this aim, information on absorption, distribution, metabolism and excretion of cyanidin-glycosides administered by main possible routes are largely insufficient. However, consisting findings allow looking at cyanidins as dietary compounds with a potential beneficial role for human health.

Keywords: Anthocyanins; Cyanidins; Cyanidin-3-glucoside; Biological properties; Antioxidant; Absorption and metabolism

1. Introduction

The anthocyanins (Greek *antos*, flower and *kyanos*, blue) are part of the very large and widespread group of plant constituents collectively known as flavonoids. They are of great nutritional interest because of the marked daily intake (180 to 215 mg/day in the United States [1], which is much higher than the intake (23 mg/day) estimated for other flavonoids, including quercetin, kaempferol, myricetin, apigenin, and luteolin [1]. They have been reported to have positive effects in the treatment of various diseases [2] and are prescribed as medicines in many countries.

The anthocyanin-health properties are due to their peculiar chemical structure, as they are very reactive towards reactive oxygen species (ROS) because of their electron deficiency. In the last years, great attention was given to the possible protection exerted by natural antioxidants present in dietary plants, particularly flavonoids and polyphenols, towards tissue injury mediated by ROS. Anthocyanins are included in the list of natural compounds known to work as powerful antioxidants. Since cyanidin and its glycosides represent one of the mayor groups of naturally occurring anthocyanins their antioxidant and biological properties have been deeply investigated and recent findings, indicating the possibility that anthocyanins are absorbed as glycosides, have renewed the interest for the studies on their bioavailability, including their absorption, metabolic fate and excretion.

* Corresponding author. Tel./fax: +39.095.351201.
E-mail address: f.galvano@unict.it (F. Galvano).

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2. Chemical structure

Anthocyanins are water-soluble glycosides of polyhydroxyl and polymethoxyl derivates of 2-phenylbenzopyrylium or flavylium salts. In fruits and vegetables there are six basic anthocyanin compounds. The differences between individual anthocyanins are the number of hydroxyl groups in the molecule; the degree of methylation of these hydroxyl groups; the nature, number, and location of sugars attached to the molecule; and the number and the nature of aliphatic or aromatic acids attached to the sugars in the molecule [1–3]. All these variables account for the large number of compounds belonging to the anthocyanin family and allow the researchers to study fingerprints of many different vegetables species, just on the basis of their anthocyanin composition.

3. Food sources

Cyanidins are considered the widest spread anthocyanin in the plant kingdom. They are largely distributed in the human diet through crops, beans, fruits, vegetables and red wines, suggesting that we daily ingest significant amounts of these compounds from plant-based diets. Cyanidin-3-glycoside (C3G), also known as kuromanin, is probably the most notorious and investigated among cyanidin-glycosides. One of the most important dietary sources of C3G is surely represented by pigmented oranges, named *Moro*, *Sanguinello* and *Tarocco*, typically growing in Sicily (Italy) [4] as well as in Florida (USA) [5].

Various authors, using different analytical methods, have reported the presence of cyanidins in many fruits and vegetables. However, almost the totality of the studies does not report quantitative estimation of cyanidin and its glycosides. Quantitative data would be obviously very useful to estimate dietary intake.

4. Metabolism and bioavailability

It was for a long time believed that anthocyanidins only, i.e., the aglycones of anthocyanins, were adsorbed by intestinal cells (being capable of passing through the gut wall) and brought into the blood stream because of the absence of a bound sugar residue. Since no specific enzymes were known to selectively hydrolyze these glycosidic bonds, it was presumed that anthocyanins were poorly absorbed. This conviction has now been changed by results of numerous studies which demonstrated in vivo absorption of glycosidic flavonoids, particularly of C3G and other cyanidin-glycosides. In several studies the presence of anthocyanins or anthocyanidins in human and/or rat plasma, after oral administration of different glycoside forms of cyanidin-glycosides, has been reported.

In a recent study on rats Youdim et al. [6] measured plasma levels of some anthocyanins, following oral supplementation of blueberry skin extract mainly containing cyanidin-galactoside, C3G, Cy-3-arabinoside and the aglycon cyanidin. Data indicated that cyanidin-glycosides are incorporated from the digestive tract into the blood stream in their intact glycosylated forms in agreement with a previous report by Tsuda et al. [7]. Similarly, Miyazawa [8], Matsumoto [9] and their colleagues also showed that cyanidin-glycosides were detected in their unaltered forms in blood of both rats and humans after oral administrations of black currant (mainly containing C3G and Cy-3,5-diglycoside) or elderberry juices (primarily composed by Cy-3-*O*-β-rutinoside).

Matsumoto et al. [9] and Netzel et al. [10, 11] demonstrated that C3G and Cy-3,5-diglycoside were excreted in their intact glycosylated forms in urines of humans, in agreement with the finding of Cao et al. [2] who detected Cy-3-sambubioside and C3G in urine and plasma of women fed with elderberry extract. These latter authors also reported the pharmacokinetics of the tested compounds: the elimination of plasma total anthocyanins appeared to follow first-order kinetics, since the urinary excretion rate had a maximum after 3 to 4 h and decreased thereafter, as also reported by Milbury et al. [12] and Murkovic et al. [13]. Further confirm of these results derives from a recent study by Felgines et al. [14] carried out on rats fed with a lyophilized blackberry powder-enriched diet. Recovery of C3G in urine as the intact glycosidic or methylated forms was observed, whereas neither aglycone nor conjugates were present. Urinary recovery of peonidin-3-glycoside was explained by authors as a probable result of hepatic methylation at the 3′-hydroxyl moiety position of C3G.

In two studies on rats Tsuda and co-workers [15] also suggested that C3G is partly hydrolyzed by the β-glycosidase reaction in the intestines, thus explaining the detection of the aglycon in the jejunum. No detection of the aglycon in the plasma was related to cyanidin instability in plasma and its consequent massive degradation to protocatechuic acid. Seeram et al. [16] confirmed that protocatechuic acid was the predominant degradation product of cyanidin in a culture cell study.

Diversely, Miyazawa et al. [8] explained no detectable amounts of cyanidin in rat plasma, liver and human plasma despite oral administrations of C3G and Cy-3,5-diglycoside, by hypothesizing that no hydrolysis of the glycosidic bond occurs in gastrointestinal tract.

Another important observation by Tsuda et al. [7] was that C3G was metabolized to methylated form in the liver and kidneys of rats, whereas glucuronides, sulfates of C3G and its metabolites were not detected in plasma and tissues. This observation was completely different with respect to the other flavonoids and the fact that C3G mainly exists as a free form would be of sure benefit to its antioxidant activity.

The detection of the anthocyanins in their unchanged glycated forms could indicate the involvement of the glu-

cose transport receptors in the absorption of these compounds in vivo [2].

The discrepancies occurring in literature about either cyanidin concentrations in plasma and urine or the kinetic of absorption might have different explanations including, in the case of rats, different strain and age of animals used, as well as administration of more than one cyanidin-glycoside. Both circumstances might have profound effects on cyanidins absorption.

With the aim to verify the absorption of C3G from red orange juice, Ciappellano et al. [17] used an in vitro model. They determined the anthocyanins transport across the mucosal epithelium of rat's everted small intestine by developing a small-scale transport device, in which isolated intestinal segments separated the "mucosal" from the "serosal" compartment. The ability of C3G to cross the intestinal wall in its intact form was confirmed by the results of this experiment. Comparing the rate of C3G absorption derived from the use of a standard solution and of red orange juice the authors also hypothesized that the kinetics of C3G absorption could be affected by the food matrix.

Another study to assess the metabolic handling of C3G was recently performed by Stumpf et al. [18] who used an isolated perfused rat small intestinal preparation. Results confirmed that C3G was absorbed into the vascular effluent and incorporated into intestinal tissue, whereas the aglycone cyanidin was not detectable. Another interesting result was the detection of glucuronate and sulfate conjugates of C3G as intestinal metabolites.

Studies by Gee et al. [19, 20], recently confirmed by similar reports by Walgren et al. [21] and by Wolffram et al. [22], suggests that quercetin glycosides are capable of interacting with the sodium dependent glucose transport receptors in the mucosal epithelium. Two potential mechanisms for the transport of quercetin glycosides by enterocytes can be supposed, namely, transport of quercetin glycosides by SGLT1, and extracellular hydrolysis by LPH, followed by passive diffusion of the aglycone. As quercetin-3-O-glucoside (isoquercitrin) and C3G share a similar basic flavonoid structure the demonstration of the presence of anthocyanins in their unchanged glycosilated forms could be indicative of the involvement of the glucose transport receptors in the absorption of these compounds in vivo.

5. Antioxidant activity

The natural electron deficiency of anthocyanins makes these compounds particularly reactive, rendering them also very sensitive to pH and temperature changes [23]. Despite some concerns related to their instability at physiological pH, anthocyanins were included in the list of natural compounds known to act as powerful antioxidants [23, 24]. Their antioxidant potency is modulated by their different chemical structure. In fact, by varying the positions and types of chemical groups on the anthocyanins aromatic rings, the capacity to accept unpaired electrons from radical molecules is varied [23, 24].

The antioxidant activity of cyanidin-glycosides raised the interest of many researchers among which it is worth to be mentioned the group of Tsuda and co-workers, who contributed with several in vivo and in vitro studies.

5.1. In vitro studies

Cyanidins in vitro antioxidant activity has been widely demonstrated by numerous studies and several methods of measurements.

Wang et al. [24] using the automated assay to measure oxygen radical absorbance capacity (ORAC) found that C3G showed the highest ORAC activity among the 14 anthocyanins tested and its activity resulted 3.5 times stronger than that of Trolox (Vitamin E analogue).

With the aim to assess the antioxidant capacities of strawberry, red plum and raspberry extracts, ORAC assay has been used also by Proteggente et al. [25] in association with the TEAC (Trolox equivalent antioxidant capacity) and the FRAP (ferric reducing ability of plasma) assays. The high antioxidant activity demonstrated in each assay has been explained by authors with the high content in anthocyanins (C3G, Cy-3-rutinoside and Cy-3-sophoroside) of the extracts.

The marked antioxidant properties of C3G was confirmed by Rice-Evans et al. [26] who observed an activity 4 times stronger than that of Trolox.

Seeram et al. [27] reported that Cy-3-glucosylrutinoside and Cy-3-rutinoside from cherries have an antioxidant activity comparable to the synthetic antioxidants tert-butylhydroquinone, butylated hydroxytoluene and butylated hydroxyanisole and superior to vitamin E.

With the aim of studying the antioxidant capacity of C3G and the daily phenolic dietary intake of French population, Landrault et al. [28] quantified these compounds for 54 varieties of wines reporting that red wines have the highest antioxidant capacity due to their higher content of cyanidins and other phenolics.

The inhibition of lipid peroxidation of a liposome system has been observed by Wang et al. [29] by evaluating the antioxidant activity of three anthocyanins (3-Cy 2"-O-β-D-glucopyranosyl-6"-O-"-L-rhamnopyranosyl-β-D-glucopyranoside, 3-Cy6"-O-"-L-rhamnopyranosyl- β-glucopyranoside, 3-Cy-O- β -D- glucopyranoside) and the aglycone cyanidin isolated from tart cherries. The inhibition of lipid peroxidation of these compounds varied from 39% to 75%. The authors also compared the antioxidant activity of these compounds with that of synthetic products (tert-butylhydroquinone and butylated hydroxitoluene) obtaining comparable values and a higher activity than Vitamin E. The values of inhibition of lipid peroxidation suggested that the aglycone form (cyanidin) has higher efficacy than its glycosides; authors hypothesized that the antioxidant activity is due to their aglycone moiety. According to these results, the

number of sugar residues at the C_3 position would be very important for the antioxidant activity, so that it would decrease by increasing the number of sugar units at the C_3. Tsuda et al. [30] tried to explain the antioxidant mechanism of C3G by reaction with an alkylperoxyl radical. Basing on the reaction products, the authors suggested that the antioxidative mechanism of C3G is different from that of alpha-tocopherol and hypothesized that C3G produces another radical scavenger, as it would break down the structure and scavenge the radicals.

Sarma et al. [31] demonstrated that oxidation of ascorbic acid by metal ions, such as Cu^{2+}, was prevented by the addition of some cyanidin-deriving anthocyanins and isolated from seeds of a cyanic cultivar of *Oryza sativa*. In addition, they provided evidence that anthocyanins not only chelate metal ions, as reported by previous studies, but also form an ascorbic acid-metal-anthocyanin complex (copigment), which could to explain the cyanidin antioxidant mechanism.

The protective effect exerted by cyanidin towards DNA was firstly reported by Sarma and Sharma [32] who showed the formation of a cyanidin-DNA copigmentation complex. The formation of the complex, prior to the exposure to hydroxyl radicals, protected both the cyanidin and DNA from the oxidative damage. When exposed individually, in contrast, they showed severe oxidative damage.

Russo et al. [33] investigated the effects of a C3G rich standardized extract from red oranges (*Citrus sinensis* varieties: Moro, Tarocco, Sanguinello) on DNA cleavage and its free radical scavenging capacity. In addition, its effect on xanthine oxidase activity and lipid peroxidation in rat liver microsomes was evaluated. The extract showed a protective effect on DNA cleavage and a dose-dependent free radical scavenging capacity; these results were confirmed by a significant inhibition of xanthine oxidase activity and an anti-lipoperoxidative capacity.

Mas et al. [34] tested C3G isolated from an aqueous extract of *Vitis vinifera* and Cy-3,5-di-β-D-glucopyranoside extracted from dried petals of rose (*Rosa gallica*). Their DNA triplex stabilization property was measured by the mean of triplex thermal denaturation experiments. The monoglycoside presented a weak but significant stabilizing effect, while the diglucoside did not modify the melting temperature. The authors indicated that the difference could be due to the supplementary sugar moiety at the 5 position of the diglucoside compound, which might represent an excessive steric hindrance inhibiting the interaction with the triplex.

Liu et al. [35] recently reported the antioxidant activity of some raspberry variety extracts by using the total oxyradical scavenging capacity (TOSC), showing a direct relation between the antioxidant activity and the total amount of flavonoids, among which cyanidin-glycosides are notoriously the main component.

A valid contribution to the knowledge of the C3G mechanism of action was given by Amorini et al. [4]. In their study, authors confirmed the remarkable antioxidant capacity of C3G in the model of Cu^{2+}-mediated human low density lipoprotein (LDL) oxidation, which was higher than both resveratrol and ascorbic acid and was independent on pH variations from 4 to 7.4. On the basis of data obtained in different ROS-generating systems, showing a direct interaction of C3G with ROS, authors hypothesized that C3G protection of LDL oxidation is due to its ROS scavenging activity rather than to a metal-chelating property. In this study, authors also determined a value of C3G redox potential that was in accordance with its powerful antioxidant capacity. Interestingly, authors also proved that C3G rich juice from pigmented oranges (named *Moro*, *Sanguinello* and *Tarocco*) was much more effective than that from blond oranges in reducing LDL oxidation.

Cyanidins antioxidant properties have been also demonstrated in several in vitro biological tests.

The ability of endothelial cells to incorporate cyanidin and its derivates has been investigated by Youdim et al. [36]. For this purpose, they used two cell models composed by bovine and human endothelial cell cultures incubated in presence of an elderberry extract containing C3G, Cy-3-sambubioside-5-diglucoside, Cy-3,5-diglucoside and Cy-3-sambubioside. Results indicated that these compounds were incorporated into the plasma membrane and cytosol of endothelial cells . Uptake within both regions appeared to be dependent from cyanidin structure, with monoglycoside cell permeation higher than that of the diglycosides in both compartments. Another important response of this study was that the incorporation of elderberry extract by endothelial cells significantly enhanced their resistance to the damaging effects caused by the following ROS-generating systems: hydrogen peroxide (H_2O_2); 2,2′-azobis(2-amidino propane)dihydrochloride and $FeSO_4$/ascorbic acid .

Wang and Mazza [37] recently reported the effects of selected compounds, among which C3G, on NO production in LPS/IFN-γ-activated RAW 264.7 macrophage cells. The reduction of NO content in cell supernatant was obtained with no toxic side effects on cell survival.

Red blood cells have been used by Youdim et al. [6] in an investigation on the potential antioxidant properties of blueberry polyphenols. Anthocyanins were extracted from the blueberry skin and some cyanidin-glycosides were identified: Cy-3-arabinoside, Cy-galactoside, C3G and cyanidin. In vitro incubation of red blood cells with increasing concentrations of these compounds afforded significant resistance to induce oxidative stress.

Biological models have been also used by Tsuda et al. [38] in order to examine the antioxidative activity exerted by C3G and its aglicone, cyanidin, in the prevention of lipid peroxidation of cell membranes induced by active oxygen radicals in living systems. For this purpose, linoleic acid, liposomes, rabbit erythrocyte membranes and rat liver microsomes were submitted to different types of ROS-mediated oxidative stress. In liposomes and rabbit erythrocyte membranes cyanidin demonstrated a stronger activity than

C3G and the same activity as a-tocopherol, whereas in rat liver microsomes cyanidin and C3G had higher antioxidant efficacy than α-tocopherol. In author's opinion data confirmed that it is possible to imagine an important role for C3G and cyanidin as dietary antioxidants. In addition, since cyanidin may be obtained from cyanidin-glycosides through the hydrolysis catalyzed by bacterial β-glycosidase occurring in the intestine, they supposed that cyanidin might be the actual antioxidant in living systems and that cyanidin-glycosides might therefore be classified as proantioxidants.

In accordance with other reports, Seeram et al. [39] recorded an inhibition by 60% of ROS-induced lipid peroxidation of liposomes exerted by Cy- 3-galactopyranoside.

Plasma LDL cholesterol is widely considered as a fundamental risk factor in coronary heart diseases occurrence. Regular consumption of red wine containing high levels of cyanidin-glycosides antioxidants is thought to account for the lower incidence of coronary artery disease in Mediterranean countries. In particular, the "French paradox", a low incidence of coronary heart disease and atherosclerosis despite of a high-fat diet, has been associated with the constant ingestion of phenolic compounds contained in red wines that may act as in vivo inhibitors of LDL oxidation [40, 41]. Evidence of the effect of red wine consumption on susceptibility of plasma and LDL to ex vivo lipid peroxidation arises from studies of Kondo [41]. In addition, Fuhrman et al. [42] reported that total polyphenols in the plasma LDL fraction were elevated 4-fold after 2 weeks of red wine consumption in humans, producing significant decrease in LDL susceptibility to lipid peroxidation.

The in vitro antioxidant activity of several anthocyanins, among which cyanidin, was tested by SatueGracia et al. [23] in both human LDL and lecithin liposomes undergoing to oxidative stress. The extent of oxidation was measured by determining the formation of conjugated dienes and exanale. In Cu^{2+}-oxidized LDL cyanidin showed a remarkable antioxidant activity, while in liposome treated with the same Cu^{2+} concentration it showed prooxidant activity.

Frankel et al. [43] investigated the inhibition by phenolic antioxidants (including cyanidin-glycosides) from grapes and 20-selected California wines of Cu^{2+}-catalyzed in vitro human LDL oxidation. They concluded that the numerous phenolic compounds found in wines are potent antioxidants and that, at the same total phenol concentration, the inhibition of LDL oxidation varied from 37 to 65% for the red wines and from 27 to 46% for white wines.

The oxidation of human LDL induced by cupric ions was significantly suppressed by the addition of a black rice extract containing C3G in a recent study by Hu et al. [44]. The authors also showed the C3G scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radical and the C3G-mediated reduction of the supercoiled DNA scission induced by either peroxyl radical or hydroxyl radical. By using human monocytic THP-1 cells, the authors also reported the reduction of the cytotoxicity and apoptosis mediated by the Fenton reaction.

Protection from oxidative damage caused by UV light was demonstrated by Tsuda et al. [45] who exposed liposomes to UVB irradiation and found inhibitory effects of lipid peroxidation by C3G and its aglycon isolated from the *Phaseolus vulgaris* L. seed coats. In addition, the authors investigated the protective activity of these compounds in both liposomes and rat liver microsomes treated with different oxidative stressors. Both pigments showed strong antioxidant capacity significantly decreasing malondialdehayde formation. In contrast with the results reported by Amorini et al. [4], the authors failed to demonstrate significant C3G scavenging activity towards hydroxyl radicals.

Very recent results on the antioxidant effects of C3G were obtained by Amorini et al. [46] in isolated Langendorff-perfused rat hearts subjected to ischemia and reperfusion and in human erythrocytes undergoing to increased oxidative stress. Myocardial reperfusion with different C3G concentrations provoked a dose-dependent decrease of ROS-mediated tissue damages, as evaluated by MDA diminution, preservation of ascorbate levels and amelioration of high-energy phosphate concentrations.

By perfusing hearts in the recirculating Langendorff mode under normoxia, authors demonstrated that significant amounts of C3G in its intact form are taken up by myocardial cells and no C3G degradation products are generated. Therefore, C3G protective effects in isolated postischemic rat heart seem related to its permeation within myocytes. In the same study, C3G was tested in human erythrocytes challenged with H_2O_2 showing better efficacy than resveratrol on biochemical parameters evaluated.

5.2. In vivo studies

Several studies by Tsuda and co-workers reported that C3G acts as a potent antioxidant in vivo when acute oxidative stress is encountered.

Authors showed that feeding rats with C3G lowered the serum thiobarbituric acid-reactive substance (TBARS) concentration and increased the oxidation resistance of the serum to lipid peroxidation provoked by 2,2′-azobis(2-amidinopropane)hydrochloride (AAPH) or Cu^{2+} [47]. In another study [48] the same authors confirmed the in vivo antioxidant activity of C3G by using hepatic ischemia/reperfusion (I/R) injury as a model of oxidative stress. I/R caused the serum elevation of the TBARS concentration and of activities of marker enzymes of liver injury and, at the same time, lowered the liver concentration of reduced glutathione. Also in this experimental model, authors reported that feeding rats with C3G significantly suppressed these changes confirming the in vivo C3G antioxidant activity. They speculated that orally administered C3G is absorbed in part by the intestine and through the blood stream is distributed to tissues, where C3G and its metabolites react with ROS decreasing tissue damages induced by hepatic I/R. The same authors recently showed that the protective effect exerted by C3G against I/R injury is also due to its capacity

Table 1
Biological properties of Cyanidin and its glycosides

Biological property	Reference
Antimutagenicity in bacterial model (*Salmonella typhimurium*)	[28, 61, 62]
Inhibition of the epidermal growth-factor receptor (EGFR) of the human vulva carcinoma cell line A431	[65]
Suppression of the incidence and multiplicity of colorectal adenomas and carcinomas	[25]
Gastric protective effects	[57–59]
Improvement of dark adaptation and transient alteration of vision	[9]
Decrease in LDL susceptibility to lipid peroxidation	[4, 30, 34, 50, 51]
Protection against oxidative damage in human erythrocytes	[23, 46]
Protection against oxidative stress caused by hepatic ischemia/reperfusion (I/R) injury in rats	[23, 54]
Protection against oxidative stress caused by heart ischemia/reperfusion (I/R) injury in rats	[46]
Improvement of plasma antioxidant capacity in rats	[58]
Protection against oxidative damage in red blood cells	[6]
Protection towards calf thymus DNA against oxidative damage	[40]
Reduction of DNA cleavage	[41]
Suppression of the toxicity in macrophage cells by reduction of NO content	[45]
Prevention of damage induced by UV light in liposome	[26]
Prevention of inflammation	[32]
Protection against human ocular diseases	[56]

to cause a decrease in the neutrophil chemoattractant production in rats [49].

The hamster cheek pouch microcirculation was the biological model used by Bertuglia et al. [50] to demonstrate the protective effects exerted by *Vaccinium Myrtillus*, that is a rich source of cyanidin-glycosides, against I/R injury.

Using Wistar rats, Toyokuni et al. [51] showed the antioxidant effect of red (c.v. Beniroman) and black rice (c.v. Okuno-murasaki) against oxidative renal injury caused by ferric nitrilotriacetate, the toxicity of which is due to Fenton-like reaction occurring in the lumina of renal proximal tubules. Even though C3G contained in colored rice was not detected in rat serum, increased serum and kidney levels of protocatechuic acid (a metabolite of C3G) may indicate, in the author opinion, that C3G was absorbed and metabolized.

The in vivo antioxidant activity of cyanidin has been recently investigated by Ramirez-Tortosa et al. [52] in rats maintained on a vitamin E-deficient diet in order to enhance susceptibility to oxidative damage and then repleted with rations containing anthocyanin-rich extracts of *Abies coreana*. C3G was one of the main anthocyanin of the extract. The consumption of the anthocyanin-reach diet significantly improved plasma antioxidant capacity and decreased the vitamin E deficiency-enhanced hydroperoxides and 8-oxodeoxyguanosine concentrations in liver. These compounds are indices of lipid peroxidation and DNA damage, respectively. Authors suggested that dietary intake of anthocyanin-rich foods may contribute to overall antioxidant status, particularly in people habitually assuming low vitamin E.

Although no specific measurement of C3G activity was performed, further indirect confirmation of its antioxidant properties is provided by studies on healthy volunteers showing a relationship between the consumption of blackcurrant and elderberry juice (two well known rich sources of C3G) and the increase of plasma antioxidant activity [10, 53].

6. Biological properties

Several in vitro and in vivo reports (Table 1) suggest that cyanidin-glycosides and their derivates have different biological properties that render them as natural compounds with possible beneficial effects in various human pathologies. Moreover, differently from other flavonoids, evidence of nil or very low order of toxicity arises from toxicological evaluation on cyanidins assessed by the Joint FAO/WHO Expert Committee on Food Additives [54].

6.1. Antimutagenic activity

A variety of mutagens and carcinogens have been detected and identified in daily foods. Some of these substances have been found to be generated during storage, cooking, and digestion of foods. Yoshimoto et al. [55–57] observed in *Salmonella typhimurium* TA 98 that the mutagenicity of some carcinogenic compounds (such as Trp-P-1 and Trp-P-2, pyrolysates of tryptofan which occur in broiled beef, or 2-amino3-methylimidazo[4,5-f] quinoline isolated from baked fish, or benzo[a]pirene-4-nitroquinoline-1-oxide and dimethhyl sulfoxide extracted from grilled beef) was reduced by sweet potato (*Ipomoea batatas*) roots. One of the four varieties of sweet potato tested, the purple-colored *Ayamurasaki* variety, strongly decreased the reverse mutations induced by all purified mutagen tested. Two pigments were purified from purple-colored sweet potato and tested in the presence of a rat liver microsomal activating system. Both compounds, one of which was 3-(6,6′-caffeylferulylsophoroside)-5-glycoside of cyanidin, effectively showed a powerful antimutagenic capacity, which was not influenced by deacylation and was stronger than peonidin derivatives.

6.2. Anticarcinogenic activity

Some studies indicating a possible anticarcinogenic activity of anthocyanins have been published in the last few years. Usually, these studies were not carried out with pure cyanidin-glycosides but with anthocyanins-rich extracts from various berries.

In a recent study on rats, Hagiwara et al. [58] tested the dietary efficacy of 'purple corn color' (PCC), a natural food colorant mainly composed by C3G and extracted from *Zea mays*. They found that PCC suppressed the incidence and multiplicity of colorectal adenomas and carcinomas induced by 1,2-dimethylhydrazine and concomitant ingestion of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. Similar results have recently been reported by Miyata et al. [59] who demonstrated in rats that grapefruit juice consumption clearly inhibited colon DNA damage induced by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, which is the most abundant of the 20 haterocyclic amines identified in cooked meat and fish producing colon tumors in male rats and being implicated in the etiology of human colon cancer.

Liu et al. [35] showed that the proliferation of HrpG2 human liver cancer cells was significantly inhibited in a dose-dependent manner after exposure to the raspberry extract, whereas Bomser et al. [60] verified the anticarcinogenic properties of four *Vaccinium* species (bilberry, cranberry, lowbush blueberry and lingonberry) by determining their ability to inhibit the induction of ornithine quinone decarboxylase, the rate-limiting enzyme in polyamine synthesis, by a specific tumor promoter.

Cyanidin ability to inhibit the growth of human tumor cells in vitro has recently been monitored by Meiers et al. [61] in the human vulva carcinoma cell line A431, where cyanidin strongly inhibited the epidermal growth-factor receptor by shutting off the downstream signaling cascades. Wang et al. [62] demonstrated for the first time that cyanidin-glycosides and anthocyanin-rich extracts induced tumor necrosis factor production and they acted as modulators of the immune response in activated macrophages.

6.3. Other properties

Auger et al. [63] showed that red wine phenolic compounds were able to significantly reduce cholesterol, triglyceride and apolipoprotein B plasma concentrations and to decrease aortic fatty streak area of hypercholesterolemic Golden Syrian hamster.

The anthocyanins of tart cherries were assayed for their anti-inflammatory efficacy because consumption of cherries had been reported to reduce arthritic pain and gout. Three cyanidin-glycosides and cyanidin were tested by Wang et al. [29] for their ability to inhibit prostaglandin endoperoxide hydrogen synthase-1 and 2. The glycosides showed little or no activity whereas the aglycone had significant inhibitory activity against both enzymes.

The gastric protective effects of a cyanidin chloride (IdB 1027) occurring in bilberries have been demonstrated by different studies. The protective effect of IdB 1027 against aspirin-induced mucosa damage in man has been reported by Barzaghi et al. [64], who found that the fall in gastric transmucosal potential difference induced by aspirin was significantly reduced by dietary administration of IdB 1027.

These data confirmed similar results obtained by Cristoni et al. [65] who showed that the gastroprotective activity of IdB 1027 is mediated by amelioration in the efficiency of gastric mucosal barrier, as evidenced by the gastric bicarbonate secretion increase. The antiulcer and gastroprotective effects of IdB 1027 observed in these experiments may also be explained by the marked increase in gastric mucosal release of prostaglandin E2 reported by Mertz-Nielsen et al. [66].

In a recent study by Matsumoto et al. [9] the oral intake of a black currant anthocyanin concentrate containing C3G and Cy-3-rutinoside, improved the darkness adaptation and the transient alteration of vision induced by long time exposure to visual display terminals in healthy human subjects. The protective effect on human vision exerted by Cy-3-O-arabinoside, Cy-3-O-galactoside and C3G contained in *Vaccinium mirtillus* has been reported by Morazzoni [67] who reviewed the beneficial clinical effects obtained in patients with different ocular disorders such as myopia, simple glaucoma, retinitis pigmentosa and other ocular diseases.

Cy-3-α-L-rhamnopyranosyl-β-D-glucopyranoside and C3G extracted by Knox et al. [68] from *Ribes nigrum* L. fruits showed potent antiviral activity against influenza viruses A and B and herpes simplex virus 1.

Joseph et al. [69] associated the ingestion of some antioxidant-rich foods, including blueberry and strawberry (both containing relevant amounts of cyanidin-glycosides), with a marked decrease of age-related declines of neuronal signal transduction and cognitive and motor behavioral deficits in rats.

7. Critical considerations and future studies

The amount of experimental data evidencing important properties of many ingredients and/or bioactive substances from plants and food plants is vast and continues to increase rapidly. The use of terms nutraceuticals, functional foods, herbal extracts, bioactive dietary constituents, phytochemicals and similar is becoming copious. In many cases marketing strategies abuse these terms and health properties are claimed although far to be scientifically demonstrated. Thus, researchers are requested of a severe scientific objectivity in evaluating health properties of food ingredients. With regards to cyanidin and cyanidin-glycosides it must be taken into account the preliminary approach research stage in this field, as the interest of scientist for these compounds is more recent than that for other flavonoids. It is possible to sustain that they are promising candidates as dietary com-

pounds with a potential beneficial role in human health associated with their constant consumption. However, some critical evaluations on literature data are necessary. Among the properties of cyanidins the antioxidant activity is certainly the most investigated, as demonstrated by 34 studies. It is important to note that the majority of studies were conducted in vitro and it is well known that antioxidant activity in vitro is particularly dependent on test system applied and some systems have no relevance to any biological system (e.g., food, blood, tissue). Besides, some in vivo studies are based on quite outdated test systems (i.e., in vivo TBARS measurement of lipid peroxidation). Thus confirms in in vivo models are necessary. With regards to the antioxidative mechanism of cyanidins there are no doubts that it is mainly related to their redox properties, which play an important role in neutralizing ROS. Another protective mechanism is probably exerted by binding of metal ions, especially Cu(II). However, it has been likely disregarded the role that cyanidins could have by suppressing radical-generating enzymes (such as cytochrome P450 and xanthine oxidase) or by protecting antioxidant vitamins (C, E) and detoxifying enzymes (i.e., gluthatione S-transferase).

Besides, it still has to be established which of the aglycone or the glycoside form has the higher antioxidant activity and verified any possible pro-oxidant property.

Another problem lies with the concentrations tested in vitro, which are often several orders of magnitude higher than the likely plasma concentration. An analogous inaccuracy is also present in some in vivo studies on metabolism and bioavailability, where administered doses were higher than the mean presumable dietary intake.

Other uncertainties derive from the fact that the referred studies report data on cyanidins considered both as single chemicals and food extracts. In some cases scarce or no information were provided about i) sources and purity of chemicals; ii) quantitative measurements of the proposed active compound; iii) methods of analysis and, iv) as particularly regards extracts, extraction procedures. Obviously, the above information are essential to enable other researchers to reproduce the experiments and to obtain comparable data.

If the antioxidant properties of cyanidins are largely supported by a conspicuous number of studies, conversely, as regard the other biological activities, the number of studies is much lower and the proposed properties quite far to be demonstrated. The antimutagenicity has been only demonstrated in bacterial assays. Data on the anticarcinogenic activity are available only from studies conducted with anthocyanin-rich extracts, whereas single or few and, in some cases, outdated studies are available on other proposed biological activity (i.e., gastric-protection, anti-inflammatory and antiviral activity). A reproduction of these latter properties in other models is certainly necessary.

Thus, with the aim to establish whether these compounds are really capable to influence positively the incidence and progression of many chronic diseases, a great deal of work in several areas is still necessary. This includes i) further studies on cyanidin-glycoside metabolism in human beings; ii) analysis of factors affecting bioavailability, including interaction with other dietary compounds (i.e., other flavonoids); iii) dietary burden and variations within and between populations; iv) epidemiological studies to evaluate the relationship between cyanidin-glycoside-rich food consumption and incidence of given pathologies.

The above suggestions refer to cyanidin-glycosides in human nutrition. When considering a possible therapeutic use of future cyanidin-based drugs the amount of work to perform is even more significant. Exhaustive information on absorption, distribution, metabolism and excretion of cyanidin-glycosides administered by main possible routes (oral, intraperitoneal, intravenous, intrathecal) are largely insufficient. Few data are available on possible cyanidin-glycoside toxic effects [54] and further information are needed on their eventual side effects and teratogenic properties. Better evaluation of the pharmacological profile of cyanidin-glycosides, with a clear-cut choice of possible human pathologies to be treated in the future with eventual cyanidin-glycoside-based drugs, is certainly needed.

8. Conclusions

The abundant mass of available data reviewed from literature allows affirming that cyanidin-glycosides have relevant antioxidant activity in vitro, often higher than that of other natural antioxidants. Structure-activity relationships need further investigations. Bioavailability, absorption, metabolism and pharmacokinetics must all be considered before attempting to extrapolate from in vitro procedures to the human in vivo situations. However, in vivo experimental studies in animals gave significant indications that cyanidin-glycoside administration and consumption are effective in diminishing ROS-mediated cell and tissue damages caused by increased oxidative stress. Promising experimental evidences of cyanidin-glycoside antitumoral effects justify further studies in this clinically relevant field. Data on absorption, metabolism and fate strongly support the evidence that cyanidin-glycosides are distributed to tissue by the blood stream in their intact, powerfully antioxidant, forms. One consideration that can be drawn from a nutritional point of view is that, as red wines are considered of strategic importance in the “Mediterranean Diet”, pigmented oranges (and their relative juice), due to their relevant C3G content, might analogously be considered fundamental as well, also taking into account their large consumption, certainly larger than that of other C3G-rich food such as berries, asparagus, etc.. In addition, the powerful antioxidant activity exerted by pigmented oranges [4] suggests that they may probably be proposed as food with protective properties against age-related diseases such as cancer or atherosclerosis.

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References

[1] Hertog MGL, Hollman PCH, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in Netherlands. Nutr Cancer 1993;20:21–9.
[2] Cao G, Muccitelli HU, Sanchez-Moreno C, Prior RL. Anthocyanins are absorbed in glycated forms in elderly women: a pharmacokinetic study. Am J Clin Nutr 2001;73:920–6.
[3] Kuhnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. World Rew Nutr Diet 1976;24: 117–91.
[4] Amorini AM, Fazzina G, Lazzarino G, Tavazzi B, Di Pierro D, Santucci R, Sinibaldi F, Galvano F, Galvano G. Activity and mechanism of the antioxidant properties of cyanidin-3-O-β-glucopyranoside. Free Radic Res 2001;35:953–66.
[5] Lee HS. Characterization of major anthocyanins and the color of red-fleshed Budd Blood orange (*Citrus sinensis*). J Agric Food Chem 2002;50:1243–6.
[6] Youdim KA, Shukitt-Hale B, MacKinnon S, Kalt W, Joseph JA. Polyphenolics enhance red blood cell resistance to oxidative stress: in vitro and in vivo. Biochim Biophys Acta-Gen Subj 2000;1523:117–22.
[7] Tsuda T, Horio F, Osawa T. Absorption and metabolism of cyanidin 3-*O*-β-D-glucoside in rats. FEBS Lett 1999;449:179–82.
[8] Miyazawa T, Nakagawa K, Kudo M, Muraishi K, Someya K. Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3, 5-diglucoside, into rats and humans. J Agric Food Chem 1999;47:1083–91.
[9] Matsumoto H, Inaba H, Kishi M, Tominaga S, Hirayama M, Tsuda T. Orally administered delphinidin 3-rutinoside and cyanidin 3-rutinoside are directly absorbed in rats and humans and appear in the blood as the intact forms. J Agric Food Chem 2001;49:1546–51.
[10] Netzel M, Strasse G, Carle E, Kesenheimer B, Janssen M, Bitsch I, Rechner A, Dietrich H, Bohm V, Bitsch R. Schwarzer Johannisbeersaft=Functional Food? Lebensmittelchemie 2000;54:84–5.
[11] Netzel M, Strasse G, Janssen M, Bitsch I, Bitsch R. Bioactive anthocyanins detected in human urine after ingestion of blackcurrant juice. J Environ Pathol Toxicol Oncol 2001;20:89–95.
[12] Milbury PE, Cao G, Prior RL, Blumberg J. Bioavability of elderberry anthocyanins. Mech Ageing Dev 2002;123:997–1006.
[13] Murkovic M, Mulleder U, Adam U, Pfannhauser W. Detection of anthocyanins from elderberry juice in human urine. J Sci Food Agric 2001;81:934–7.
[14] Felgines, Texier O, Besson C, Fraisse D, Lamaison JL, Rémésy C. Blackberry anthocyanins are slightly bioavailable in rats. J Nutr Environ Med 2002;12:19-34.
[15] Tsuda T, Horio F, Osawa T. The role of anthocyanins as an antioxidant under oxidative stress in rats. Biofactors 2000;13:133–9.
[16] Seeram NP, Bourquin LD, Nair MG. Degradation products of cyanidin glycosides from tart cherries and their bioactivities. J Agric Food Chem 2001;49:4924–29.
[17] Ciappellano, S, Brusamolino, A, Drissner, M, Galvano, G. Intestinal absorption of phenolic compounds, Proceeding of 53rd National Congress of Italian Society of Physiology. 26-29 September, Ferrara, Italy, 2002.
[18] Stumpf, C, Frank, K, Andlauer, W, Furst, P. Metabolic handling of cyanidin-3-*O*-β-D-glucoside, Proceeding of 5th Karlsrue Nutritional Congress. 22-24 October 2000.
[19] Gee JM, DuPont SM, Day AJ, Plumb GW, Williamson G, Johnson JT. Intestinal transport of quercetin glycosides in rats involves both deglycosilation and interaction with hexose transport pathway. J Nutr 2000;130:2765–71.
[20] Gee JM, DuPont MS, Rhodes MJP, Johnson IT. Quercetin glucosides interact with the intestinal glucose transport pathway. Free Radic Med 1998;25:19–25.
[21] Walgren RA, Lin JT, Kinne RKH, Walle T. Cellular uptake of dietary flavonoid quercetin 4'– β-glucoside by sodium-dependent glucose transporter SGLT1[1]. J Pharmacol Exp Ther 2000;294:837–43.
[22] Wolffram S, Block M, Ader P. Quercetin-3-Glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. J Nutr 2002;132:630–5.
[23] SatueGracia MT, Heinonen M, Frankel EN. Anthocyanins as antioxidants on human low-density lipoprotein and lecithin-liposome systems. J Agric Food Chem 1997;45:3362–7.
[24] Wang H, Cao GH, Prior RL. Oxygen radical absorbing capacity of anthocyanins. J Agric Food Chem 1997;2:304–9.
[25] Proteggente AR, Pannala AS, Pagana G, Van Buren L, Wagner E, Wiseman S, Van De Put F, Dacombe C, Rice-Evans CA. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radic Res 2002;36: 217–33.
[26] Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res 1995;22:375–83.
[27] Seeram NP, Momin RA, Nair MG, Bourquin LD. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. Phytomedicine 2001;8:362–9.
[28] Landrault N, Poucheret P, Ravel P, Gasc F, Cros G, Teissedre PL. Antioxidant capacities and phenolics levels of French wines from different varieties and vintages. J Agric Food Chem 2001;49:3341–8.
[29] Wang H, Nair MG, Strasburg GM, Chang YC, Booren AM, Gray JI, DeWitt DL. Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. J Nat Prod 1999;62:294–6.
[30] Tsuda T, Ohshima K, Kawakishi S, Osawa T. Oxidation products of cyanidin 3-*O*-β-D-glucoside with a free radical initiator. Lipids 1996; 31:1259–63.
[31] Sarma AD, Sreelakshmi Y, Sharma R. Antioxidant ability of anthocyanins against ascorbic acid oxidation. Phytochemistry 1997;45: 671–4.
[32] Sarma AD, Sharma R. Anthocyanin-DNA copigmentation complex: mutual protection against oxidative damage. Phytochemistry 1999; 52:1313–8.
[33] Russo A, Bonina F, Acquaviva R, Campisi A, Galvano F, Ragusa N, Vanella A. Antioxidant activity and protective effect on DNA cleavage of a red orange extract. J Food Sci 2002;67:2814–8.
[34] Mas T, Susperregui J, Berke B, Cheze C, Moreau S, Nuhrich A, Vercauteren J. DNA triplex stabilization property of natural anthocyanins. Phytochemistry 2000;53:679–87.
[35] Liu M, Qi Li X, Weber C, Lee CY, Brown J, Liu RH. Antioxidant and antiproliferative activities of raspberries. J Agric Food Chem 2002; 50:2926–30.
[36] Youdim KA, Martin A, Joseph JA. Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress. Free Radic Biol Med 2000;29:51–60.
[37] Wang J, Mazza G. Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN-gamma-activated RAW 264.7 macrophages. J Agric Food Chem 2002;50: 850–7.
[38] Tsuda T, Watanabe M, Ohshima K, Norinobu S, Choi SW, Kawakishi S, Osawa T. Antioxidative activity of the anthocyanin pigments cyanidin 3-*O*-β-D-glucoside and cyanidin. J Agric Food Chem 1994; 42:2407–10.
[39] Seeram NP, Schutzki R, Chandra A, Nair MG. Characterization, quantification, and bioactivities of anthocyanins in *Cornus* species. J Agric Food Chem 2002;50:2519–23.

[40] Renaud S, Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 1992;339:454–7.
[41] Kondo K, Matsumoto A, Kurata H, Tanahashi H, Koga H, Amachi T, Itakura H. Inhibition of low density lipoprotein with red wine. Lancet 1994;344:1152.
[42] Fuhrman B, Lavy A, Aviram M. Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. Am J Clin Nutr 1996;61:549–54.
[43] Frankel EN, Waterhouse AL, Teissedre PL. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. J Agric Food Chem 1995;43:890–4.
[44] Hu, C, Zawistowski, J, Kitts, DD. Phytochemical characterization and bioactivities of anthocyanins from pigmented rice, 2002 Annual Meeting and Food Expo – Anaheim, California. Session 84, Nutraceuticals & Functional Foods.
[45] Tsuda T, Shiga K, Ohshima K, Kawakishi S, Osawa T. Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from *Phaseolus vulgaris* L. Biochem Pharmacol 1996;52:1033–103952.
[46] Amorini AM, Lazzarino G, Galvano F, Fazzina G, Tavazzi B, Galvano G. Cyanidin-3-*O*-*β*-D-glucopyranoside protects myocardium and erythrocytes from oxygen radical-mediated damages. Free Radic Res 2003;37:453–60.
[47] Tsuda T, Horio F, Osawa T. Dietary cyanidin 3-*O*-*β*-D-Glucoside increases ex vivo oxidation resistance of serum in rats. Lipids 1998; 33:583–8.
[48] Tsuda T, Horio F, Kitoh J, Osawa T. Protective effects of dietary cyanidin 3-*O*-*β*-D-Glucoside on liver ischemia-reperfusion injury in rats. Arch Biochem Biophys 1999;368:361–6.
[49] Tsuda T, Horio F, Kato Y. Cyanidin 3-O*β*D-glucoside attenuates the hepatic ischemia-reperfusion injury through a decrease in the neutrophil chemoattractant production in rats. J Nutr Sci Vitaminol 2002; 48:134–41.
[50] Bertuglia S, Malandrino S, Colantuoni A. Effect of *Vaccinium myrtillus* anthocyanosides on ischemia reperfusion injury in hamster cheek pouch microcirculation. Pharmacol Res 1995;31:183–7.
[51] Toyokuni S, Itani T, Morimitsu Y, Okada K, Ozeki M, Kondo S, Uchida K, Hiai H, Tashiro T. Protective effect of colored rice over white rice on Fenton reaction-based renal lipid peroxidation in rats. Free Radic Res 2001;36:583–92.
[52] Ramirez-Tortosa C, Andersen OM, Gardner PT, Morrice PC, Wood SG, Duthie SJ, Collins AR, Duthie GG. Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. Free Radic Biol Med 2001;31:1033–7.
[53] Janssen M, Netzel M, Strasse G, Kesenheimer B, Herbst M, Carle E, Bitsch I, Bohm V, Bitsch R. Increased plasmatic antioxidant capacity in humans after ingestion of black currant and elderberry juice. In: Martens S, Treutter D, Forkmann G, editors. Polyphenols communications. Munich: Technische Universitat Munchen, 2000. p. 367–368.
[54] http://www.inchem.org/documents/jecfa/jecmono/v17je05.htm.
[55] Yoshimoto M, Okuno S, Kumagai T, Yoshinaga M, Yamakawa O. Distribution of antimutagenic components in colored sweetpotato. JARQ 1999;33:143–8.
[56] Yoshimoto M, Okuno S, Yoshinaga M, Yamakawa O, Yamaguchi O, Yamada J. Antimutagenicity of sweetpotato (*Ipomoea batatas*) roots. Biosci Biotechnol Biochem 1999;63:537–41.
[57] Yoshimoto M, Okuno S, Yamaguchi M, Yamakawa O. Antimutagenicity of deacylated anthocyanins in purple-fleshed sweetpotato. Biosci Biotechnol Biochem 2001;65:1652–5.
[58] Hagiwara A, Miyashita K, Nakanishi T, Sano M, Tamano S, Kadota T, Koda T, Nakamura M, Imaida K, Ito N, Shirai T. Pronounced inhibition by a natural anthocyanin, purple corn color, of 2-amino-1-methyl-6-phenylimidazo[4, 5-*b*]pyridine (PhIP)-associated colorectal carcinogenesis in male F344 rats pretreated with 1, 2-dimethylhydrazine. Cancer Lett 2001;171:17–25.
[59] Miyata M, Takano H, Takahashi K, Sasaki YF, Yamazoe Y. Suppression of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine-induced DNA damage in rat colon after grapefruit juice intake. Cancer Lett 2000;183:17–22.
[60] Bomser J, Madhavi DL, Singletary K, Smith MAL. In vitro anticancer activity of fruit extracts from *Vaccinum* species. Planta Med 1996;62:193–288.
[61] Meiers S, kemeny M, Weyand U, Gastpar R, von Angerer E, Marko D. The anthocyanidins cyanidin and delphinidin are potent inhibitors of the epidermal growth-factor receptor. J Agric Food Chem 2001; 49:958–62.
[62] Wang J, Mazza G. Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor alpha in LPS/IFN-gamma-activated RAW 264.7 macrophages. J Agr Food Chem 2002; 50:4183–9.
[63] Auger C, Caporiccio B, Landrault N, Teissedre PL, Laurent C, Cros G, Besancon P, Rouanet JM. Red wine phenolic compounds reduce plasma lipids and apolipoprotein B and prevent early aortic atherosclerosis in hypercholesterolemic Golden Syrian hamster (*Mesocricetus auratus*). Nutr Metab 2001;131:2837–42.
[64] Barzaghi N, Gatti G, Crema F, Perucca E. Protective effect of cyanidin (IdB 1027) against aspirin-induced fall in gastric transmucosal potential difference in normal subjects. Ital J Gastroenterol 1991;23: 249–52.
[65] Cristoni A, Malandrino S, Magistretti MJ. Effect of a natural flavonoid on gastric mucosal barrier. Arzneimittelforschung 1989;39: 590–2.
[66] Mertz-Nielsen A, Munck LK, Bukhave K, Rask-Madsen J. A natural flavonoid, IdB 1027, increases gastric luminal release of prostaglandin E2 in healthy subjects. Ital J Gastroenterol 1990;22:288–90.
[67] Morazzoni P. Vaccinium Myrtillus anthocyanosides, In *Eppos* – Rivista Italiana, Actes des 13èmes Journèes Internationales Huiles Essentielles. 1-3 September Digne-Les-Bains, France 515-54.
[68] Knox YM, Hayashi K, Suzutani T, Ogasawara M, Yoshida I, Shiina R, Tsukui A, Terahara N, Azuma M. Activity of anthocyanins from fruit extract of *Ribes nigrum* L. against influenza A and B viruses. Acta Virol 2001;45:209–15.
[69] Joseph JA, Shukitt-Hale B, Denisova N, Bielinski D, Martin A, McEwen JJ, Bickford PC. Reversals of age-related declines in neuronal signal trasduction, cognitive, and motor behavioral deficits whit blueberry, spinach, or strawberry dietary supplementation. J Neurosci 1999;19:8114–21.