



COMPARATIVE ASSESSMENT OF THE BIOCHEMICAL COMPOSITION OF FRUIT OF THE *OXYCOCCUS* *MACROCARPUS* (AIT.) PERS. CULTIVARS INTRODUCED IN BELARUS

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The article describes the findings of a comparative study of the sugar-acid index, content of free organic, ascorbic, and hydroxycinnamic acids, dry, tanning, and pectic substances, soluble sugars, and the main groups of bioflavonoids in fruit of six newly introduced *Oxycoccus macrocarpus* (Ait.) Pers. cultivars in Belarus — Stevens (st), Bain Favorite, Holliston, Hollister Red, Stankovich, and WSU 108. It is established that the *Hollister Red* cultivar has the highest integrated level of nutritive and vitamin value of fruit based upon the combination of properties analyzed, from 1.8 to 11 times the level observed in the other cultivars, whereas the *WSU 108* and especially the *Bain Favorite* cultivars have the lowest levels.

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Gantsevichy District of the Brest Region, in the central agro-climatic region of the country with light sandy sodpodzolic soils and dried high peatbogs.

Weather conditions in the area were characterized by higher-than-normal temperature settings and relatively favorable precipitation pattern throughout the season.

Six cultivars of the *Oxycoccus macrocarpus* variety had been selected as objects of the study: Stevens(st), Bain Favorite, Holliston, Hollister Red, Stankovich, and WSU 108.

The comparative assessment of the biochemical composition of their fruit was conducted based upon a broad range of indicators associated with various classes of active substances. Fresh averaged samples of ripe fruit were used to determine the content of (i) dry matter in accordance with GOST (State Standard) 28561-90,¹⁵ (ii) ascorbic acid (Vitamin C) by the standard indophenol method,¹⁶ and (iii) titratable acids (total acidity) by the volumetric method.¹⁶ Samples of plant materials dried at a temperature of 60 °C were used to determine the content of hydroxycinnamic acids (in terms of chlorogenic acid) by the spectrophotometric method,¹⁷ soluble sugars by the accelerated semi-micro method,¹⁸ pectic substances by the calcium pectate method,¹⁶ the amount of anthocyanin pigments according to Swain and Hillis¹⁹ with a calibration curve made based upon crystalline cyanidin derived from black chokeberry fruit and purified according to Skorikova and Shaftan,²⁰ true anthocyanins and the amount of catechins (with the use of the vanillin reagent) by the photolorimetric method,^{16,21} the amount of flavonols (in terms of rutin) by the spectrophotometric method¹⁶ and tannins by Leventhal's titrimetric method.²² All analytical determinations were made with a three-time biological repeatability. The data were statistically processed using the Excel software programme. The data showed statistically significant differences from the reference (standard) cultivar Student's *t*-test at $p < 0.05$.

Introduction

Data on the biochemical composition of fruit of American/large cranberry (*Oxycoccus macrocarpus* (Ait.) Pers.) are currently widely available in international academic literature.¹⁻⁷ The 45 years' worth of studies of introduced varieties carried out by the Central Botanical Garden of the National Academy of Sciences of Belarus resulted in a significant amount of scientific data on the issue, including several major monographs.⁸⁻¹⁴

Over the past few years, new varieties of large cranberry have been added to the Botanical Garden's extensive collection. With a view to identifying the most promising cultivars for regional assignment and selection, a comparative monitoring study was conducted for these cultivars for the first time to determine the content of the most physiologically valuable compounds, based upon an integrated assessment of not only breeding and bioproductive parameters, but also nutritive and vitamin value of fruit. The study made it possible to identify the new cultivars of the introduced variety with the highest level of nutritive and vitamin value of fruit.

Experimental

Research was carried out in 2015–2016 at the Experimental Station of the Central Botanical Garden of the National Academy of Sciences of Belarus located in the

Table 1. Content of dry substances and organic acids (in dry matter) in fruit of introduced *Oxycoccus macrocarpus* cultivars.

Cultivar	Dry substances, %		Organic acids					
			titratable, %		ascorbic, mg %		hydroxycinnamic, mg %	
	$\bar{x} \pm m_x$	t_{St}	$\bar{x} \pm m_x$	t_{St}	$\bar{x} \pm m_x$	t_{St}	$\bar{x} \pm m_x$	t_{St}
<i>Stevens(st)</i>	14.0±0.2		22.5±0.1		370.5±6.9		651.0±12.2	
<i>Bain Favorite</i>	12.7±0.3	-4.0	24.4±0.1	34.9	476.2±3.9	13.3	589.0±14.1	-3.3
<i>Holliston</i>	15.7±0.1	9.2	17.2±0.1	-76.6	345.5±6.3	-2.8	667.2±13.9	0.9
<i>Hollister Red</i>	13.3±0.1	-4.0	22.4±0.1	-0.3	426.1±4.8	6.6	710.8±25.5	2.8
<i>Stankovich</i>	12.0±0.1	-9.5	25.2±0.1	46.3	568.2±2.8	26.6	549.8±14.0	-5.4
<i>WSU 108</i>	13.0±0.1	-5.8	25.3±0.1	35.9	445.1±2.5	10.2	563.7±13.9	-4.7

The identification of large cranberry cultivars with the highest integrated level of nutritive and vitamin value of fruit based upon the combination of analyzed properties was performed on the basis of the patented plant ranging method.²³

Results and discussion

The late-ripening *Stevens* cultivar previously released in Belarus as the standard was adopted as the benchmark for the biochemical screenings of the new cultivars of the *Oxycoccus macrocarpus* variety.

According to our findings (Table 1), the content of dry matter in the fruit of the cultivars of large cranberry in question varied from 12.0 % and 15.7 %. The content of free organic acids in their dry matter was within the range of 17.2 to 25.3 %, of ascorbic acid from 345.5 mg % to 568.2 mg %, of hydroxycinnamic acids from 549.8 mg % to 710.8 mg %, which was commensurable with the findings of our earlier studies of other cultivars of this *Ericaceae* variety.

Table 2. Content of soluble sugars and pectic substances in the dry matter of fruit of introduced *Oxycoccus macrocarpus* cultivars

Cultivar	Soluble sugars		Sugar-acid index		Pectic substances	
	$\bar{x} \pm m_x$	t_{St}	x^*	t_{St}	x^*	t_{St}
<i>Stevens (st)</i>	26.5±0.1		1.21		10.0	
<i>Bain Favorite</i>	26.5±0.1	0	1.1	-7.6	9.4	-5.2
<i>Holliston</i>	29.7±0.3	9.5	1.71	26.7	8.1	-14.0
<i>Hollister Red</i>	29.3±0.3	8.5	1.3	6.9	7.6	-20.8
<i>Stankovich</i>	31.5±0.5	10.0	1.3	3.4	5.2	-39.7
<i>WSU 108</i>	28.5±0.5	4.0	1.1	-3.2	6.8	-27.7

*St=±m_x

The total content of soluble sugars in the fruit of the cranberry cultivars under study varied within a comparatively narrow range of very low values of 26.5 to 31.5 % of dry matter (Table 2). Because of the high content of titratable acids indicated above, they were characterized by extremely low sugar-acid index indicators that remained within 1.1 to 1.7, which attested to their extremely sour taste.

At the same time, cranberry fruit was characterized by the relatively high parameters, comparable to those that we have determined for highbush blueberry and *Vaccinium vitis-idaea* (lingonberry, cowberry)¹³, of the accumulation of pectic substances that varied within the range of cultivars from 5.2 % to 10.0 % of dry matter (Table 2).

As is known, *Oxycoccus macrocarpus* fruit is extremely rich in bioflavonoids that have a marked P-vitamin effect.²⁴ According to our findings (Table 3), their total amount in the dry substance of the fruit of the cultivars under analysis varied within the range from 7461.0 to 11032.2 mg 100 g⁻¹. Anthocyanin pigments dominated in the bioflavonoid complex, as in all representatives of the *Ericaceae*, with total content varying from 5581.3 to 8463.0 mg 100 g⁻¹ and accounted for 74-77 % of the total. Leucoanthocyanins were the prevailing fraction in these compounds, as in the *Vaccinium*,²⁵ with content in the fruit of the cultivars under analysis between 3878.0 and 5908.0 mg /100 g⁻¹, 2.3-3.2 times the content of true anthocyanins (at 1,703.3 to 2,555.0 mg 100 g⁻¹), and the gap was the smallest in the *Bain Favorite*, *Hollister Red*, and *WSU 108* cultivars and the biggest in *Holliston*.

Catechin content in dry matter of large cranberry fruit varied between 1016.2 and 1827.6 mg 100 g⁻¹, with fluctuations of contributions of these restored compounds to the composition of the bioflavonoid complex within a range from 13 % in *WSU 108* to 18 % in *Stankovich*. Flavonols were characterized by the lowest contribution to the P-vitamin complex of cranberry fruit, varying from 7 % in *Hollister Red* to 12 % in *Bain Favorite* within the taxonomic range, with content between 771.8 and 1092.8 mg /100 g⁻¹. The content of tannins in cranberry fruit was quite high, at 2.62-3.37 % of the dry mass (Table 3).

The cultivars of large cranberry under analysis showed distinct differences from the benchmark *Stevens* cultivar when it came to the biochemical composition of fruit (Table 4). The *Bain Favorite*, *Stankovich*, and *WSU 108* cultivars showed the highest content of titratable acids, which exceeded the benchmark by 8 to 12 %. At the same time, *Hollister Red* was characterized by the accumulation of free organic acids comparable to that in *Stevens*, whereas in *Holliston*, their content was 24 % lower than in the benchmark. The fruit of all of the tested cultivars (except *Holliston*) proved to have a higher-than-benchmark content of ascorbic acid, by 15-53 %, whereas *Stankovich* showed little difference.

Table 3. Content of phenol compounds in the dry matter of fruit of introduced *Oxycoccus macrocarpus* cultivars.

Cultivar	Bioflavonoids, mg 100 g ⁻¹							
	True anthocyanins		Leucoanthocyanins		Amount of anthocyanin		Catechins	
	$\bar{x} \pm m_x$	t_{St}	$\bar{x} \pm m_x$	t_{St}	$\bar{x} \pm m_x$	t_{St}	$\bar{x} \pm m_x$	t_{St}
<i>Stevens(st)</i>	2041.7±42.1		5268.7±122.2		7310.3±80.3		1524.3±13.1	
<i>Bain Favorite</i>	1703.3±11.7	-7.8	3878.0±71.8	-9.8	5581.3±80.3	-15.2	1016.2±15.2	-25.3
<i>Holliston</i>	1761.7±30.9	-5.4	5609.3±69.1	2.8	7371.0±52.5	0.6	1456.0±52.5	-1.3
<i>Hollister Red</i>	2555.0±20.2	11.0	5908.0±32.3	5.1	8463.0±52.5	12.0	1774.5±26.3	8.5
<i>Stankovich</i>	1948.3±5.8	-2.2	5559.2±31.5	2.8	7507.5±26.3	2.8	1827.6±20.1	12.6
<i>WSU 108</i>	2070.8±35.5	0.5	4921.0±11.3	-2.8	6991.8±40.1	-3.5	1243.7±54.7	-5.0
Cultivar	Bioflavonoids, mg/100 g						Tannins,%	
	Flavonols		Flavonols / Catechins		Amount		$\bar{x} \pm m_x$	t_{St}
	$\bar{x} \pm m_x$	t_{St}	$\bar{x} \pm m_x$	t_{St}	$\bar{x} \pm m_x$	t_{St}		
<i>Stevens(st)</i>	1092.8±33.3		0.7±0.03		9927.3±103.8	59.9	3.12±0.02	
<i>Bain Favorite</i>	863.5±33.3	-4.9	0.9±0.03	3.0	7461.0±45.9	-32.7	2.62±0.02	-15.3
<i>Holliston</i>	1054.6±26.5	-0.9	0.7±0.01	0.3	9881.6±26.5	-0.7	2.99±0.01	-5.6
<i>Hollister Red</i>	794.7±7.6	-8.7	0.4±0.01	-9.3	11032.2±79.2	11.1	3.16±0.01	1.7
<i>Stankovich</i>	771.8±42.6	-5.9	0.4±0.03	-7.6	10106.9±26.5	2.8	3.37±0.02	7.7
<i>WSU 108</i>	985.8±35.0	-2.8	0.8±0.01	2.6	9221.3±125.6	-5.1	2.95±0.02	-5.2

Table 4. Relative differences (in percentage terms) between the introduced *Oxycoccus macrocarpus* cultivars and the standard *Stevens* cultivar by the content of active substances.

Indicator	<i>Bain Favorite</i>	<i>Holliston</i>	<i>Hollister Red</i>	<i>Stankovich</i>	<i>WSU 108</i>
Dry matter	-9.3	+12.1	-5.0	-14.3	-7.1
Free organic acids	+8.4	-23.6	-	+12.0	+12.4
Ascorbic acid	+28.5	-6.7	+15.0	+53.4	+20.1
Hydroxycinnamic acids	-9.5	-	+9.2	-15.5	-13.4
Soluble sugars	-	+12.1	+10.6	+18.9	+7.5
Sugar-acid index	-8.3	+41.7	+8.3	+8.3	-8.3
Pectic substances	-6.0	-19.0	-24.0	-48.0	-32.0
True anthocyanins	-16.6	-13.7	+25.1	-	-
Leucoanthocyanins	-26.4	+6.5	+12.1	+5.5	-6.6
Anthocyanin pigments	-23.7	-	+15.8	+2.7	-4.4
Catechins	-33.3	-	+16.4	+19.9	-18.4
Flavonols	-21.0	-	-27.3	-29.4	-9.8
Bioflavonoids	-24.8	-	+11.1	+1.8	-7.1
Tannins	-16.0	-4.2	-	+8.0	-5.4

However, in most of the new cultivars, the content of hydroxycinnamic acids was from 10 % to 16 % lower than in *Stevens*; it was only higher in *Hollister Red*, by 9 %, whereas *Holliston* showed no valid difference.

All of the new large cranberry cultivars, except *Bain Favorite*, were characterized by 8-19 % higher content of soluble sugars compared to the fruit of the standard *Stevens* cultivar. In *Hollister Red*, *Stankovich* and especially *Holliston*, the sugar-acid index of soluble sugars was from

8 % to 42 % higher than in *Stevens*, which results in a sweeter taste of berries compared to the benchmark (Table 4). At the same time, the same parameter in *Bain Favorite* and *WSU 108* was 8 % below the benchmark. Unlike soluble sugars, all of the tested cranberry cultivars showed 6 % to 48 % lower content of pectic substances in their fruit as against the benchmark. The most significant difference was observed in *Stankovich*. Mixed trends were observed in the nature of differences of the tested *Oxycoccus macrocarpus* cultivars from the *Stevens* benchmark in the context of

bioflavonoid content in fruit, only *Hollister Red* showed a significant difference (11 % higher), whereas *Stankovich* showed an insignificantly (within 2 %) yet reliably higher level than the benchmark. Unlike the two cultivars above, overall P-vitamin content in the *WSU 108* and especially *Bain Favorite* cultivars was lower than in *Stevens*, by 7 and 25 %, respectively, and *Holliston* showed no difference from the standard cultivar.

Also mixed were such differences in the accumulation in cranberry fruit of certain groups of bioflavonoids. Only in *Hollister Red* fruit was the content of the most valuable bioflavonoid group, true anthocyanins, higher than that in the benchmark cultivar, by 25 %, whereas the *Bain Favorite* and *Holliston* cultivars, on the contrary, showed anthocyanin content 14 % and 17 % lower, respectively, while *Stankovich* and *WSU 108* showing no significant difference. The content of leucoanthocyanins in *Holliston*, *Hollister Red*, and *Stankovich* fruit was 6 % to 12 % above that in *Stevens*, whereas in *WSU 108* and *Bain Favorite*, it was 7 % and 26 % lower, respectively (Table 4).

Because of the dominating position of leucoanthocyanins in the composition of the P-vitamin complex of cranberry fruit, similar trends in the nature of differences of the tested cultivars from *Stevens* were observed when it comes to the content of anthocyanin pigments, as well as the content of catechins, which are close to anthocyanins in their chemical nature. Only the fruit of the *Hollister Red* and *Stankovich* cultivars showed catechin content higher than that in the benchmark cultivar, by 16 and 20 %, respectively, whereas in *WSU 108* and *Bain Favorite*, catechin content was 18 and 33 % lower, respectively. The *Holliston* cultivar showed no significant difference. As for the flavonols, their content proved to be lower in all of the tested cultivars, except for *Holliston*, compared to that in *Stevens*, by 10 to 29 %. The content of tannins in the fruit of new cranberry cultivars was in most cases lower than that in the benchmark cultivar by 4 % to 16 %, and only *Stankovich* showed a higher-than-benchmark content, by 8 %.

As a result of biochemical screening of the fruit of the *Oxycoccus macrocarpus* cultivars under analysis, taxa with the highest and lowest parameters of accumulated active substances of various chemical nature were identified. *Holliston* was the leading cultivar by the content of dry matter in fruit, the *Stankovich* and *WSU 108* cultivars led in terms of the content of free organic substances, *Stankovich* was the leader by the content of ascorbic acid, *Hollister Red* in terms of hydroxycinnamic acids, *Stankovich* by the content of soluble sugars, *Holliston* by the content of sugar-acid index, *Stevens* in terms of pectic substances, *Hollister Red* in terms of bioflavonoids, including true anthocyanins and leucoanthocyanins, *Stankovich* by the content of catechins and tannins, and *Stevens* and *Holliston* in terms of flavonols.

Despite the diversity of the advantages of any given *Oxycoccus macrocarpus* cultivar in terms of the content of compounds of various chemical nature in their fruit, it is hard to identify the taxa with the highest integrated level of nutritive and vitamin value. To this end, we made use of our own, patented²³ method, based upon the juxtaposition of the relative sizes, amplitudes, and ratios of statistically valid positive and negative deviations in the cultivars in question from the reference values of analyzed characteristics of the

biochemical composition of their fruit. The scope of the combined amplitude of deviations revealed, irrespective of their sign (plus or minus), can serve as an indicator to decide on the distinctiveness of differences in each of the entities under analysis from the *Stevens* cultivar by the combination of analyzed properties, which makes it possible to rank them by the degree of these differences in the descending order. The correlation of the relative ranges of the scopes of positive and negative deviations with the reference content of active substances in fruit became an assessment criterion of the integrated level of their nutritive and vitamin value in each of the tested cranberry cultivars, based upon the premise that all of the analyzed properties are equally important for the assessment of the quality of fruit.

The data presented in table 5 that are based upon those in table 4 and characterize the direction and the significance of shifts in the biochemical composition of the fruit of the new tested *Oxycoccus macrocarpus* cultivars in reference to the *Stevens* benchmark show pronounced genotypic differences in the direction and magnitude of the said shifts. With amplitude of these differences in the cultivar range between 139.6 and 237.7 %, they appeared to be the least distinct in *Holliston*, whereas the *Bain Favorite* and *Stankovich* cultivars showed the most striking contrast. In two cranberry cultivars, *Bain Favorite* and *WSU 108*, the relative scope of negative differences from the benchmark *Stevens* cultivar in terms of the combination of analyzed properties prevailed over those positive, whereas for the rest of the cultivars, the situation was the opposite.

Table 5. Relative scopes, amplitudes, and correlations of variously oriented differences, in percentage terms, in the biochemical composition of fruit of newly introduced *Oxycoccus macrocarpus* cultivars from the standard *Stevens* cultivar.

Cultivar	Relative scale of differences, %			
	+	-	amplitude	+/-
<i>Bain Favorite</i>	36.9	194.9	231.8	0.2
<i>Holliston</i>	72.4	67.2	139.6	1.1
<i>Hollister Red</i>	123.6	56.3	179.9	2.2
<i>Stankovich</i>	130.5	107.2	237.7	1.2
<i>WSU 108</i>	40.0	112.5	152.5	0.4

The most objective account of the integrated level of nutritive and vitamin value of the fruit of each of the newly introduced *Oxycoccus macrocarpus* cultivars can be provided with the help of the multiple scale of the correlation of the relative amounts of positive and negative deviations of the combination of analyzed properties with the reference value. It turned out that only in three cranberry cultivars this scale exceeded 1.0, which attested to a higher integrated level of nutritive and vitamin value of their fruit compared to the standard *Stevens* cultivar. Below is the sequence of tested cultivars in the descending order of the degree of their advantages with reference to the benchmark cultivar, based upon the above criterion:

Hollister Red > *Stankovich* > *Holliston* > *Stevens* > *WSU 108* > *Bain Favorite*

It can be ascertained that *Hollister Red* is the breakaway leader in the group of large cranberry cultivars, whereas the least valuable cultivars in terms of the biochemical

composition of their fruit are *WSU 108* and especially *Bain Favorite*, both of which are at the end of the sequence.

Based upon the comparison of the scale of the correlation under analysis, a quantitative assessment was provided for the degree of decrease, as against the *Hollister Red*, the leader in the taxonomic range, in the integrated level of nutrient and vitamin value of the fruit of the remaining cranberry taxa. As one would expect, that degree was highest in *Bain Favorite*, which was 11 times behind the leading cultivar in terms of this feature. The gap was significantly narrower for the rest of the cultivars, between 1.8x and 5.5x.

Conclusion

As a result of the comparative examination of sugar-acid index, content of free organic, ascorbic, and hydroxycinnamic acids, dry substances, tannins, and pectic substances, soluble sugars, and main groups of bioflavonoids in fruit of six *Oxycoccus macrocarpus* cultivars, *Stevens(st)*, *Bain Favorite*, *Holliston*, *Hollister Red*, *Stankovich*, *WSU 108*, that have been newly introduced in Belarus, it has been established that the *Hollister Red* cultivar was characterized by the highest level of nutritive and vitamin value of fruit in terms of the combination of properties under analysis, which proved to be 1.8x to 11x above that in the other cultivars, whereas *WSU 108* and especially *Bain Favorite* showed the lowest levels.

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