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CHARACTERISTICS OF THE PIGMENTS FROM ANTHOCYAN-CONTAINING FOOD PLANTS, RAW MATERIAL FOR PRODUCTION OF BIOFLAVONOID DYES

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*Data are presented on the composition of anthocyans of the fruits and pulp of the food plants bilberry (*Vaccinium myrtillis *L.), high-bush cranberry (*Viburnum opulus *L.), elderberry (*Sambucus nigra *L.), cherry (*Cerasus vulgaris *Mill.), crowberry (*Empetrum nigrum *L.), cranberry (*Oxycoccus palustris *Pers.), currant (*Ribes nigrum *L.), and mulberry (*Morus nigra *L.) growing in the Ukraine. The anthocyan pigments differ qualitatively and quantitatively owing to genetic peculiarities in the different species of fruits and the localization of the pigments in them.*

Key words: anthocyan pigments, *Vaccinium myrtillis*, *Viburnum opulus*, *Sambucus nigra*, *Cerasus vulgaris*, *Empetrum nigrum*, *Oxycoccus palustris*, *Ribes nigrum*, *Morus nigra*.

The quality of various types of food products can be significantly improved by coloring them in order to give them a color close to the natural one. The list of approved synthetic dyes decreases every year owing to their foreign nature and toxic action on the human organism [1]. The food industry needs natural organic dyes that are stable to various industrial processes; act as vitamins, antioxidants, and antimutagens; and prolong the shelf-life of food products. Such dyes, in particular, anthocyans, are heterocyclic compounds based on oxonium. Anthocyans are biologically and physiologically active P-vitamins. Therefore, the qualitative and quantitative characteristics of the pigments in the pulp of various types of food plants and their processed products in preserving and fruit-processing plants are important.

Table 1 presents data for the pigment content in food plants of the Ukraine.

Crowberry has the maximal and cranberry the minimal amount of natural anthocyan pigments. Bilberry and black elderberry contain significant quantities of anthocyans. The remaining studied plants have much lower contents. It is noteworthy that anthocyans are located primarily in the skin of most food plants with the exception of mulberry. The distribution of pigments in cranberry was very even.

Leucoanthocyans, which have the general structure C6–C3–C6 and convert to anthocyans upon dehydrogenation, accumulate in small quantities compared with anthocyans. The minimal concentration of leucoanthocyans is found in high-bush cranberry and cranberry. Their fraction compared with that of anthocyans is from 6.8 to 48.9%. The fraction of leucoanthocyans in bilberry, elderberry, and crowberry, which are rich in pigments, is less (6.8-15.0%) compared with the other studied plants. The maximal amount of these compounds is found in cherry, 21.2-48.9%. However, leucoanthocyans are one of the most important groups of polyphenolic compounds that make up the organoleptic and biological value of fruits during their processing [2, 3].

Table 2 presents results from the study of the anthocyan components in fruits and pulp of the aforementioned specimens.

An analysis of the results suggests the following conclusions. The concentration of polyphenolic compounds and their composition are due to the species-specific features of the plants. The qualitative composition of the anthocyans depends also on the variety and habitat. In particular, two compounds (cyanidine-monoglucoside and cyanidine-gentiobioside) were identified in elderberry from central Russia and the Krasnodar region [2] whereas four pure compounds were obtained from plants growing in the Ukraine. A wider spectrum of anthocyan pigments was observed in bilberry, 5 and 7, and cranberry, 4 and 5, growing in Russia and the Ukraine, respectively.

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TABLE 1. Concentration of Anthocyan Pigments in Fruits and Pulp

Specimen	Polyphenol fraction, 10^{-3}%.			
	anthocyans		leucoanthocyans	
	fruit	pulp	fruit	pulp
Bilberry	1580-2070	1690-2605	107-215	135-296
Black elderberry	1450-1600	1770-2790	125-240	180-265
Black currant	490-955	510-1105	67-230	79-254
Crowberry	1720-2170	1840-3180	185-320	196-397
High-bush cranberry	186-645	227-693	29-97	47-120
Cherry	366-1475	492-1820	179-324	211-385
Mulberry	469-1327	411-1235	79-231	53-202
Cranberry	234-542	230-559	49-67	47-70

TABLE 2. Composition of Anthocyan Pigments of Food Plants and Pulp, % of Total

Anthocyan	Elderberry		Currant		Bilberry		Crowberry		High-bush cranberry		Cherry		Mulberry		Cranberry	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Cyanidine-3-glucoside	40.6	41.8	24.2	25.1	7.3	6.8	10.1	8.6	44.5	48.1	24.1	25.0	56.2	55.1	-	-
Delphinidine-3-glucoside	-	-	15.2	14.8	43.1	41.4	-	-	-	-	-	-	28.3	29.2	7.9	11.4
Cyanidine-3-rutinoside	-	-	8.4	9.2	-	-	-	-	-	-	6.4	5.1	-	-	-	-
Delphinidine-3-rutinoside	-	-	11.6	12.1	-	-	-	-	-	-	-	-	-	-	-	-
Cyanidine-3-diglucoside	-	-	10.1	9.0	-	-	-	-	-	-	12.2	13.1	-	-	-	-
Delphinidine-3-diglucoside	-	-	30.5	29.8	-	-	-	-	-	-	-	-	-	-	-	-
Delphinidine-5-glucoside	-	-	-	-	19.7	21.2	-	-	-	-	-	-	-	-	-	-
Delphinidine-3-rhamnosylglucoside	-	-	-	-	16.1	15.7	-	-	-	-	-	-	-	-	-	-
Cyanidine-3-arabinoside	-	-	-	-	2.5	3.8	8.5	11.3	-	-	7.5	6.8	-	-	27.2	29.6
Petunidine-3-glucoside	-	-	-	-	3.8	5.6	0	2.4	-	-	-	-	-	-	-	-
Malvidine-3-glucoside	-	-	-	-	7.5	4.3	-	-	5.8	1.7	-	-	-	-	-	-
Peonidine-3-glucoside	-	-	-	-	-	1.2	-	-	-	-	30.0	32.6	-	-	31.1	32.8
Delphinidine-3-arabinoside	-	-	-	-	-	-	9.6	12.8	-	-	-	-	-	-	-	-
Peonidine-3-arabinoside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23.4	20.9
Petunidine-3-arabinoside	-	-	-	-	1.2	1.8	-	-	-	-	-	-	-	-	-	-
Malvidine-3-arabinoside	-	-	-	-	1.6	1.3	-	-	-	-	-	-	-	-	-	-
Delphinidine-3-galactoside	-	-	-	-	-	-	50.3	46.5	-	-	-	-	-	-	-	-
Cyanidine-3-galactoside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.9	7.0
Malvidine-3-galactoside	-	-	-	-	-	-	20.1	15.9	-	-	-	-	-	-	-	-
Cyanidine-3-xylosylarabinoside	-	-	-	-	-	-	2.4	2.0	-	-	-	-	-	-	-	-
Cyanidine-3-sambubioside	46.4	44.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyanidine-3-sambubioside-5-glucoside	10.1	10.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyanidine-3,5-diglucoside	2.9	3.4	-	-	-	-	-	-	-	-	-	-	10.1	9.8	-	-
Cyanidine-3-arabinosylglucoside	-	-	-	-	-	-	-	-	49.7	50.2	-	-	-	-	-	-
Cyanidine-3-sophoroside	-	-	-	-	-	-	-	-	-	-	8.7	8.3	-	-	-	-
Cyanidine-3-rhamnoglucoside	-	-	-	-	-	-	-	-	-	-	11.2	9.1	-	-	-	-
Malvidine-3-diglucoside	-	-	-	-	-	-	1.4	2.5	-	-	-	-	-	-	-	-
Pelargonidine-3-monoglucoside	-	-	-	-	-	-	-	-	-	-	-	-	5.4	5.9	-	-

Fruit (1) and pulp (2).

Differences in the coloration and processing properties of the fruits explain the data for the qualitative and quantitative characteristics of the anthocyans.

Table 1 shows that the pigment concentration in the pulp is higher for the studied plant species. This indicates that part of the pigments apparently is absorbed on subcellular structures or is located in the epidermal layer and remains in the solids, the pulp, of the food plants after pressing out the juice regardless of the original location in the vacuoles and their solubility in the cellular fluid.

The qualitative compositions of dyes from the pulp and fruit are identical (Table 2), with the exception of petunidine-3-glucoside in crowberry. This is probably due to the low concentration in the berries. The quantitative characteristics of the individual anthocyan pigments in percent of their total in the juice and pulp varies little. Considering the differences in the total concentration of anthocyans in juice and pulp, the high content of the studied compounds in the pulp is noteworthy. This explains the advantage of using them in the processing of food plants containing anthocyans as a valuable raw material for producing bioflavonoid dyes.

EXPERIMENTAL

Fruits of the food plants bilberry (*Vaccinium myrtillis* L.), high-bush cranberry (*Viburnum opulus* L.), elderberry (*Sambucus nigra* L.), cherry (*Cerasus vulgaris* Mill.), crowberry (*Empetrum nigrum* L.), cranberry (*Oxycoccus palustris* Pers.), currant (*Ribes nigrum* L.), and mulberry (*Morus nigra* L.) were collected when ripe in Poltava and Odessa districts of the Ukraine in 1996-1998. They were studied immediately after collection or stored as necessary for more than 8 h in a refrigerator at 0-4°C and relative humidity 90-95%. The berries were preliminarily washed, inspected, pressed on a laboratory press to obtain the pulp, and stored for less than 0.5 h. The total amounts of anthocyans and leucoanthocyans were determined by the literature method [7].

The concentrations of pure pigments were determined by chromatographic separation and subsequent quantitative determination by a colorimetric method [2, 7]. Anthocyans were separated by two-dimensional preparative paper chromatography on Whatman No. 2, grade M, using CH_3CO_2H—HCl (conc.)—H_2O (15:3:82, first direction) and *n*-pentanol—CH_3CO_2H—H_2O (2:1:1, second direction). The phenolic compounds were also prepared on L50 (Merck) silica-gel plates [8, 9].

The studied solution was spotted (0.05 cm^3) on each chromatographic band using a micropipette. The spots were visualized using sodium carbonate. Anthocyans give a color from red to violet.

The parts of the paper chromatograms with the anthocyans were cut out; of the thin-layer chromatograms, scraped off. The compounds were eluted with aqueous alcohol (1:1) acidified with HCl (1%). The absorption was determined at the pigment absorption maximum of 460-500 nm. The standard was an acidified solution of ethanol (1:1). The mass concentration was found from a calibration curve constructed using cyanidine [2, 8].

Anthocyans were identified by using literature data for the R_f values and comparing the mobilities of the studied compounds with standards and by using spectral and chemical methods. UV and visible spectroscopy of anthocyan pigments were recorded on a Specord UV-Vis instrument using a methanol solution of conc. HCl (0.01%) as a standard.

Structural Studies. Preparative isolation of phenolic compounds from the studied material by paper (PC) and thin-layer chromatography (TLC) yielded 30-50 mg for each parameter studied. Anthocyans that were isolated by PC and TLC for structural studies were hydrolyzed with acid, degraded with base, and cleaved by oxidation with hydrogen peroxide according to the literature [8, 10]. Acid hydrolysis was used to determine the type and molar ratio of the aglycone and monosaccharides. Selective hydrolysis was carried out with 30% H_2O_2. This enabled the glycoside to be separated from the third C atom of the acylated anthocyans [2, 10]. Basic degradation was performed with 10% KOH. Aglycones of the studied anthocyans were identified in the decomposition products using PC.

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