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Plant Fruits Anthocyanins of the Belgorod Region

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ABSTRACT

In this paper the results of investigations of Belgorod flora Rosaceae plants fruits anthocyanins were summarized as well as the evaluation of anthocyanins content in the fruits currently available in the Belgorod market (RF) was presented. It has been established that all plant fruits anthocyanins are based mainly on cyanidin background while the difference appears as a consequence of position 3 glycosylation leading to mono-, di- and triglycosides; the pattern being characteristic within Rosaceae plant species. A wide group of *Rosaceae* family plant fruits is available to the Belgorod population and it is an important nutrition source of anthocyanins.

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INTRODUCTION

Anthocyanins belong to the most important water-soluble natural antioxidants. Antioxidant activity of these compounds provides them with the high biological activity that can be used by human organism for disease treatment or/and prevention [1, 2]. Plant-synthesized anthocyanins should penetrate the human organism with plant foods; therefore, a correctly mad up diet, including a necessary quantity of principally important nutrients, can contribute to a higher lifestyle quality. The role of anthocyanins as antioxidants is emphasized by the "French paradox" [3] or the use of bilberry anthocyanins in the solution of ophthalmological problems. According to contemporary data up to one thousand of various anthocyanins species have been found in nature [2] while the antioxidant activity of anthocyanins depends on their chemical structure [4]. It may be taken into account that the differences in anthocyanins specific composition can be used in plant selection as well as in plant chemosystematics, for establishing the authenticity and quality of plant products.

The aim of the present investigation is a generalization of results of determination of Rosaceae plant fruit anthocyanins tupes and evaluation of common content of anthocyanins in fruits currently available in the Belgorod (RF) market.

Methods:

Sample preparation:

Fresh plant fruits were bought in the market, obtained from private gardens and the Botanic Garden of the Belgorod State Research University. The anthocyanins were obtained by exhausting extraction from blender-ground fruits in successive portions of 0.1 M HCl water solution. The concentrate was separated from the residual by centrifugation and filtered through a paper filter. The filtrate was directly used for spectrophotometrical determination of the sum of anthocyanins. For the HPLC determination of specific composition, the residual was purified from attendant substances with the solid-phase extraction method (SPE). For this purpose, syringe cartridges DIAPACK C18 (BioChemMack ST, Moscow, RF) were activated by 5 ml of acetone, conditioned by 10 ml 0.1 M HCl water solution. Antocyanins were sorbed from the concentrate onto the cartridge sorbent, the cartridge was washed with 1ml of HCl, and anthocyanins were re-extracted by the eluent containing 30 vol. % CH_3CN and 30 vol.% HCOOH in water/ Before HPLC analysis; the re-extract was diluted with distilled water at ratio 1:2.

Determination of total anthocyanins content:

The differential spectrophotometrical method [5] was used to determine the total of anthocyanins accumulation level. The results were calculated in cyanide-3-glycoside chloride equivalent.

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HPLC determination of anthocyanin complexes specific composition. Mobile phases:

Reversed-phase HELC in eluents of the acetonitrile-formic acid-water system was used in the isocratic modein eluents from 6 to 10 vol. % acetonitrile and 10 vol. % of formic acid in water — depending on the stationary phase species and complexity of anthocianin complexes. Gradient modes of different profiles were used in eluents of the same system for more complicated compositions (extracts containing acylated anthocyanins or/and products of partial and complete anthocyanins hydrolisis).

Stationary phase:

Columns 4.6Ч250 mm Kromasil Eternity 100 C18 and Reprosil-Pur C18-AQ, 5 mcm were used in the present contribution. The columns were thermostatted at 40^{o}C.

Equipment:

Agilent Infinity 1200 with a diode-matrix and MS (ESI-mode) detectors were used.

Anthocyanins identification:

For anthocyanins complexes components identification electronic and mass-spectra were utilized as well as the method of chromatographic relative retention analysis [6 - 8].

RESULTS AND DISCUSSION

Genus Rubus:

To present any fruits anthocyanin complex we propose to analyze indices of the enzymes relative activity. According to our data, the main components of athocyanin fruits complexes of all the investigate red raspberry fruits may be: cyanin-3-sophoroside (Cy3Sopho), cyanidin-3-(2”-glucosyl-rutinoside) (Cy3^{2Glu}Rut), cyanidin-3-glycoside (Cy3Glu) and cyanidin-3-rutinoside (Cy3Rut). The following is obvious:

1) All anthocyanins are formed on cyanidin-3-glycoside.

Considering the known way of anthocyanins biosynthesis [9], it is possible to assert that:

2) The activity of flavanol-3’-hydroxilase, *F3’H*, that provides, finally, synthesis of cyanidin and peononidin («cyaniding» series anthocyanins) is high. In the presence of appreciable concentrations of pelargonidin derivatives, it could be possible to evaluate this enzyme relative activity according to equation:

$$A(F3'H) = \frac{\sum[Cy^*]}{\sum[Cy^*] + \sum[Pg^*]}, \quad (1)$$

where $\Sigma[Cy^*]$ – sum of all cyanidin derivatives concentrations,

$\Sigma[Pg^*]$ – sum of all pelargonidin derivatives concentrations.

However, in case of red raspberry fruits, this parameter is very high (more than 95%) and, thus, it is out of interest.

3) Because of the absence of aglycon (cyanidin), the activity of glucosyl-3-transferase (*Glu3T*) is very high (100%).

4) and 5) The rest kinds of anthocyanins are determined by activity of two enzymes – ramnosyl-6”-transferase (*R6”T*) that provides *Cy3Rut* synthesis which, affected by glucosyl-2”-transferase (*G2”T*), can be transformed into Cy3^{2Glu}Rut. The latter can be produced in a different way: first, Cy3Glu is transformed into Cy3Sopho affected by *G2”T* and, then, affected by *R6”T* -into Cy3^{2Glu}Rut. Hence, the evaluation of the indicated enzymes relative activity may be calculated as:

$$A(R6''T) = \frac{[Cy3Rut] + [Cy3^{2G}Rut]}{\sum[Cy^*]}, \quad (2)$$

$$A(G2''T) = \frac{[Cy3Sopho] + [Cy3^{2G}Rut]}{\sum[Cy^*]}. \quad (3)$$

where square-bracketed are concentrations of the corresponding anthocyanins determined on peaks areas of these compounds on the chromatogram.

Finely, the activity of one more enzyme (transferring xylosyl moiety instead of glucosyl one, xylosyl-2”-transferase, *X2”T*) was found in the case of *Rubus occidentalis*; it may be estimated according to equation:

$$A(G2''T) = \frac{[Cy3XylGlu] + [Cy3^{2X}Rut]}{\sum[Cy^*]}. \quad (4)$$

The results of anthocyanins complexes investigation of fruits of *Rubus* species together with other Rosaceae are summarized in Table 1.

Genus Cerasus:

Just as in case of raspberry, a large diversity of this plant cultivars is being grown in the local parts. Anthocyanin complexes of different cherry fruits species and cultivars are determined by the activity of the same enzymes as those of red raspberry, synthesized anthocyanin species being also considerably different in

the ratio; but, in this case, *R6"T* relative activity is moderately high at a possible decrease to zero of *G2"T* [10] activity. In case of cherry, species with a high anthocyanins accumulation level (e.g. fruits of *Cerasus mahaleb* (L.) Mill [10]) are also known, though the content of anthocyanins in cherry is usually comparable to that of raspberry (see table).

In the case of *Cerasus avium* (L.) Moench $Cy3^{2Glu}Rut$ is known to be found only in a trace amounts – thus *G2"T* activity is practically completely suppressed. Hence, a set of the fruits anthocyanins is presented only by the pair of Cy3Glu and Cy3Rut.

Table 1: Characteristics of fruits anthocyanins complexes of some plants of the *Rosaceae* family.

№	Plant species	Total anthocyanins content, g/100 g*	Relative enzyme activity		
			R6"T	*G2"T*	*X2"T*
1	*Rubus idaeus*	0.120 ± 0.012	4.4	51.8	3.7
2	*Rubus occidentalis*	0.740 ± 0.056	80.5	0	30.5
3	*Rubus fruticosus*	0.085 ± 0.012	0	0	0
4	*Cerasus vulgaris*	0.130 ± 0.015	93.7	73.2	0
			94.6	4.7	0
5	*Cerasus avium*	0.070 ± 0.015	96.6	0	0
6	*Prunus domestica*	< 0.010	66.2	0	0
7	*Prunus spinosa*	< 0.010	65.0	0	0
8	*Prunus persica*	< 0.010	3.5	0	0

* - as cyanidin-3-glycoside chloride.

Genus Prunus:

Plants of genus *Prunus* accumulate antjcyanins only in thin fruits skin; all anthocyanin complexes have the same specific composition as sweet cherry fruits and some sour cherries, relative *R6"T* activity being variable within a wide range [11] for various *P. domestica* L. cultivars. One more distinctive feature is that the activity of a new enzyme, methyltransferase (*MT*) transforming OH-groups in position 3' of ring B into CH_3O-groups, can manifest itself in case of a number of plum cultivars: i.e. peonidin derivatives may add to those of cyanidin in case of plums. Note that, according to the commonly accepted systematization, apricots, *P. armeniaca* L. and *P. cerasifūra* Ehrh. belonging to Prunus genus, are also popular in the Central Chernozem Area. And, indeed, these plant species anthocyanins are like those of plums, but a new anthocyanin - cyanidin-3-galactoside (Cy3Gala) - is found in cherry plum: thus at the early stages of anthocyanins synthesis galactosyl-3'-transferase (*Gala3T*) may be expressed instead that of *Glu3T*.

Tribe Maloideae:

The characteristic feature of fruits anthocyanin complexes is a simple cyanidin position-3 monoglycosylation by galactose (*Gala3T*), glucose (*Glu3T*), arabinose (*Ara3T*) and xylose (*Xyl3T*) with qualitatively the same types of anthocyanins though with different quantitative ratios, table 2. The relative activity of Gala3T is the highest in all cases. The most reach anthocyanins sources (accumulating more than 0.8 g per 100 g of fresh fruits) are fruits of *Aronia michurinii* A. Skvorts. et Yu. Maitullina, selected by Michurin, with is commonly wrongly named as *Aronia melanocarpa* (Michx.) Elliott. It should be mentioned that in some black-colored *Crataegus* sp. and *Amelanchier* sp. fruits the level of anthocyanins accumulation may exceed that for blackcurrants fruits.

Table 2: Relative enzyme activity in fruits of some *Maloideae.*

№	Plant species	Enzymes relative activity, %			
		Gala3T	*Glu3T*	*Ara3T*	*Xyl3T*
1	*Aronia michurinii*	59.9	2.2	33.2	2.7
2	*Crataegus chlorosarca*	28.4	52.4	8.1	<0.1
3	*Crataegus pentagyna*	61.2	31.5	2.1	1.4
4	*Sorbocotoneaster*	93.7	1.1	3.2	<0.1
5	*Amelanchier* sp.	69.8	12.5	8.7	4.3
6	*Malus domestica*	83.0	0.7	2.7	3.0

Genus Fragaria:

It is a favorite early fruit in Belgorod region though it is not a rich source of anthocyanins (less than 0.100 g per 100 g). A great variety of cultivars are grown up everywhere but anthocyanin complex of the fruits of *Fragaria* Ч *ananassa* (Weston) Duchesne ex Rozier proves to be quite simple and constant (62-67% of pelargonidine-3-glucoside, 6-7 % of Cy3Glu and acylated glycosides). The main anthocyanin is pelargonidin-3-glucoside (Pg3Glu – 62-67 %) with a small quantities of Cy3-Glu (6-7 %). So in strawberry fruits activity of *F3'H* is highly suppressed; though it is more pronounced in fruits of the varieties, selected with a participation of *Fragaria moschata* Weston (71.6 % of Cy3Glu, 16.4 % of Pg3Glu and 9.5 % of peonidin-3-glucoside). Moreover, the level of anthocyanins acylation by acetic acid (up to 25 %) is out of features for the other Rosaceae plants.

Conclusion:

The Rosaceae fruits are a good source of anthocyanin for human nutrition in Belgorod region. Their anthocyanin complexes are rather specific for each genus of the plant family; the complexes may be described by enzymes relative activities. Therefore, it may be used in chemosystematics and for estimation of the quality and authenticity of foodstuffs as well as for adulteration detection.

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