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COMPOSITION AND QUANTIFICATION OF ANTHOCYANINS IN HEALTHY-DIET POTATO (*Solanum tuberosum* L.) VARIETIES FOR BREEDING AND GROWING IN THE RUSSIAN FAR EAST

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Abstract

Potato (*Solanum tuberosum* L.) has been the focus of dietary research in recent decades due to its ability to accumulate phenolic substances (anthocyanins) in tubers. In Russia, such varieties have been created for a number of regions, but in the Far East, the program to increase the content of anthocyanins in potato tubers has recently begun. In the presented study using liquid chromatography and second-order mass spectrometry methods, we profiled anthocyanins and quantified their accumulation for potato varieties of different origin (Russia, Ukraine, Belarus, Kazakhstan, Germany, the Netherlands), which had not previously been characterized by this feature. For the first time, four dietary Russian varieties with an increased content of anthocyanins have been identified. This work aimed to determine the profiles of anthocyanins and their content in tubers in the conditions of the Russian Far East. We used 22 varieties selected for early maturity, productivity, low starch content and different colors of tubers and flowers. The anthocyanin profile was double-identified, by the retention time on a C18 reverse phase column with detection of absorbance at wavelength range 400-700 nm and ESI-MS/MS in positive ionization mode. Anthocyanins were quantified spectrophotometrically. The anthocyanins identified in the tubers were delphinidin, petunidin, malvidin, cyanidin, and pelargonidin. Delphinidin and cyanidin were found in mono- and diglycosylated forms. It was revealed that petunidin-3-glucoside is the most common anthocyanin which is present in almost all of the studied varieties. Depending on varietal specificity, there were from 1 to 5 anthocyanins of those found. Varieties with yellow skin and pink spots on the yellow tuber skin were characterized by a low content of anthocyanins. The pink and dark pink color of the skin positively correlates with the content of pelargonidin-3-glucoside, while petunidin-3-glucoside and cyanidin-3-glucoside give purple and blue-violet color to the skin of the tuber. The anthocyanin level was the highest in Phioletovii variety (310 mg/kg cyanidin-3-glucoside, 50 mg/kg malvidin, 30 mg/kg delphinidin), Vasilyok variety (150 mg/kg petunidin), and in Mayak (95 mg/kg pelargonidin) and Kuznechanka (78 mg/kg pelargonidin) varieties. In the Phioletovi variety, the cyanidin-3-glucoside prevailed. We recommend to involve varieties with pink, dark pink, purple and blue-purple tuber skin in selection for an increased content of anthocyanins.

Keywords: *Solanum tuberosum* L., potatoes, dietary varieties, anthocyanins, delphinidin, petunidin, malvidin, cyanidin, pelargonidin, mass spectrometry, HPLC

Potato (*Solanum tuberosum* L., *Solanaceae*) is the fourth most important food crop after wheat, rice, and corn, and the first among tuber and root crops in terms of grown areas [1]. The nutritional value of potatoes is largely due to the high content of carbohydrates, a significant amount of ascorbic acid and anthocyanins, highly digestible proteins, as well as potassium, calcium, and magnesium salts [2].

Anthocyanin pigments are synthesized in the skin and flesh of potato tubers. In addition, flowers, leaves, stems, and eyes can be colored. In the potato as a food crop, the content of anthocyanins in the edible parts, i.e., in the flesh of tubers, should be increased [3].

Anthocyanin pigments are used in the food and pharmaceutical industries. These antioxidants are water-soluble natural dyes that color food in different shades of red. They are approved by Russian sanitary rules and regulations for coloring cheeses, wines, soft drinks, canned vegetables, breakfast cereals (up to 200 mg/kg), jams, jellies, and marmalades, and serve as an alternative to carcinogenic azo dye carmoisine [4, 5].

The anthocyanins which possess capillary-strengthening, anti-oxidant, antibacterial and anticarcinogenic properties are used for treating and preventing various diseases, for example, type II diabetes and some types of tumors [6-9].

Development and application of DNA markers for target genes involved in anthocyanin biosynthesis facilitate breeding for red and purple potato varieties. It is known that the chalcone synthase (CHS), chalconflavanone isomerase (CHI), dihydroflavonol-4-reductase (DFR), flavonone-3-hydroxylase (F3H), flavonoid-3′-hydroxylase (F3′H), flavonoid-3′,5′-hydroxylase (F3′5′H), and anthocyanidin synthase (ANS) genes are involved in biosynthesis of anthocyanins [10, 11]. *S. tuberosum* also has gene loci with regulatory properties. Locus *D* (developer, designated in the diploid potato *S. rybinii* Juz. & Bukasov as *I*, the inhibitor), located on chromosome 10, encodes the R2R3 MYB transcription factor (TF), which is highly similar to the product of the previously detected petunias (*Petunia hybrid*) gene PhAN2 [12]. In plants, the R2R3 MYB gene family is the largest group of TF genes which play an important role in the biosynthesis of anthocyanins [13-15].

Data on the genes controlling anthocyanin pigmentation in *S. tuberosum* tubers and flowers facilitate combination of crossing pairs to select hybrids with colored flowers, stems, leaves, and tubers [16-18].

Pigmented *S. tuberosum* cultivars are rich in anthocyanins, in particular, their acylated derivatives [19]. Potato genotypes differ greatly in the content of bioactive substances, including anthocyanins. Pigmented varieties have 1.5-2.5 times higher phenolic activity, 2-3 times higher antioxidant capacity and accumulate higher levels of anthocyanins than non-pigmented genotypes. Anthocyanins were not found in unpigmented potatoes [20].

In the USA, dietary potato breeding has been carried out since the early 2000s. As a result, red and purple potato varieties with a high level of carotenoids and anthocyanins have been produced [4]. In Russia, breeding potato varieties for dietary nutrition is also being in focus. The varieties that have the highest pigmentation index and a 5-fold content of antioxidants compared to white potatoes have been identified.

The program of potato breeding for high anthocyanin content has been performed in the Far East since 2018. Varieties of various origins from Russia, the Netherlands, Germany, Kazakhstan, Ukraine, and Belarus have been assessed to reveal donors of commercial and dietary traits (high yield, red and purple tuber skin, red-violet color of the corolla), which were further crossed [22]. To proceed, it is necessary to study the dietary antioxidant properties of the varieties, the composition and content of anthocyanins in tubers and the relationship between these traits and anthocyanin coloration of other organs, the skin and corolla.

Here, we applied high-performance liquid chromatography (HPLC) and second-order mass spectrometry to identify and quantify anthocyanins in 22 potato varieties of various origins that had not previously been characterized by this trait. Four varieties of Russian origin rich in anthocyanins have been revealed and recommended for dietary use.

This work aimed to profile and quantify anthocyanins in tubers of dietary potato varieties promising for growing and breeding in the Russian Far East.

Materials and methods. The study included varieties of various origins from the VIR World Collection (the Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg), a collection of the Lorkh All-Russian Research Institute of Potato Farming (Moscow Province), and those bred at the Chaika Federal Research Center of Agricultural Biotechnology of the Far East (FSC ABFE). A total of 22 varieties selected for early maturity (440-650 g tubers per plant on days 60 and 70 after planting) and the tuber color, the Bashkirsky, Vasilek, Dachny, Kazachok, Krepysh, Kuznechanka, Matushka, Mayak, Meteor, Ognivo, Olsky, Pamyati Kulakova, Sarma, Sirenevyi tuman, Fioletovii, Yantar (Russia), Sante (the Netherlands), Vitesse (Germany), Tamyr (Kazakhstan), Povin, Shchedrik (Ukraine), and Manifest (Belarus) were tested at a collection nursery (FSC ABFE, 2016-2018).

The composition and accumulation of anthocyanins were analyzed following a method described by Lewis et al. [20]. Anthocyanins was measured in the tuber flesh together with the skin. The potatoes were collected and stored until the analysis (no more than 2 weeks) in a cool place without direct sunlight. The tubers were rinsed in cold water, weighed, crushed, and placed in a 40% ethanol + 1% formic acid (5 g of the biomass + 25 ml of the resulting solution) mixture, frozen, thawed, disintegrated ultrasonically to destroy the walls and membranes of cells and organelles. Anthocyanins were extracted for 90 min at 40 °C in closed vessels to prevent the access of atmospheric oxygen. The extract was centrifuged (CM-6M, Elmi, Latvia) at 3500 g for 30 min, the supernatant was filtered through syringe filters (pore size 0.45 μm). The extract was stored in a freezer at –20 °C.

Anthocyanins in the extracts were separated and quantified using HPLC analysis (a 5 μl aliquot of each extract, a liquid chromatograph LC-20AD equipped with a high-pressure gradient pump and a CTO-20A column thermostat, Shimadzu, Japan; a Shodex C18-4E reverse phase column 250×4.6 mm I.D., 5 μm sorbent particles, Shodex, Japan; column temperature 50 °C; the rate of mobile phase A:B 0.58 ml/min). Eluent A was acetonitrile (AppliChem GmbH, Germany), eluent B was 1% formic acid solution (Sigma-Aldrich, USA). During gradient elution, B concentration decreased from 100% to 92% (0.00-5.00 min), from 92% to 80% (5.00-45.00 min), and from 80% to 10% (45.00-45.01 min). Peaks were detected at 300-600 nm UV/VIS (a spectrophotometric detector SPD-20A, Shimadzu, Japan). The analysis was performed in 66 biological and 3 analytical replicates. For cultivars Phioletovii and Vasilek, the absorption coefficient of malvidin-3-glucoside (3.02×10^4 at 300-600 nm, a molecular weight of 493.3 g/mol) was used to recalculate the content of anthocyanins, for other varieties, the molar extinction coefficient of pelargonidin-3-glucoside was applied (2.73×10^4 at 300-600 nm, molecular weight of 433.3 g/mol).

Anthocyanins purified by HPLC were identified by direct-injection second-order mass spectrometry method using an amaZon SL trap (Bruker, Germany) equipped with an electrospray ionization source. Detection was carried out in the modes of positive and negative ions, with a mass scanning range from 150 to 2200 u, maximum scanning speed 32000 Da/s, the nebulizing capillary voltage 4500 V, nebulizer pressure 29 psi, dry gas flow 10 l/s, and capillary temperature 180 °C. Ions were fragmented using a 1.5 eV electron beam. The analysis was arranged in 66 biological and 3 analytical replicates.

MS Excel 2007 and Statistica 8 (StatSoft, Inc., USA) were used for data processing, the mean (M) and $t_{0,05}\times$SEM were calculated.

Results. The tested varieties were low-starchy (8.0-12.0%) and had pink or purple tuber skin. The low-starchy varieties are considered dietary [23].

Anthocyanin coloration of different parts of plants is an important trait that can be effectively used in breeding for a higher level of phenolic pigments [5]. The studied varieties differed in morphological traits (Table 1).

1. Morphological traits of potato (*Solanum tuberosum* L.) varieties involved in the study (a collection nursery, Chaika Federal Research Center of Agricultural Biotechnology of the Far East, 2016-2017)

Varieties	Color		
	tuber flesh	corolla	tuber skin
Sante, Kazachok, Vitesse, Sarma, Meteor	Yellow	White	Yellow
Krepysh	White	Pale red-violet with white brush-stroke markings	
Dachnyi, Shchedrik	White	White	
Yantar	Bright yellow	White	
Tamyr	Light yellow	White	
Olskii	Cream	White	Yellow pink-spotted
Pamyati Kulalova	White	White	
Kuznechanka, Bashkirsky	White	Pale red-violet	Pink
Sirenevyi tuman	Light yellow	Pale red-violet with white brush-stroke markings	
Povin	Yellow	Pale red-violet	
Matushka	Cream	White	
Ognivo	Cream	Red-violet with white brush-stroke markings	
Mayak	Cream	Pale red-violet	Dark pink
Manifest	Cream	Red-violet with white brush-stroke markings	
Vasilek	Cream	Blue-violet with white brush-stroke markings	Violet
Fioletovii	Violet	Blue-violet with white brush-stroke markings	Blue-violet

Five varieties (Kazachok, Meteor, Sarma, Sante, and Vitesse) have white-colored corolla, but yellow skin and flesh. In other varieties, the pigmentation (yellow, shades of red-violet, violet, pink, etc.) of plant parts varied. The morphological features in our studies (the color of the generative organs and the skin of the tubers during the flowering period) correspond to the descriptions [24].

The HPLC-MS/MS detected five different anthocyanins in tubers of the examined potato varieties (Table 2) identified as delphinidin, petunidin, malvidin, cyanidin, and pelargonidin. The biochemical nature of anthocyanins in *S. tuberosum* is the same as in cultured diploid species [25] which anthocyanins include pelargonidin, peonidin, petunidin, and malvidin.

2. HPLC-MS/MS tuber anthocyanin profiles of potato (*Solanum tuberosum* L.) varieties involved in the study (a collection nursery, Chaika Federal Research Center of Agricultural Biotechnology of the Far East, 2018)

Anthocyanin	Molecular ion $[M+H]^+$	HPLC retention time, min	Variety
Delphinidin-3-glucoside	465.3; 303.2	25	Mayak, Povin, Kuznechanka, Manifest, Fioletovii
Delphinidin-3-rhamnosyl-5-glucoside	627.3; 465.3; 303.2	17.5	Mayak, Povin, Kuznechanka, Vasilek, Manifest
Malvidin-3-glucoside	493.3; 331.3	44	Fioletovii
Pelargonidin-3-glucoside	433.3; 271.1	37.5	Mayak, Povin, Kuznechanka, Fioletovii, Vasilek
Petunidin-3-glucoside	479.3; 317.2	35	Vasilek, Manifest, Matushka, Povin, Mayak, Sirenevyi tuman, Pamyati Kulalova, Olskii
Cyanidin-3-glucoside	449.2; 287.2	27.5	Fioletovii
Cyanidin-3-rhamnosyl-5-glucoside	611.3; 499.3; 287.2	19	Matushka, Vasilek, Fioletovii, Povin

The anthocyanins dolphinin and cyanidin were found in both monoglycosylated and diglycosylated forms. The most common anthocyanins were petunidin-3-glucoside and pelargonidin-3-glucoside. They were identified in almost all the samples (Table 3).

Research publications recognize petunidin and pelargonidin as the main anthocyanins in potatoes. Petunidin causes purple color, and pelargonidin causes red-orange color [20, 26]. Pelargonidin-3-glucoside prevailed in the varieties with pink and dark pink tubers, and petunidin-3-glucoside prevailed in violet and blue-violet varieties. In the yellow-skinned varieties anthocyanin compounds were either not detected or detected in an insignificant amount.

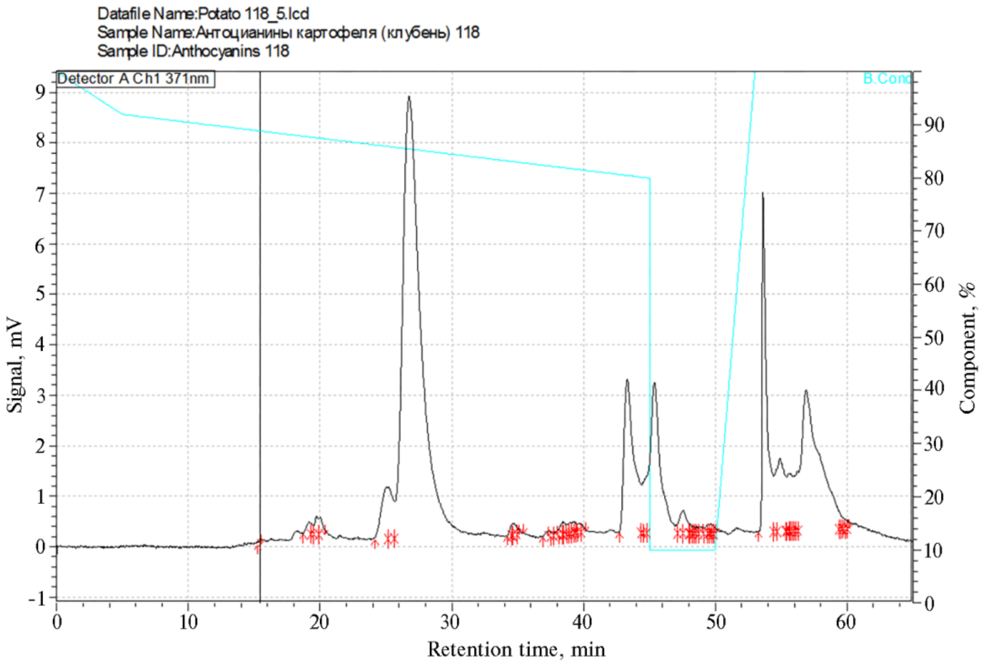
3. Concentration of anthocyanins (mg/kg) **in tubers of potato (*Solanum tuberosum* L.) varieties involved in the study** (n = 3, $M \pm t_{0,05} \times$SEM, a collection nursery, Chaika Federal Research Center of Agricultural Biotechnology of the Far East, 2018)

Variety	1	2	3	4	5	6	7
Yellow-skinned tubers							
Sante	< 0.5	< 0,5	1,0±0,1	< 0,5	< 0,5	< 0,5	< 0,5
Kazachok	< 0.5	< 0,5	< 0,5	< 0,5	2,1±0,1	< 0,5	< 0,5
Vitessa	< 0.5	< 0,5	< 0,5	0,9±0,1	< 0,5	< 0,5	< 0,5
Sarma	< 0.5	< 0,5	< 0,5	2,2±0,1	< 0,5	< 0,5	< 0,5
Meteor	< 0.5	< 0,5	1,1±0,1	1,8±0,1	< 0,5	< 0,5	< 0,5
Dachnyi	< 0.5	< 0,5	1,0±0,1	< 0,5	< 0,5	< 0,5	< 0,5
Shchedrik	< 0.5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5
Yantar	< 0.5	< 0,5	< 0,5	< 0,5	1,8±0,1	< 0,5	< 0,5
Tamyr	< 0.5	< 0,5	< 0,5	< 0,5	2,0±0,1	< 0,5	< 0,5
Krepysh	< 0.5	< 0,5	< 0,5	1,2±0,1	< 0,5	< 0,5	< 0,5
Yellow pink-spotted tuber skin							
Olskii	< 0.5	< 0.5	< 0.5	5.3±0.1	< 0.5	< 0.5	< 0.5
Pamyati Kulalova	< 0.5	< 0.5	< 0.5	3.2±0.1	< 0.5	< 0.5	< 0.5
Pink-skinned tubers							
Kuznechanka	4.1±0.1	9.7±0.1	< 0.5	78.4±0.3	< 0.5	< 0.5	< 0.5
Bashkirsky	0.9±0.1	3.1±0.1	< 0.5	24.8±0.2	2.3±0.1	< 0.5	< 0.5
Sirenevyi tuman	2.7±0.1	< 0.5	12.1±0.1	5.0±0.1	< 0.5	< 0.5	< 0.5
Povin	3.0±0.1	7.3±0.1	<0.5	24.8±0.2	5.0±0.1	< 0.5	10.2±0.1
Matushka	< 0.5	1.1±0.1	<0.5	22.2±0.2	0.7±0.1	< 0.5	< 0.5
Ognivo	1.3±0.1	< 0.5	<0.5	1.9±0.1	< 0.5	< 0.5	< 0.5
Dark pink-skinned tubers							
Mayak	2.1±0.1	2.1±0.1	<0.5	94.6±0.3	9.9±0.1	< 0.5	< 0.5
Manifest	1.0±0.1	1.2±0.1	<0.5	20.1±0.2	1.1±0.1	< 0.5	< 0.5
Violet-skinned tubers							
Vasilek	< 0.5	5.0±0.1	<0.5	38.2±0.2	149.8±0.4	< 0.5	2.0±0.1
Blue-violet-skinned tubers							
Fioletovyi	30.4±0.2	< 0.5	50.1±0.2	< 0.5	5.1±0.1	310.0±0.4	8.4±0.1

Note. 1 — delphinidin-3-glucoside, 2 — delphinidin-3-rhamnosyl-5-glucoside, 3 — malvidin-3-glucoside, 4 — pelargonidin-3-glucoside, 5 — petunidin-3-glucoside, 6 — cyanidin-3-glucoside, 7 — cyanidin-3-rhamnosyl-5-glucoside.

The highest anthocyanin levels were characteristic of the varieties Fioletovyi, Vasilek, Mayak, and Kuznechanka. The variety Fioletovyi with blue-violet potato skin and flesh was distinguished by a significant content of four anthocyanins, the cyanidin-3-glucoside (310 mg/kg), malvidin-3-glucoside (50 mg/kg), delphinidin-3-glucoside (30 mg/kg), and cyanidin-3-rhamnosyl-5-glucoside (8 mg/kg). The varieties Mayak and Kuznechanka have pink and dark pink tubers with the highest pelargonidin concentration, 95 and 78 mg/kg, respectively. The variety Vasilek with purple-skinned tubers outstands due to the highest petunidin-3-glucoside content (150 mg/kg). These varieties can be used as a functional food product with high antioxidant properties.

Cyanidin-3-glucoside was found to be the main in the tuber anthocyanin profile of the Fioletovyi variety (Fig.). The most intense signal at 371 nm (retention time 27.5 min) corresponds to the main anthocyanin of this variety, the cyanidine-3-glucoside; in addition, there are significant amounts of malvidin-3-glucoside (peak at 44 min) and dolphinin-3-glucoside (small peak at 25 min). The elution profiles revealed a relationship between the content of specific anthocyanins and the color of tubers, as well as their varietal specificity. Available publications confirm that the composition of anthocyanins, as a rule, is specific for a particular plant species and is quite stable. However, it depends on the varietal characteristics and growing conditions, which determine the activity of the enzymes involved in synthesis of certain components of the anthocyanin complex [19, 27].



Elution profile of the anthocyanins from **tubers of potato (*Solanum tuberosum* L.) variety Fioloetovyi** (*n* = 3, a collection nursery, Chaika Federal Research Center of Agricultural Biotechnology of the Far East, 2018). HPLC analysis, a Shodex C18-4E reverse phase column 250×4.6 mm I.D., 5 μm sorbent particles, Shodex, Japan. Eluent A is acetonitrile (AppliChem GmbH, Germany), eluent B is 1% formic acid (Sigma-Aldrich, USA), 0.00-5.00 min gradient (see *Materials and methods*). Peaks at 27.5 min, 44 min, and 25 min are cyanidin-3-glucoside, malvidin-3-glucoside, and delphinidin-3-glucoside, respectively.

Varieties with tuber skin of different shades of purple and pink were distinguished by the tuber anthocyanin concentration compared to varieties with yellow tubers. Therefore, the color of the tuber skin (pink, dark pink, blue-violet, purple) can serve as a visual trait in breeding dietary varieties rich in anthocyanins.

So, a second-order mass spectrometry identified anthocyanins delphinidin, petunidin, malvidin, cyanidin, and pelargonidin in the studied potato varieties. The most common petunidin-3-glucoside was found in almost all the samples. The highest content of anthocyanins was characteristic of the varieties Fioletovyi, Vasilek, Mayak, and Kuznechanka. The Fioletovyi variety with blue-violet tuber skin and flesh outstands for a significant content of four anthocyanins, the delphinidin-3-glucoside (30 mg/kg), malvidin-3-glucoside (50 mg/kg), cyanidin-3-glucoside (310 mg/kg), and cyanidin-3-rhamnosyl-5-glucoside (8 mg/kg). Cvs. Mayak and Kuznechanka have pink and dark pink tubers and an increased content of pelargonidin in flesh, 95 and 78 mg/kg, respectively. Variety Vasilek with purple-skinned tubers has the highest petunidin-3-glucoside content, 150 mg/kg. These findings show that the color of the tuber skin can be a trait for selection and breeding varieties with a high level of anthocyanins. Pink- and dark pink-skinned tubers contain pelargonidine-3-glucoside. Petunidin-3-glucoside and cyanidin-3-glucoside give violet and blue-violet color to the skin. The selected samples of dietary potatoes are now involved in breeding. To this end, we plan to further study the heritability of the desired anthocyanin pigmentation in the produced potato varieties and determine genes responsible for the anthocyanin composition and content of tubers.

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