UDC 543.54:547.973

ANTIOXIDANTS OF BELGOROD STATE UNIVERSITY BOTANICAL GARDEN PLANTS: RIBES AUREUM FRUITS ANTHOCYANINS

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Ribes aureum Pursh. (syn. R. odoratum H. Wendl.) of Belgorod National Research University of Botanical Garden collection produces edible berries about 1 cm in diameter differing (for different varieties) in color from greenish-yellow to shiny black. The four main anthocyanins are the same as for black currant fruits: delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside; the peaks assignment has been confirmed by spectrophotometrical and mass spectrometrical detections. The overall anthocyanin accumulation depends upon fruit color intensity, differing in the broad range from 0.065 to 0.980 g cyaniding-3-glucoside chloride equivalent per 100 g of fresh fruits. Mode of anthocyanins biosynthesis in fruits of some species of Ribes genus is discussed on the basis of relative activity of corresponding enzymes.

Keywords: Ribes aureum, anthocyanins, HPLC, relative activity, enzymes

Antioxidants of natural origin are commonly recognized as the substances being necessary for the men health care [1]. Anthocyanins are known as one of the most effective water-soluble antioxidants [2]; so they have an advantage for being natural colorants with health-promoting properties [3]. That is why fruits enriched with anthocyanins are reported to reveal diverse biological activities. Among them black currant (Ribes nigrum L.) fruits having the dark color due to skin enrichment with anthocyanins were found to improve vision dark adaptation [4] and even to afford chemoprevention of carcinogenesis in rats [5]. Thus black currant fruit anthocyanins are included in «Антоциан Форте» – Russian biologically active supplement.

Genus Ribes includes about 150 species; on appearance the species may be roughly divided into two groups according to a degree of anthocyanin accumulation – with red and nearly black fruits. Anthocyanin complex of black currant (R. nigrum L.) fruits being the most popular Ribes subject for investigations worldwide was found to be composed of the four main species – delphinidin-3-glucoside (D3G), delphinidin-3-rutinoside (D3R), cyanidin-3-glucoside (C3G) and cyanidin-3-rutinoside (C3R), though sometimes petunidin derivatives are also present in not negligible concentrations as well as some another minor components may be detected [6-10]. Our investigations as well as literature data reveal the fruit anthocyanin complexes to be rather constant and independent upon black currant variety, thus the fruits may be used to extract four natural anthocyanins as standards for anthocyanins HPLC determination [11]. On the other hand anthocyanin complexes of red colored fruit currants differ from the black currants significantly though the literature data for the former as well as for another Ribes species are rather restricted [12] The aim of the present investigation is to determine anthocyanin composition of fruits of Ribes aureum Purch. of National Research Belgorod State University Botanical Garden collection.

Materials and methods of investigation. Fruits of Ribes aureum plants were harvested in National Research Belgorod State University Botanical Garden (BG) in the seasons of 2008 and 2012. Anthocyanins were extracted from fresh crushed fruits by 0.1 M water HCl solutions for 3-6 hours under periodical stirring. Extracts were filtered through paper filters and purified by solid-phase extraction (SPE) on «ДИАПАК C18» (BioChemMack ST, Moscow) syringe cartridges. For the extraction the cartridge was activated by passing of 2-5 ml of acetone, than it was conditioned by passing of 15-20 ml of 0.1 M water HCl solution. The extract was applied at the volumes before appearance of colored filtrate. The anthocyanins were re-extracted from cartridge by 2 ml of mixture of formic acid – acetonitrile – water (30 : 30 : 40 vol.%) and diluted by two volumes of water before storage in refrigerator (4°C).

Anthocyanins were separated by reversed-phase LC using a Agilent 1260 Infinity liquid chromatograph (Agilent Technologies Inc., Wilmington, DE), equipped with a photodiode array detector and mass-spectrometric (ESI mode) detector. 250×4.6 мм Reprosil-Pur C18-AQ (5 mcm) column was explored in the isocratic elution mode in a mobile phase of 7 vol.% of CH_3CN and 10 vol.% of HCOOH in water, 1 ml/min. Column temperature was maintained at 40°C. Fresh purified extract of black currant fruits were utilized as reference anthocyanin mixture.

The level of anthocyanin accumulation in fruits was determined by the conventional pH-differential method [13] before SPE.

Results and discussions. Ribes aureum Pursh. (syn. *R*. odoratum H. Wendl.) produces edible berries about 1 cm in diameter differing (for different varieties) in color: there were plants with fruit colors from greenish-yellow to shiny black in Ribes collection of BG. HPLC analysis of shiny black fruit (as well as for fruits with red color) extract has shown that the same four anthocyanins being common for blackcurrants fruits are predominant for the samples under investigation, Fig.1.

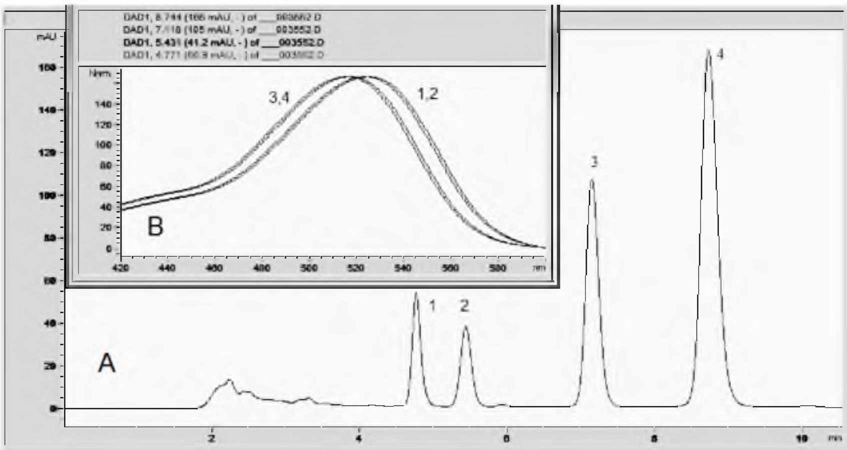

Fig.1. HPLC chromatograms of shiny black *Ribes* aureum fruit anthocyanins with peaks electronic spectra
A – chromatogram; B – electronic spectra of anthocyanins. 1 – D3G, 2 – D3R, 3 – C3G, 4 – C3R.

The retention times of the four anthocyanin components were equal to that for black currant fruits extracts. The peaks have characteristic electronic spectra. The spectra of peaks 3 and 4 were typical for cyanidin derivatives (λ_{max} = 515 nm, Fig.2) [14]. The local maxima in region of 430 nm indicated the absence of carbohydrate radicals in 5-position of the anthocyanidin backbone. The bathochromic shifts (for ~ 7 nm) of absorption maxima for addition of OH-group to cyanidin to form delphinidin derivatives as well as for addition of rhamnose moiety to 6”-position of glucoside radical to form rutinosides (for ~ 1.5 nm) let it to propose anthocyanins compositions: delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside. The peaks assignment has been confirmed by mass spectrometrical detection [15]. The quantitative difference between composition of anthocyanin complexes of the two Ribes species was obvious indicating that the activity of flavanon-3’5’-hydroxylase (F3’,5’H) in the case of R. aureum is rather suppressed, causing a decrease of delphinidin derivatives accumulation, Tab.1. It makes cyanidin derivatives predominant anthocyanins instead of delphinidin derivatives being somewhat more abundant in R. nigrum fruits. The decrease of delphinidin derivatives concentration is also characteristic for R. grossularia with dark colored fruits. By the way according to our investigations the same anthocyanins pattern have fruit extracts of R. americanum and R. lacustre, Tab.1.

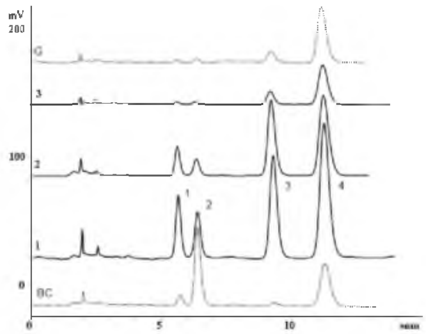

Fig.2. HPLC chromatograms of some Grossulariaceae species fruit anthocyanins
Species: BC – R. nigrum, GCh – R. grossularia, 1, 2, 3 – R. aureum varieties. Anthocyanins: – D3G, 2 – D3R, 3 – C3G, 4 – C3R.

Moreover, the difference is also obvious for the relative activities of anthocyanin rhamnose transferase (ART), being responsible for anthocyanidin-3-glucoside transformation towards anthocyanidine-3-rurinoside. The relative enzyme activities were calculated by equations 1 and 2 using a relative concentrations (calculated by peak areas on the chromatograms) of the four anthocyanins species.

$$\alpha(F3'4'H) = \frac{[D3G]+[D3R]}{[D3G]+[D3R]+[C3G]+[C3R]} \cdot 100 \%, \quad (1)$$

$$\alpha(ART) = \frac{[D3R]+[C3R]}{[D3G]+[D3R]+[C3G]+[C3R]} \cdot 100 \%. \quad (2)$$

Table 1

Anthocyanins of some Grossulariaceae species fruits

Varieties	Anthocyanins, peak area %				Enzyme relative activity, %	
	D3G	D3R	C3G	C3R	a(F3'5'H)	a(ART)
R. aureum						
1	12.6 ± 0.5	10.7 ± 0.5	28.9 ± 0.9	47.8 ± 0.9	23.3 ± 0.5	58.5 ± 0.7
2	9.6 ± 0.4	6.3 ± 0.4	35.6 ± 1.0	48.5 ± 0.7	15.9 ± 0.4	54.8 ± 0.9
3	3.9 ± 0.2	3.4 ± 0.1	17.3 ± 0.6	68.8 ± 0.6	7.3 ± 0.3	77.2 ± 0.5
R. nigrum						
«Orlovia»	5.4 ± 0.2	47.7 ± 0.6	2.2 ± 0.2	41.3 ± 0.7	53.1 ± 0.5	92.2 ± 0.9
«Lent'iai»	5.2 ± 0.3	48,8	1.9 ± 0.2	39.8 ± 0.7	53.9 ± 0.6	92.6 ± 0.5
R. americanum						
1	11.2 ± 0.4	13.1 ± 0.5	24.2 ± 0.6	57.9 ± 0.8	24.3 ± 0.4	66.7 ± 0.7
R. lacustre						
1	13.5 ± 0.4	19.2 ± 0.6	18.7 ± 0.5	55.0 ± 0.8	32.7 ± 0.5	69.8 ± 0.6
R. grossularia						
«Chernomor»	1.6 ± 0.2	3.3 ± 0.3	12.7 ± 0.4	73.1 ± 0.7	4.9 ± 0.2	84.2 ± 0.8
«Chernyi Negus»	1.6 ± 0.2	4.4 ± 0.2	13.1 ± 0.4	81.5 ± 0.8	6.0 ± 0.3	85.4 ± 0.8

According to relatively low ART activity anthocyanin biosynthesis in R. aureum fruits of some plant samples resembles that for R. americanum and R. lacustre, though the relative activity of the enzyme appears to be greater for the others with more dark colored fruits.

The quantitative parameters of anthocyanins accumulation in fruits of R. aureum are also diverse – the difference is very pronounced: from 0.065 to 0.980 g cyaniding-3-glucoside chloride (C3G) equivalent per 100 g of fresh fruits, Tab.2. The results for the fruits of 2012 year harvesting can be overestimated because of fruits storage in refrigerator before analysis.

Table 2

The overall anthocyanins accumulation in fruits of Ribes aureum

Harvest year	Anthocyanin content in fruits with colors of increased intensity, expressed as cyaniding-3-glucoside chloride equivalent, g per 100 g				
	1	2	3	4	Mean
2008	0,079±0,015	0,100±0,020	0,244±0,044	0,481±0,063	0,226
2012	0,081±0,008	0,132±0,077	0,410±0,075	0,872±0,051	0,373

It is obvious that the varieties of R. aureum may be chosen with fruit anthocyanin accumulation exceeding that for ordinary black currant varieties (0.250 g per 100 g of C3G-equivalent fresh fruits for ordinary varieties [15]). It makes R. aureum a very interesting plant with fruits high content of biologically active anthocyanins.

Conclusions. Ribes aureum is a promising plant for production of fruits enriched with anthocyanins.

Acknowledgment. Исследование выполнено при финансовой поддержке Министерства образования и науки РФ «Государственное задание вузу на 2013 г, проект № 3.1785.2011».

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АНТИОКСИДАНТЫ БОТАНИЧЕСКОГО САДА НАЦИОНАЛЬНОГО ИССЛЕДОВАТЕЛЬСКОГО БЕЛГОРОДСКОГО ГОСУДАРСТВЕННОГО УНИВЕРСИТЕТА: АНТОЦИАНЫ ПЛОДОВ RIBES AUREUM

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Ribes aureum Pursh. (син. R. odoratum *H. Wendl.*) Ботанического сада Национального исследовательского Белгородского государственного университета дает съедобные плоды диаметром около 1 см, различающиеся (для различных сортов) по цвету от зеленовато-желтого до блестяще черного. Четыре основные антоциана являются теми же, что и в плодах черной смородины: дельфинидин-3-глюкозид, дельфинидин-3-рутинрозид, цианидин-3-глюкозид и цианидин-3-рутинозид; отнесение пиков подтверждено анализом электронных и масс-спектров. Суммарное накопление антоцианов зависит от интенсивности окраски в широком диапазоне: от 0.065 до 0.980 г на 100 г свежих плодов (в пересчете на цианидин-3-глюкозид хлорид). Характер биосинтеза антоцианов в плодах некоторых видов рода Ribes обсуждается с позиций относительной активности соответствующих ферментов.

Ключевые слова: Ribes aureum, антоцианы, ВЭЖХ, относительная активность, ферменты