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ANTHOCYANINS IN INDIGENOUS AND CULTURED FRUIT IN POLIMLJE, NORTH-EAST OF MONTENEGRO[*]

ABSTRACT

Investigations determined the qualitative content of anthocyanins in cultivated apples, plums, cherries and sour cherries, and raspberries, strawberries, blackberries, myrobalans and cornelian cherries which grow spontaneously in the Polimlje area.

The qualitative analysis of anthocyanins was performed using the one-dimensional ascending thin-layer chromatography method, while the relative content vas evaluated using a numeric scale of 0-5. The total European blueberry (*Vaccinium myrtillus* L.) anthocyanin content was investigated according to the procedure described in the European Pharmacopeia 6.0. Anthocyanin aglycones were analysed using high performance liquid chromatography (HPLC).

The contrasts in genotype stipulated qualitative and quantitative variations in the content of anthocyanins between different species, and between the same species and genotypes.

Altogether, seventeen anthocyanins were identified, whereof eleven were from cultured fruit and eight from indigenous fruit.

Different fruit species contained the same anthocyanins: *cyanidin-3-abinoside* was identified in apples and cornelian cherries, *cyanidin-3-glycoside* in plums, myrobalans and raspberries, and *peonidin-3-rutinoside* in plums and myrobalans.

Key words: apple, sour cherry, plum, myrobalan, raspberry, cornel, bilberry, anthocyanin

INTRODUCTION

Anthocyanins are pigments which give fruit and vegetables blue, purple or dark red colours. They are strong antioxidants which are materials that prevent the development of heart disease, cancer and other diseases. The consumption of

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synthetic antioxidants is very popular, however their influence is not very high, and they can be toxic as well. Antioxidants in foods are more effective and are not toxic because fruit does not contain heavy metals in amounts that are harmful to human health (Mladenović-Antić et al., 2009).

The consumption of fruit and vegetables is the best way to provide useful antioxidants to organisms. Lately, the accent has been on the potential advantages of the consumption of blue, purple and dark red food (blueberries, blackberries, plums, apples, cherries, sour cherries, raspberries, strawberries, cornels, etc.). Anthocyanins can also be used in the pharmaceutical industry (Balijagić et al., 2010), colour and polish industry, and conservation industry, which contributes to their demand in the world market.

Because of their content of tannins, anthocyanins, flavonoids, plant sugars and pectin, blueberry and raspberry fruits are used in standard and alternative medicine. From fresh raspberries pharmacies are preparing Raspberry Syrup (*Syrupus Rubi Idaei*). And according to Balijagić et al. (2010) bilberry fruit (*Myrtilli fructus*) is in the official pharmacopoeia (DAC 1986; ÖAB9; Helv VII).

Cells with red coloured vacuoles are distributed in the epidermis and sub-epidermis of the fruit. Since the properties of the sub-epidermal layer cells are inherited, this research is important for genetics and pomology, because it contributes to a better knowledge of the way to inherit the anthocyanin disposition in the epidermis of fruit (Mišić and Tešović, 1970; 1972). Considering the various values in coloured fruit usage, these investigations are also important for technology. Furthermore, anthocyanins are very interesting even as chemotaxonomic markers.

Previous investigations determined the qualitative content of anthocyanins in James Grieve, Delicious and Jonathan apples (Mišić and Tešović, 1970; 1972), Krstovača apples (Jovančević and Popović, 2002), Požegača and Ruth Gerstätter plums (Marić et al., 2007), sour cherries and May Duke, Richmorency, and Heimanns Konservenweichsel cherries, which are cultivated (Tešović, 1976; 1985).

The fruits of 316 wild red raspberry seedlings (*Rubus idaeus* L.) from Mounts Maljen and Željin (Serbia), Visitor and Hajla (Montenegro**)** and Đeravica (Tešović, 1988), European blueberry genotypes (*Vaccinium myrtillus* L.) from 11 sites (Mounts Bjelasica and Lisa and the Koritsko plateau) (Jovančević et al., 2010) and 16 cornelian cherry genotypes (*Cornus mas* L.), which grow spontaneously in the Polimlje area (Jovančević et al., 2009), were also evaluated in this study.

MATERIALS AND METHODS

The qualitative analysis of anthocyanins was performed using the one-dimensional ascending thin-layer chromatography method according to Tešović (1988). The identification of anthocyanins was done based on the Rf value, while the relative content was evaluated using a numeric scale 0-5 chromatograph (HPLC). The total anthocyanin content in European blueberries (*Vaccinium* *myrtillus* L.) was investigated according to the procedure described in the European Pharmacopeia 6.0. Anthocyanin aglycones were analysed using high performance liquid chromatography. The cellular content and distribution of anthocyanins in the first three layers of the fruit epidermis were examined using histological methods (Mišić and Tešović, 1970).

RESULTS AND DISCUSSION

The differences in genotypes caused qualitative and quantitative differences in the content of anthocyanins in cultivated and spontaneously grown fruits in the Polimlje area. The differences in the number of anthocyanins were manifested within the species, therefore all the cultivars and genotypes do not contain anthocyanins typical for actual species.

The appearance of the red colour of the cell juice in the epidermis of the apple fruit is caused by the anthocyanin *cyanidin-3-galactoside* as the primary pigment, and *cyanidin-3-arabinoside* and *cyanidin-7-arabinoside* as the secondaries. All three anthocyanins are present in the fruit of the Jonathan, Delicious (Mišić and Tešović, 1970) and James Grieve apples (Mišić and Tešović, 1972), and only two (*cyanidin-3-galactoside* and *cyanidin-3-arabinoside*) in the fruit of Krstovača apples (Jovančević and Popović, 2002).

The percentage of cells with anthocyanin varies in the various cultivars from 31.6 (in Richard) to 30.4 (in Jonathan), while in the fruit skin of the Golden Delicious no anthocyanin has been found. The tendency of anthocyanin distribution in the cells of the fruit skin is as follows: sub-epidermis, epidermis and third layer. In Richard apples 53.8 % of the cells of the sub-epidermis had anthocyanin, and in Jonathan apples 49.0 % did. We found the following percentages in the epidermis of the cells with anthocyanin: 31.2 % in Richard and 32.3 % in Jonathan. The third layer of cells contains the following quantities of anthocyanin: 9.9 % in Richard and 10 % in Jonathan. The inheritance of the anthocyanin content in the skin of F1 hybrid apples is polygenic (Tešović, 1976). In the cellular distribution of anthocyanins in the fruit skin of apple cultivars parenting is important, since the properties of the sub-epidermal layer are transmitted to the offspring (Crane and Lawrence, 1956).

Bulatović (1978) isolated in the Ruth Gerstätter plum cultivar three anthocyanins (*cyanidin-3-rutinoside, cyanidin-3-glucoside* and *peonidin-3-rutinoside*) and in the Požegača four anthocyanins (*cyanidin-3-rutinoside, cyanidin-3-glucoside, peonidin-3-rutinoside* and *peonidin-3-glucoside*). The identified anthocyanins were present in the analysed material to a varying degree, so that *cyanidin-3-glucoside* was found in the fruit skin of the Stanley, Čačanska Najbolja, Čačanska Rodna and Požegača plums; *peonidin-3-glucoside* in the Stanley and Čačanska Rana plums; *peonidin-3-rutinoside* in the Stanley, Čačanska Rana, Čačanska Najbolja, Čačanska Rodna and Požegača plums; and *cyanidin-3-rutinoside* in the Stanley, Čačanska Rana, Čačanska Najbolja, Čačanska Rodna and Požegača plums (Marić et al., 2007). The relative average content of the identified anthocyanins in the analysed plum cultivars was: 4.0 for *peonidin-3-rutinoside,* 2.2 for *peonidin-3-glucoside,* 2.2 for *cyanidin-3-rutinoside* and 0.8 for *cyanidin-3-glucoside.*

Table 1: Anthocyanins in indigenous and cultured fruit in Polimlje

Anthocyanins \ fruit	*Malus silvestris* L. Miller	*Prunus domestica* L.	*Prunus cerasifera* Ehrh.	*Cerasus vulgaris* Mill. (*Prunus cerasus* L.)	*Rubus idaeus* L.	*Cornus mas* L.
cyanidin-3-galactoside	+					
cyanidin-3-arabinoside	+					+
cyanidin-7-arabinoside	+					
cyanidin-3-gentibioside				+		
cyanidin-3-glucoside		+	+		+	
cyanidin-3-rutinoside		+				
cyanidin-3-rhamnoglucoside				+		
cyanidin-3-monoglucoside				+		
cyanidin-3-diglycoside				+		
cyanidin-3-sophoroside					+	
cyanidin-3-glucosylrutinoside					+	
cyanidin-3-rutinoside					+	
delphinidin-3-glucoside						+
peonidin-3-rutinoside		+	+			
peonidin-3-glucoside		+				
peonidin-3-arabinoside						+
peonidin-3-rhamnoside						+

From the epidermis of the cherry plum Jovančević and Božović (2001) isolated two anthocyanins (*cyanidin-3-glucoside* and *peonidin-3-rutinoside*) in the cell juice, while Pančev and Vasilev (1973) isolated four anthocyanins in *Prunus cerasifera* Ehrh. sp. Since the plum cultivars differ in biological properties, the differences were noticed in the anthocyanin content as well. There is variability even in the population of cherry plums, because in the Polimlje area there are all tones, from light yellow to dark red and dark blue colours of the fruit. Therefore, with the investigation of a higher number of biologically different genotypes there would be a higher probability of determining all the named anthocyanins. However, the low temperatures that occurred on 14 April 2001 (-4.3 ^{0}C) and 16 April 2001 (- 6.0 ^{0}C) during flowering, carried out a selection between genotypes, so that it was a rare tree in which both genotypes were observed in the yield (Jovančević and Božović, 2001).

The colour of the fruit is one of the most important criteria for sour cherry selection. Different tones of the red colour of the sour cherry fruits are conditioned by the number and quantity of anthocyanins present. In the cultivar Richmorency, Tešović (1985) isolated two anthocyanins (*cyanidin-3-rhamnoglucoside* and *cyanidin-3-gentiobioside*). The cultivar Heimanns Konservenweichsel contains four anthocyanins (*cyanidin-3-gentiobioside, cyanidin-3-rhamnoglucoside*, *cyanidin-3-monoglucoside* and *cyanidin-3-diglycoside*), and May Duke contains three anthocyanins (*cyanidin-3-gentiobioside,* *cyanidin-3-rhamnoglucoside* and *cyanidin-3-diglycoside*) (Tešović, 1987).

The fruit of 12.5% of the seedlings from the progeny of Heimanns Konservenweichsel x May Duke contained 4 anthocyanins (*cyanidin-3-rhamnoglucoside, cyanidin-3-gentiobioside, cyanidin-3-monoglucoside* and *cyanidin-3-diglycoside*), the fruit of 28.1% of seedlings had 3 anthocyanins *(cyanidin-3-rhamnoglucoside*, *cyanidin-3-gentiobioside* and *cyanidin-3-monoglucoside*), the fruit of 28.1% of the seedlings had two combinations, each containing two anthocyanins (18.7% *cyanidin-3-rhamnoglucoside* + *cyanidin-3-gentiobioside* and 9.4% *cyanidin-3-rhamnoside* + *cyanidin-3-diglycoside*), and the fruits of 31.3% contained one anthocyanin (18.8% *cyanidin-3-rhamnoglucoside* and 12.5% *cyanidin-3-gentiobioside*) (Tešović, 1987).

In the fruit of the wild red raspberry (*Rubus idaeus* L.), there's a big difference between seedlings which come from different populations in the content quantity of *cyanidin-3-glucoside, cyanidin-3-reutinoside, cyanidin-3-sophoroside* and *cyanidin-3-glucosilrutinoside.* The origin of the seedlings also influences the absence of particular anthocyanins in raspberry fruits (Tešović, 1988). The greatest number of seedlings (3.2%) which have only traces of *cyanidin-3-glucoside* (19.0%), or do not contain it at all originate from Mount Maljen. Most of the seedlings with a low content of this pigment (28.3%) are from Mount Hajla, and with a high content (54.2%) are from Mount Vizitor. The greatest number of seedlings (28.5%) which do not contain *cyanidin-3-rutinoside* originate from Mount Đeravica, with a small amount from Mount Vizitor (31.7%), and the high (14.9%) and very high content (2.2%) originate from Mount Hajla. The greatest number of seedlings (3.3%) which have only traces of *cyanidin-3-sophoroside* (8.3%), or do not contain it at all originate from Mount Željin. The highest percentage of seedlings (20.6%) with a low content of this pigment are from Mount Vizitor, with a medium content (35.1%) from Mount Hajla.

The highest percentage of seedlings (37.5%) which do not contain *cyanidin-3-glucosylrutinoside* originate from Mount Đeravica, with a small amount from Vizitor (25.5%), and with a very high content from Mount Hajla (10.5%). A high content of *cyanidin-3-glucoside* was found in the greatest number of seedlings (54.2%) from Mount Vizitor, *cyanidin-3-rutinoside* in those from Mount Hajla (37.4%), *cyanidin-3-sophoroside* in the greatest number of seedlings (54.2%) from Mount Đeravica and *cyanidin-3-glucosylrutinoside* in the greatest number of seedlings (50.0%) from Mount Željin.

In cornel fruit there are also differences in the quality and quantity of anthocyanins depending on the properties of the genotypes. Jovančević et al. (2009), in 8 genotypes from Berane and 9 genotypes from Bijelo Polje, isolated the following anthocyanins: *delphinidin-3-glucoside* (Rf=18), *cyanidin-arabinoside* (Rf=27), *peonidin-3-arabinoside* (Rf=36), *peonidin-3-rhamnoside* (Rf=47) and an unidentified anthocyanin (Rf=7.5). Depending on the genotype of the cornel fruit, the anthocyanins *delphinidin-3-glucoside* and *cyanidin-3-arabinoside* are quantitatively the most presented ones, therefore they determine the shade of red in the fruit in the natural population.

The amount of total anthocyanins from 11 different localities in the mountainous region of Montenegro varied between samples from 0.27 to 0.46 % (Jovančević et al., 2011). The quantification of five characteristic anthocyanin aglycones (*cyanidin*, *delphinidin*, *peonidin*, *malvidin* and *petunidin*) was done after the acid hydrolysis and HPLC chromatogram. The amounts of anthocyanin aglycones varied between samples: *delphinidin* from 0.33 to 0.75 mg/g fw, *cyanidin* from 0.25 to 0.75 mg/g fw, *peonidin* from 0.05 to 0.18 mg/g fw, *malvidin* from 0.11 to 0.52 mg/g fw and *petunidin* from 0.08 to 0.21 mg/g fw.

CONCLUSION

The subject of this study was anthocyanin pigments in different fruit. Analysis was done using the one-dimensional ascending chromatography method on apples, plums, cherries, sour cherries, raspberries, strawberries, blackberries, myrobalans and cornelian cherries.

The contrasts in genotype stipulated qualitative and quantitative variations in the content of the anthocyanins between different species, and between the same species and genotypes.

Altogether, seventeen anthocyanins were identified, whereof eleven were from cultured fruit and eight from indigenous fruit.

Different fruit species contained the same anthocyanins: *cyanidin-3-abinoside* was identified in apples and cornelian cherries; in plums, myrobalans and raspberries *cyanidin-3-glycoside* was identified; and *peonidin-3-rutinoside* was identified in plums and myrobalans.

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ANTOCIJANI U PLODOVIMA SAMONIKLIH I GAJENIH BILJAKA U POLIMLJU

IZVOD

Analizirani su antocijani, metodom jednodimenzionalne uzlazne tankoslojne hromatografije u plodovima jabuka, šljiva, višanja, malina, džanarike i drijena, a u plodovima borovnice po Evropskoj farmakopeji 6.0. i HPLC. Ćelijski sadržaj i raspored antocijanau prva tri sloja pokožice ploda ispitan je primjenom histološke metode.

U voćnim vrstama identifikovani su različiti antocijani, mada su iste antocijane sadržavale i različite voćne vrste. I unutar jedne voćne vrste bio je varijabilan je broj indetifikovanih antocijana.

Ukupno je identifikovano 17 antocijana, od čega 11 u gajenom a 13 u samoniklom voću. U jabuka su identifikovana tri, šljiva četiri, višnja četiri, drijena pet, džanarika četirii, malina četiri i borovnice pet antocijana.

Ključne riječi: jabuka, šljiva , višanja, malina, džanarika, drijena, borovnica, antocijanin.