

## **Anthocyanins: occurrence, bioactivity and bioavailability, with special reference to the anthocyanins of raspberries (a review)**

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Anthocyanins are the most abundant flavonoid constituents of vegetables and fruits, especially berries. Human consumption of anthocyanins is among the highest of all flavonoids. Epidemiological studies have suggested that the consumption of anthocyanins lowers the risk of cardiovascular disease, diabetes, arthritis and cancer.

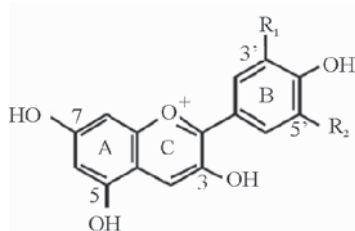
Therefore, the evaluation of berry genetic resources for the presents of bioactive compounds, such as anthocyanins, and investigation of their biological properties is doubtless significant and of great benefit for breeders, food and pharmaceutical industries.

The anthocyanin content and composition of raspberries and their absorption, metabolism, and biological activity are reviewed in this article.

**Key words:** anthocyanins, antioxidants, berries, health properties, metabolism.

**Introduction.** Anthocyanins are the most abundant flavonoid constituents of fruits and vegetables. They comprise the largest and most important group of water-soluble pigments in nature.

Anthocyanins are conjugated anthocyanidins, which are accumulated in cell vacuoles and are responsible for diverse pigmentation from orange to red, purple and blue in flowers, fruits and vegetables (Szajdek, Borowska, 2008). Anthocyanins have a C6 (A ring)-C3 (C ring)-C6 (B ring) flavonoid skeleton (Fig. 1). Approximately 95 % of all anthocyanins are derived from six anthocyanidins (aglycones): cyanidin, peonidin, pelargonidin, malvidin, delphinidin, and petunidin (Fig. 1) (Beattie et al., 2005; He, Giusti, 2010). Glucose and rhamnose are the more common sugar moieties attached to the aglycone, but galactose, arabinose, xylose, rutinose, sambubiose, and other sugars are also frequently found (He, Giusti, 2010). Anthocyanins are usually present in a coloured flavylium cation form, but may also be in an uncoloured form depending on the pH. This characteristic is an important issue when analyzing anthocyanins and should be considered when interpreting data in the literature from the technological point of view and with respect to health-related studies (Pascual-Teresa, Sanchez-Ballesta, 2007).



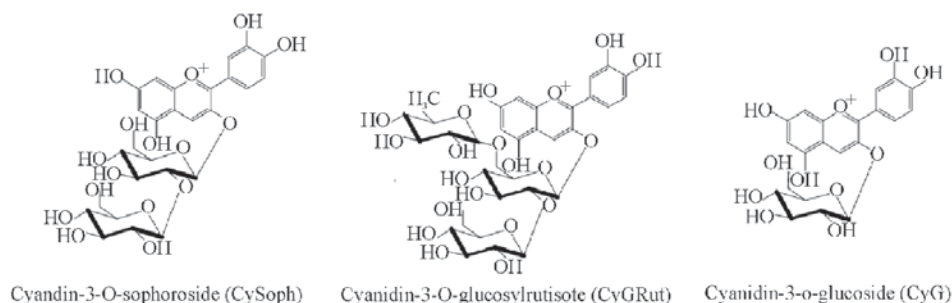
Anthocyanidin Antocianidinas	R <sub>1</sub>	R <sub>2</sub>	Colour Spalva	λ max (nm) in HCl acidified MeOH λ max (nm) HCl parūgštintame MeOH
Pelargonidin Pelargonidinas	H	H	Orang-red Oranžinė-raudona	520
Cyanidin Cianidinas	OH	H	Red Raudona	535
Delphinidin Delfinidinas	OH	OH	Pink Rausva	546
Peonidin Peonidinas	OCH <sub>3</sub>	H	Bluish purple Melsvai purpurinė	532
Petunidin Petunidinas	OCH <sub>3</sub>	OH	Purple Purpurinė	543
Malvidin Malvidinas	OCH <sub>3</sub>	OCH <sub>3</sub>	Redish purple Rausvai purpurinė	542

**Fig. 1.** Structures of the major anthocyanidins  
**1 pav.** Pagrindinių antocianinų sandara

Anthocyanins in plants are synthesized from precursors through two biosynthetic pathways: shikimate, producing phenylalanine; and that generating malonyl-CoA. These two precursors are linked by chalcone synthase via a polyketide folding mechanism, to form an intermediate chalcone, a substrate for chalcone isomerase generating prototype pigment naringenin, subsequently oxidized by a series of enzymes (flavanone 3-hydroxylase, flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase) to leucoanthocyanidins, and finally converted to anthocyanidins by leucoanthocyanidin dioxygenase. Unstable anthocyanidins are coupled to a small range of sugars by activities such as UDP-glucose/flavonoid 3-O-glucosyltransferase and O-methyltransferase to yield anthocyanins (Kassim et al., 2009).

**Anthocyanins in raspberries.** Among berry phenolics, anthocyanins are the best studied (Seeram, 2008). The major anthocyanins in raspberries and blackberries are derivatives of cyanidins, which commonly exist in non-acylated forms. Red raspberries contain a wide spectrum of anthocyanins with the major constituents being cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside and cyanidin-3-glucoside (Fig. 2) with smaller quantities of cyanidin-3-xylosylrutinoside, cyanidin-3,5-diglucoside, cyanidin-3-rutinoside, cyanidin-3-sambubioside, pelargonidin-3-sophoroside, pelargonidin-3-glucosylrutinoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside (Beekwilder et al., 2005 a; Perez-Vicente et al., 2004; Mullen et al.,

2002; Kassim et al., 2009). In general, pelargonidin glycosides comprise the minority of the total anthocyanin content in red raspberries (typically less than 2). When the inheritance of anthocyanins in various *Rubus* species was studied, it was noted that some degree of orange colour is imparted to progenies when pelargonidin pigments are incorporated (Hall et al., 2009).

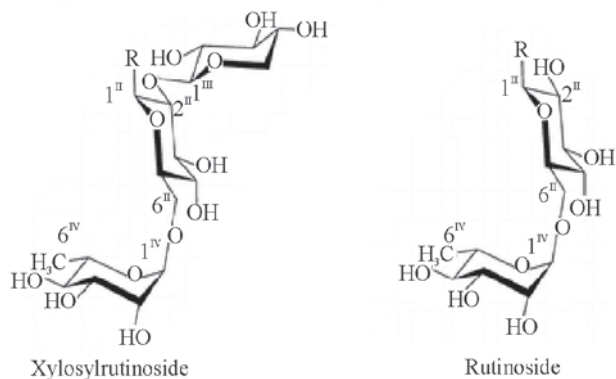


**Fig. 2.** The major anthocyanins present in red raspberries  
**2 pav.** Pagrindiniai antocianinai, aptinkami raudonosiose avietėse

Significant differences have been reported within raspberry cultivars with respect to relative amounts of individual anthocyanins (Beekwilder et al., 2005 a; Borges et al., 2010). Moreover, significant year-to-year variations in the contents of anthocyanins have been noted in raspberries by different authors (Koponen et al., 2007; Kassim et al., 2009). Low and high temperatures have long been considered to promote and reduce, respectively, anthocyanin synthesis in fruits and berries.

The total anthocyanin content is one of the main criterions used to differentiate between the species of *Rubus idaeus* and *Rubus occidentalis*. Black raspberries accumulate considerably higher amounts of anthocyanins than red raspberries (Bobinaite et al., 2012; Viskelis et al., 2010; Cheplick et al., 2007; Weber et al., 2008), blackberries (Wada, Ou, 2002; Wang, Lin, 2000) and some black currant and blueberry genotypes (Moyer et al., 2002). Anthocyanins in black raspberry consist mainly of cyanidin-3-xylosylrutinoside and cyanidin-3-rutinoside (Fig. 3) with smaller amounts of cyanidin-3-glucoside, cyanidin-3-sambubioside and pelargonidin-3-rutinoside (Tian et al., 2006; Tulio et al., 2008).

The concentration of anthocyanins in raspberries is low at the beginning of fruit ripening (green fruit) with only cyanidin-3-glucoside present and some traces of cyanidin-3-rutinoside. When raspberry fruits turn pink, small amounts of cyanidin-3-sophoroside and cyanidin-3-glucosylrutinoside are produced. By the time the fruit is red, the quantity of these anthocyanins sharply increases, and pelargonidin glycosides begin to form the last (Beekwilder et al., 2005 a).



**Fig. 3.** The major glycosides of cyanidins in black raspberries  
**3 pav.** Pagrindiniai juodosios avietės aptinkami cianidinų glikozidai

Anthocyanin contents greatly vary depending on raspberry variety (Bobinaite et al., 2012). Overall, the total anthocyanin content of red raspberries is typically between 20 and 100 mg in 100 g of fresh fruit, and the total anthocyanin content of black raspberries is between 200 and 600 mg in 100 g of fresh fruit (Szajdek, Borowska, 2008; Borges et al., 2010; Weber et al., 2008; Wang, Lin, 2000; Moyer et al., 2002).

Anthocyanins are the second largest group after ellagitannins of phenolic antioxidants found in red raspberry fruits (Määttä-Riihinen et al., 2004; Beekwilder et al., 2005 b).

It should be noted that the between-study variation in reported concentrations of anthocyanins in raspberries is much less than that of ellagitannins.

Beekwiler and co-workers reported that the contribution of anthocyanins to the total antioxidant activity of red raspberries is approximately 25, while the contribution of ellagitannins – 55 (Beekwilder et al., 2005 b). In a similar study, Borges and co-workers reported that the contribution of anthocyanins to the total antioxidant capacity of red raspberries is only 16.5 (Borges et al., 2010).

In black raspberries, two major anthocyanins, namely cyanidin-3-rutinoside and cyanidin-3-xylosylrutinoside, have been reported as the most important phenolic antioxidants (Tulio et al., 2008).

**Biological effects and putative health benefits of anthocyanins.** Epidemiologic studies have suggested that the consumption of anthocyanins lowers the risk of cardiovascular disease, diabetes, arthritis and cancer (Prior, Wu, 2006).

Although most of the protective effects of anthocyanins are attributed to their ability to scavenge reactive oxygen species (ROS) (Prior, Wu, 2006), they also function by chelating metals and by direct binding to proteins (Kong et al., 2003). The radical scavenging activity of the anthocyanidins (aglycons) is superior to their respective anthocyanins (glycosides), and it decreases as the number of sugar moieties increase (Wang, Stoner, 2008). Anthocyanin cyanidin, with B ring 3',4'-dihydroxy substituents

and conjugation between A and B rings, have antioxidant potentials four times higher than that of a vitamin E analogue (Rice-evans et al., 1995).

Cancer cell growth is dependent on the balance between proliferation and apoptosis. Unregulated cell proliferation and suppression of apoptosis are key steps in the initiation and progression of cancer.

The following potential cancer chemopreventive activities of anthocyanins have been revealed from *in vitro* studies: radical scavenging activity; stimulation of phase II detoxifying enzymes; reduction of cell proliferation, inflammation, angiogenesis and invasiveness; and induction of apoptosis and differentiation. Anthocyanins modulate the expression and activation of multiple genes associated with these cellular functions (Wang, Stoner, 2008). *In vivo* studies have shown that dietary anthocyanins inhibit cancers of the gastrointestinal tract and that topically applied anthocyanins inhibit skin cancer (He, Giusti, 2010). Regular consumption of raspberry anthocyanins is reported to improve brain function, age-related degeneration of eyesight and influence cardiovascular disease (Stone et al., 2007).

Wang et al. recently emphasised the importance of black raspberry anthocyanins as preventive agents against some degenerative diseases, and their *in vivo* study showed that the anthocyanin-rich extract of black raspberries is nearly as effective in preventing oesophageal cancer in rats as whole black raspberries containing the same concentration of anthocyanins (Wang et al., 2009). However, in the same study, they mentioned that another diet containing an alcohol insoluble (residue) fraction of black raspberries (containing only trace amounts of anthocyanins) was also nearly as effective as the anthocyanin-rich fraction in preventing tumourigenesis. The authors suggested that the residue fraction comprised high levels of ellagitannins.

**Bioavailability and metabolism of anthocyanins.** Human consumption of anthocyanins is among the highest of all flavonoids (He, Giusti, 2010). The daily intake of anthocyanins and other flavonoids in the U. S. diet is estimated to be 190 mg/d, but the intake of anthocyanins and other flavonoids in Finland is estimated to be somewhat lower at 80 mg/d (Ovaskainen et al., 2008).

The absorption of anthocyanins differs from the other flavonoids because they are mainly absorbed as intact glycosides. However, glucuronidated and methylated anthocyanin metabolites have also been reported in human plasma (Cerdá et al., 2005).

The absorption of anthocyanins occurs quickly following consumption. The maximum plasma concentration is reached between 45 min to 4 h after the ingestion of an anthocyanin-containing meal depending on the conditions of the experiment (Pascual-Teresa, Sanchez-Ballesta, 2007). These observations have led to the suggestion that anthocyanins are absorbed not only by the upper part of small intestine but also by the stomach (McGhie, Walton, 2007; Passamonti et al., 2003). A variety of anthocyanins appear in urine after supplementation with berries or berry extracts but in low concentrations (usually 0.1 or lower of the ingested dose). Kay and co-workers reported that the recovery of anthocyanins in urine after consumption is in a range from 0.03 to 4. (Kay et al., 2004).

Because the absorption of anthocyanins is low, most of the anthocyanins pass into the colon intact and may be degraded into phenolic acids by gut microflora followed by absorption (Aura et al., 2005; Freschhut et al., 2006). Protocatechuic acid has been

identified as a major metabolic by-product of anthocyanins produced by human faecal bacteria (Freschhut et al., 2006).

In general, the absorption, metabolism, and recovery of anthocyanins are dependent on the structure of aglycone and the sugar moiety (Pascual-Teresa, Sanchez-Ballesta, 2007).

**Conclusions.** Berries are one of the richest dietary sources of anthocyanins, with black raspberries having one of the highest anthocyanin contents among them. Red raspberries as compared to certain fruits and berries accumulate quite low amounts of anthocyanins, but if genes underlying production were determined marker assisted breeding strategies could be employed to enhance their anthocyanins content.

The research experience and knowledge during the last years concerning berry anthocyanins have increased a lot. Anthocyanins have been demonstrated to have a wide range of bioactivities. It was demonstrated by a large number of *in vitro* and *in vivo* studies. However in most of the interventions of anthocyanins in human health, details on the mechanisms of action for bioactivity, transformations that these compounds undergo *in vivo*, whole body distribution and tissue localization are still not fully elucidated. Therefore, it is clear that more research is needed to understand the mechanism of action and effectiveness of anthocyanins *in vivo*.

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**Aviečių antocianinai: paplitimas, biologinis aktyvumas,  
biologinis prieinamumas**

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*Santrauka*

Antocianinai yra labiausiai paplitę iš flavonoidų grupės junginių, aptinkamų daržovėse, vaisiuose, ypač uogose. Iš flavonoidų grupės junginių žmonės daugiausia suvartoja būtent antocianinų. Epidemiologinių tyrimų duomenimis, antocianinų vartojimas mažina riziką susirgti širdies ir kraujagyslių ligomis, diabetu, artritu bei vėžiu. Todėl uogų genetinių išteklių įvertinimas, identifikuojant jose esančius biologiškai aktyvius junginius, tokius kaip antocianinai, ir tų junginių biologinių savybių tyrimas yra neabejotinai svarbūs ir naudingi tiek selekcijos tikslais, tiek maisto ir farmacijos pramonei.

Šiame straipsnyje apžvelgiama aviečių uogų antocianinų kokybinė ir kiekybinė sudėtis, jų absorbcija, metabolizmas ir biologinis poveikis.

**Reikšminiai žodžiai:** antioksidantai, antocianinai, metabolizmas, poveikis sveikatai, uogos.