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Biochemical contents of highbush blueberry fruits grown in the Western Forest-Steppe of Ukraine

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Abstract. The study of the physical and consumption qualitative indices of the highbush blueberries (the cultivars 'Reca', 'Elizabeth' and 'Bluegold') in the Western Lisosteppe of Ukraine has shown that the biggest fruit mass was accumulated by the berries of two last mentioned vs (1.54–1.50 g respectively). 'Bluegold' has this indicator as the least variable (V = 10.6%). The accumulation of fruit mass of all studied cultivars was facilitated by a moderately humid period of their development in 2018 with a hydrothermal coefficient of 1.1–1.2. The favorable year for the intensive synthesis of the dry matter and soluble solids was 2017 when the precipitation amount did not exceed 44 mm. The biggest number of the mentioned substances during the research period was accumulated by fruits of 'Reca' - 17.48 and 13.24%, respectively. It was established that the dry matter content had high level of stability (V = 8.2%), and middle was for soluble solids (V = 19.5%). The amount of monosaccharides from which glucose and fructose were investigated in fruits varied from 6.11 ('Bluegold') to 7.85 ('Reca'), it was slightly lower in 'Elizabeth' berries - 7.78%. 'Bluegold' fruits were characterized by high stability (B = 9.6%) of the biggest content of titrated acids among the studied cultivars (2.42%). The dry weather with low number of precipitation in 2017 (hydrothermal coefficient 0.3–0.4) was favourable for the accumulation of both the mentioned acids and vitamin C in fruits of highbush blueberries in the period of their formation and growth. The amount of ascorbic acid in fruits in the specified year varied from 20.00 mg 100 g^{-1} WM ('Reca') to 27.00 mg 100 g^{-1} WM ('Elizabeth') with an intermediate value of 22.50 mg 100 g^{-1} WM ('Bluegold'). The latest of the mentioned varieties had the most constant index (V = 7.0%). The content of polyphenolic substances was slightly dependent on weather conditions during the period of fruit growth, the coefficients of variation were 6.2% ('Reca'), 7.0% ('Elizabeth') and 5.8% ('Bluegold'). The fruits of the last mentioned cultivars were characterized with the biggest anthocyanins and chalcones content (68 and 13 mg 100 g^{-1} WM, respectively). The substantial indirect dependence of the content of the nutritive substances and anthocyanins on the berry mass was revealed in the 'Reca' fruits.

Key words: *Vaccinium corymbosum* L., average mass, nutritional and bioactive substances, correlation.

INTRODUCTION

The popularity of highbush blueberries has increased over the last ten years, and now these are grown in many countries around the world. In 1990, *V. corymbosum L.* was cultivated in only 10 countries (Statistics Division, 2015), while in 2011 its commercial cultivation was introduced in 27 ones (Evans & Ballen, 2014). World production of highbush blueberry fruit increased from 262 t in 2006 to 556 t in 2016 (Aliman et al., 2020). According to the FAOSTAT data, the leaders of world blueberry production are the United States (269 tons) and Canada (179 tons). In Ukraine, over the past 10 years, tall blueberries from a little-known niche culture have become one of the main berry species. In Ukraine, during the last 10 years, *V. corymbosum* L. from a little-known niche crop have turned in to one of the main small fruit crops. Since 2017, Ukraine has become among five biggest blueberry fruit producers in Europe. In a period from 2007 to 2017 the total area of highbush blueberry plantations in Ukraine has dramatically increased from 130 ha to more than 1,500 ha (end of 2018 year) and it is expected to be 2,000 ha circa now. The rapid increase in the area under *V. corymbosum L.* was due to the high demand of the population for its fruit, which was formed due to significant consumer, dietary and medicinal properties, which is confirmed in the research by well-known scientists (Basu et al., 2010; Krikorian et al., 2010; Carey et al., 2014; Nil & Park, 2014; Singh, 2018).

Blueberry fruits contain large amount of organic and inorganic substances, which is mostly determined by the variety genotype, growing conditions, including ecological and technological, as well as the state of berry ripeness (Gündüz et al., 2015). Polish scientists established that content of *V. corymbosum L.* fruits is, %: water - 84, carbohydrates - 9.7, albumins - 0.6, fats - 0.4 (Michalska & Łysiak, 2015); other components are polyphenols, antioxidants, vitamins, minerals and fiber (Ścibisz & Mitek, 2007). The energetic value of a 100 g fresh berry portion is estimated at 192 kilojoules. Fruits of blueberry are also a good source of nutritive fibre accounting for 3–3.5% of their mass. In addition to good taste, the main interest in these berries is due to the moderate content of vitamin C, as 100 g of blueberries provide, on average, 10 mg of ascorbic acid, equal to 1/3 of the recommended daily norm (Prior et al., 1998; NHMRC, 2015). According to many scientists, polyphenolic and antioxidant compounds of blueberries provide positive effect on human health; reduce the risk of various diseases (Ramata-Stunda, 2020). These substances can also prevent neurodegenerative disorders (Ramassamy, 2006; Correa-Betanzo et al., 2014; Giacalone et al., 2015; Diaconeasa et al., 2015; Alibabić et al., 2019). Among berry species, highbush blueberries are distinguished by the presence of various types of anthocyanins (Gao & Mazza, 1994), in particular, such as: malvidin, delphinidin, petunidine, cyanidin and peonidine with sugar fragments of glucose, galactose and arabinose. According to some researchers, malvidin and delfinidine are the main components and amount to nearly 75% of all detected anthocyanins (Scibisz & Mitek, 2007).

The main world originators of *V. Corymbosum* L. varieties are the United States (Lyrene, 2007; Brevis et al., 2008), however breeding programs that aim to create new varieties adapted to the conditions of the region are the task of breeders from other countries (Scalzo et al., 2013), including Ukraine. Creation of varieties with high standards of marketable and consumer qualities, stable to the growing conditions of Ukraine, can be realized only in a case of selection of appropriate parental forms.

The aim of our research was to study the biochemical contents of highbush blueberry small fruits grown in the climatic conditions of the Western Forest-Steppe of Ukraine, however it is very important to find out the nutritional value of the introduced varieties. Knowledge of the degree of correlation between the biochemical components themselves and with physical ones will allow breeders to select parent pairs for conducting the breeding process and creating varieties with a stable content of consumer and biologically active substances. Our research will be the basis for the breeding process aimed at creating varieties of *V. corymbosum* L. with a significant polyphenolic complex, and in particular its anthocyanin component, which makes the fruits of this crop a trendy food product.

MATERIALS AND METHODS

The research was conducted during 2017–2019. The experiment included cultivars with different ripening time: ‘Reca’ - early season (origin - New Zeland), ‘Elizabeth’ and ‘Bluegold’ - mid season (origin - the USA). Fruit samples were taken on the plots of the Institute of Horticulture of NAAS which is situated in the Western Forest-Steppe of Ukraine, altitude is 100–140 m. Climate of the region is moderate continental the minimum of average air temperature is 3.2 °C, maximum - 21.3 °C, average year temperature is 10 °C, annual precipitation is 619 mm. Plot`s soil is grey forest slightly loam. The plantation was created in 2016; the distance between the bushes in a row was 1 meter, and 3 meters between the rows. Rows were mulched with sawdust and there was turf between rows.

Analytical research was performed in the laboratory on the storage and processing of fruits and berries of the IH NAAS. Fruits of highbush blueberries with a characteristic shape and color were selected at the stage of consumer ripeness in the amount of at least one kilogram, according to the ‘Methods for assessing the quality of fruit and berry products’ (Kondratenko et al., 2008). The average berries mass was determined by weighing of 50 fruits on laboratory scales. Crushed analytical samples of fruits to determine the content of nutrients and biologically active substances were prepared using a homogenizer. The sample was weighed on analytical scales. The biochemical components such as: the content of total dry matter (drying samples method by the temperature of 98–100 °C - due to the national standard 7804:2015), soluble solids (Brix) - refractometrically (NS 8402:2015), titrated acids and vitamin C content by titration (NS 7803:2015), sugar and polyphenolic substances content - spectrophotometrically (NS ISO 4954:2008) were analyzed. Anthocyanins and chalcones were determined by the method of Kryventsov (Kryventsov, 1982). The sugar-acid index was calculated by dividing the total amount of sugars by the content of titrated acids. The experiments were conducted in 3-time replications.

Meteorological data for the trial evaluation years were obtained at the Vantage Pro2 Plus weather station. The hydrothermal coefficient (SCC Selyanova) was calculated by dividing the amount of precipitation in mm by the sum of active temperatures of 10 °C and above for the period of growth and development of fruits. The obtained data were decreased 10 times.

Statistical analysis

Statistical data processing was carried out with the use of the program STATISTICA 13/1 (StatSoft, Inc., USA). The results are presented as mean values with their standard errors, as average ± standard error (x ± SE). The differences between repetitions, as well as average inter-varietal value were determined using ANOVA. The research results were presented at the level of $P < 0.05$. Two-factor analysis of variance of the significant impact of variety genetic and climatic conditions into the content of nutritional and biologically active substances of highbush blueberry fruits and the correlation analysis were conducted in Excel.

RESULTS AND DISCUSSION

Weather conditions for three-year research in the period of plants and fruits development (varieties - 'Reca', 'Elizabet', 'Bluegold') were different, both in temperatures and precipitations. 2018 turned out the wettest, the amount of precipitation for this period was higher than 125 mm, the least fell in 2017 (43.9 mm). The average daily temperature varied from 18.0 °C in 2017 to 23.6 °C in 2019. The sum of active temperatures above 10 °C during the period varied from 1,396 to 1,424 °C. The ratio of precipitation and the sum of active temperatures of 10 °C and above was in the range from 0.3 in 2017 to 1.2 in 2018 (Table 1).

Table 1. Weather indices during the period of *V. corymbosum* L. fruits growth and development (2017–2019)

Cultivars		Amount of days from flowering to ripening	Average daily temperature, °C	Sum of effective temperature > 10 °C	Precipitation, mm	Humidity, %	HTC
'Reca'	2017	62	18.0	1,095	39.2	48.6	0.4
	2018	60	20.0	1,180	125.1	49.7	1.1
	2019	65	22.2	1,424	94.1	55.7	0.7
	Average for 3 years	62	20.1	1,233	86.1	51.3	0.7
'Elisabeth'	2017	62	20.2	1,277	36.9	49.0	0.3
	2018	54	20.0	1,082	126.8	49.9	1.2
	2019	57	23.6	1,348	73.1	51.1	0.5
	Average for 3 years	58	21.3	1,236	78.9	50.6	0.7
'Bluegold'	2017	70	18.2	1,257	43.9	48.3	0.3
	2018	58	19.6	1,139	136.0	50.0	1.2
	2019	62	22.8	1,396	87.6	55.2	0.6
	Average for 3 years	63	20.2	1,264	89.2	51.2	0.7

According to Spanish scientists, mass of highbush blueberry fruits must have more than 0.75 g in order to be acceptable for market (Molina et al., 2008). The average berry weight grown in Bosnia ranged from 1.12 to 2.11 g (Aliman et al., 2020), and Portuguese berries weighed 1.4–2.4 g (Correia et al., 2016). The fruit mass from Macedonia was approximately the same (Arsov et al., 2010), from Serbia it was 1.86−1.94 g

(Zorenc et al., 2016), and from Korea - 1.83–2.21 g (Kim et al., 2013). The weight of fruits studied in our laboratory was: 1.29 g for ‘Reca’, 1.50 - ‘Bluegold’, 1.54 - ‘Elizabeth’. The heaviest mass fruits had in 2018, in particular at ‘Reca’ - 1.60; ‘Bluegold’ - 1.70 and ‘Elizabeth’ - 1.80 g. ‘Bluegold’ was characterized as the variety with the most stable fruit weight over the years of observation with the coefficient of variation of 10.6% (Table 2).

Table 2. Physical and consumption indicators of the *V. corymbosum* L. fruits quality (2017–2019) (*n* = 9)

		2017	2018	2019	Average for 3 years	Coefficient variation, %
Average mass, g	‘Reca’	1.13 ± 0.07	1.60 ± 0.08	1.13 ± 0.08	1.29 ± 0.27	18.6
	‘Elizabeth’	1.62 ± 0.09	1.80 ± 0.06	1.20 ± 0.10	1.54 ± 0.31	17.8
	‘Bluegold’	1.40 ± 0.06	1.70 ± 0.05	1.40 ± 0.09	1.50 ± 0.18[a]	10.6
	SE				0.17	
	x				1.44	
Dry matter, %	‘Reca’	19.10 ± 0.45	16.00 ± 0.96	17.33 ± 0.38	17.48 ± 1.63	8.2
	‘Elizabeth’	18.40 ± 0.45	16.00 ± 0.84	17.33 ± 0.51	17.24 ± 1.30	6.7
	‘Bluegold’	16.80 ± 0.91	14.83 ± 0.49	15.07 ± 0.16	15.57 ±1.17[b]	6.7
	SE				0.94	
	x				16.76	
Soluble solids, %	‘Reca’	16.64 ± 0.44	11.80 ± 0.57	11.28 ± 0.37	13.24 ± 2.92	19.5
	‘Elizabeth’	16.24 ± 0.30	11.20 ± 0.29	11.48 ± 0.59	12.97 ± 2.80	19.1
	‘Bluegold’	12.63 ± 0.63	11.00 ± 0.24	11.48 ± 0.31	11.70 ±0.90[b]	6.8
	SE				1.44	
	x				12.64	
Sugars, %	‘Reca’	13.45	9.44	9.02	10.64 ± 2.77	23.0
	‘Elizabeth’	13.10	9.10	9.40	10.53 ± 2.52	21.0
	‘Bluegold’	10.14	9.10	9.30	9.51 ± 0.62	5.80
	SE				0.70	
	x				10.23	
Glucose and fructose, %	‘Reca’	9.52 ± 0.54	6.97 ± 0.20	7.06 ± 0.51	7.85 ± 1.47	16.6
	‘Elizabeth’	8.54 ± 0.59	6.02 ± 0.30	8.78 ± 0.66	7.78 ± 1.57	17.9
	‘Bluegold’	5.89 ± 0.44	5.56 ± 0.18	6.89 ± 0.84	6.11 ± 0.75[b]	10.9
	SE				0.91	
	x				7.25	
Titrated acids, %	‘Reca’	1.68 ± 0.11	1.23 ± 0.11	1.63 ± 0.07	1.51 ± 0.26	15.0
	‘Elizabeth’	2.65 ± 0.18	1.23 ± 0.15	1.98 ± 0.23	1.95 ± 0.71	32.2
	‘Bluegold’	2.60 ± 0.12	2.20 ± 0.22	2.46 ± 0.25	2.42 ± 0.26	9.6
	SE				0.36	
	x				1.96	

Note: [a]; [b] are values of indicators that differ significantly from the average (x) for the studied group at $P < 0.05$.

The dry matter content in different fruits and small fruit species, according to our data, ranges from 7 to 26%, however content of it in lowbush blueberries, according to Canadian scientists, varies from 14.03 to 16.23% (Kalt & McDonald, 1996). In our research the biggest amount of dry matters in blueberry fruits was acquired in 2017, when the precipitation sum did not exceed 44 mm, and it was 16.80, 18.40 and 19.10% for varieties ‘Bluegold’, ‘Elizabeth’ and ‘Reca’ respectively. The lowest content of these substances, for varieties 'Reca', 'Elizabeth' (16.00) and 'Bluegold' (14.83%), was observed in 2018. That year the precipitation index was more than 1.1. Bigger content of DM comparing with average data for the studied varieties was observed for 'Reca' (17.48%) and 'Elizabeth' (17.24%). The stability of the DM content in highbush blueberry fruits obtained high; the variation coefficient (V) did not exceed 8.2% (Table 2).

The amount of soluble solids (SS), according to Colombian scientists, determines fruit sweetness (Cortés-Rojas et al., 2016). The content of soluble solids was bigger in fruits of highbush вlueberries grown in the highland, what is explained by the fact that this area is characterised by higher level of sunlight than elsewhere (Fischer et al., 2012). Hot and rainless weather of temperate climates (Naumann & Wittenburg, 1980) accelerates the rate of photosynthesis (Taiz & Zeiger, 2010), this leads to an increase of the soluble solids concentration (Jifon & Syversten, 2001). This fact was confirmed by our research. The biggest SS amount of high-bush blueberry fruits (12.63–16.64%) was in 2019, when hydro-thermal coefficient was 0.3–0.4. The least level of SS was in 2018 from 11.00% (Bluegold variety) to 11.80% (Reca variety), when the precipitation amount was less than average, by 89.3 mm than in the analogous period of 2017 and by 65.8 mm than in 2019. The *V. corymbosum* L. fruits cultivated in Belorussia contained 13–15.3% of SS (Zenkova & Pinchykova, 2019), those from the Northern-eastern Turkey - 13.3% (Celik et al., 2018). The highbush blueberry fruits cultivated in the Western Lisosteppe of Ukraine accumulated from 11.70% ('Bluegold' variety) to 13.24% ('Reca' variety) (Table 2), which corresponded to the data obtained by scientists from neighbouring countries. The most stable SS amount among the researched varieties was determined for 'Bluegold' (V = 6.8%), for 'Reca' and 'Elizabeth' the coefficients of variation corresponded to the average value of 19.5 and 19.1%, respectively (Table 2).

In highbush blueberry fruit, sugars determine their organoleptic quality (Li et al., 2020), in particular, taste (Okan et al., 2018). It was reported by Gündoğdu (2019) that environmental factors and cultural practices (rootstock, irrigation, etc.) were effective on biochemical compounds such as sugar, phenolics and organic acids in fruits. The content of the mentioned substances in *V. corymbosum* L. fruits grown in Bosnia and Herzegovina was 9.73–9.94% (Aliman et al., 2020), in Russia it was at a level of 10.15–14.8% (Kirina et al., 2020). The biggest sugar content of high-bush blueberry fruits cultivated in the Western Lisosteppe of Ukraine was determined for 'Reca' variety (6.97–9.52%). The varieties Elizabeth and Bluegold were characterized by having lower sugar amount 6.02–8.78% and 5.56–6.89% respectively. The tendency of sugars accumulation in highbush blueberry fruits of the studied varieties was identical with the dynamics of dry matter and soluble solids accumulation. None of the studied varieties was determined as stable according to the sugar content; the coefficients of variation were higher than 10.9%, but lower than 20.0% (Table 2). Two-factor analysis of variance established the significance of the influence of weather and climatic conditions, at the level of 72.7%, on the sugar content in the fruits of the studied varieties, while genetic features determined their sugar content by only 9.3% (Fig. 1).

Besides the taste quality, sugars determine the calorific value of fruit, which is usually insignificant. Their dietetic properties are based on a high content of simple carbohydrates, namely glucose and fructose (Skrovankova et al., 2015), which are the main highbush blueberry sugars (Kalt & McDonald, 1996). Turkish researchers state that the ratio of glucose and fructose in high-bush blueberry is almost the same (Ayaz, 2001), and range from 2.9 to 7.1% - fructose and from 2.7 to 6.9% - glucose, and their total number is from 9.1 to 9.9% (Hirvi & Honkanen, 1983). Other researchers emphasize that the amount of suitable substances is from 2.64 to 4.65% to 2.29–4.31%, respectively, and the total amount is 6.95–8.96% (Aksic, 2019).

Our research proved the presence of simple sugars in fruits of the high-bush blueberry at the level of 7.25% with the average minimum 6.11% and maximum 7.85%. The amount of glucose and fructose in fruits differed depending on the conditions of the year. The level of those substances ranged from 9.52% in 2017 at Reca variety, when the precipitation number was about 44 mm, to 5.65% in 2018 at Bluegold, with the precipitation over 125 mm in a fruits growing period. These facts prove the influence of the weather conditions to the accumulation and content of sugars (Table 2). The two- factor disperse analysis found the dependence of the content of these substances on varietal characteristics at 37.1%, and on weather and climatic factors - 34%.

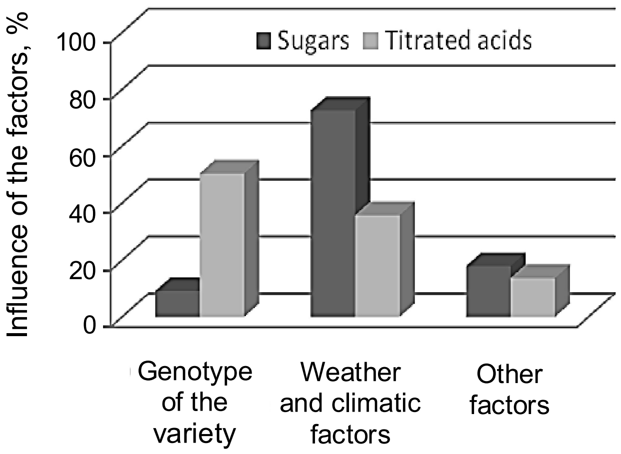

Figure 1. Particles of factors of significant influence that determine the content of sugars and titrated acids in the *V. corymbosum* L. fruits.

According to Estonian scientists, the content of titrated acids (TA) in V. *corymbosum* L. fruits is genetically fixed and to some extent adjusted by growing conditions (Starast et al., 2007), that is confirmed by our research data. Thus, the two-factor analysis of variance has shown that variety by 72.2% determines the acidity of berries and only by 35.8% it depends on weather and climatic factors (Fig. 1). Although other researchers emphasize the variability of titrated acids from growing conditions (Gerçekçioğlu & Esmek, 2005). In our research the highest amount of titrated acids was accumulated by Bluegold berries 2.20–2.60%), slightly less (in Elizabeth (1.23–2.65%) and Reca (1.23–1.68%), which corresponds with the data obtained by Belarusian researchers, the content of these substances was 1.10–2.05% (Zenkova & Pinchykova, 2019). The comparison of weather conditions with titrated acids of fruits showed the increase of the mentioned substances in 'Elizabeth' variety to 2.65% in the year of 2017, when the number of precipitation was low, with HTC - 0.3. In the other two varieties in the same year, there was also an increased amount of organic acids, in particular 2.60% in 'Bluegold' and 1.68 in 'Reca'. The most stable number of TA was in fruits of 'Bluegold' variety - with a coefficient variation 9.6%, whereas the most unstable with high dependence of the TA amount from the weather conditions was 'Elizabeth' variety (32.2%) (Table 2).

Organoleptic of berries depends on the number of titated acids and sugars (Ehlenfeldt et al., 1994). In our research we have established that high index of titrated acids and sugars correlation determines better taste of blueberry fruits. The sugar-acid index (SAI) of fruits of studied varieties was: 'Bluegold' - 3.9, 'Elizabeth' - 5.7, 'Reca' - 7.1 (Fig. 1). The fruits of the researched varieties were the sweetest according to the sugar-acid index in 2018, when the hydro-thermal coefficient was 1.1–1.2. In particular, SAI of vr 'Reca' fruits was 7.7, 'Elizabeth' - 7.4, 'Bluegold' - 4.1. Higher than in other years of research, the average daily temperatures in the period of fruit formation and ripening in 2019 (Table 1) negatively affected the taste of blueberries. The lowest taste coefficient (3.8) appeared in 'Bluegold' variety, 'Elizabeth' had it not much higher (4.7), the fruits of 'Reca' had it 5.5 (Fig. 2).

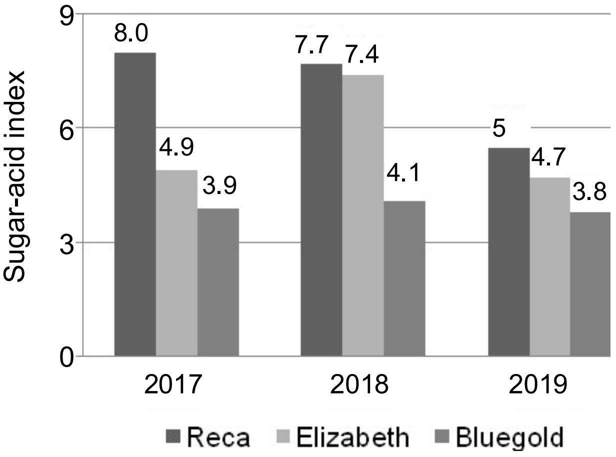

Figure 2. Sugar-acid index of the highbush blueberries (2017–2019).

Ascorbic acid relates refers to bioactive compounds that function in the human body as antioxidants (Celik et al., 2018). The amount of vitamin C in blueberry fruits was not the same in research of foreign scientists, for example data from the Baltics was 11.8 mg 100 g^{-1} (Rupasova et al., 2009), Belarus (60.5–72.2 mg 100 g^{-1}) (Zenkova & Pinchykova, 2019) from Northeast Turkey - 39 mg 100 g^{-1}. Portuguese scientists claim that the different content of vitamin C in blueberries is a varietal trait and can be adjusted to the conditions of the year and range from 6 to 162 mg 100 g^{-1} (Correia, et al., 2016). The amount of vitamin C in blueberry fruits grown in the Western Lisosteppe of Ukraine is little higher than in those from Baltic countries, but lower than in Belarusian ones, however, it is within the vitamin potency declared by Portuguese sciences. In our research the biggest vitamin C number was accumulated in fruits of the highbush blueberries in 2017, in particular, by 'Reca' - 20.00 mg 100 g^{-1}, 'Bluegold' - 22.5 and 'Elizabeth' 27.0 mg 100 g^{-1}. The fruits of such varieties as 'Elizabeth' and 'Bluegold' contained 20.17 and 20.90 mg 100 g^{-1} of the ascorbic acid respectively that was higher than the average content for the studied group of varieties (19.46 mg 100 g^{-1}). The content of vitamin C in the fruits of 'Bluegold' and 'Reca' varieties (7.0 and 12.3%, respectively) can be considered stable in terms of the coefficient of variation, and 'Elizabeth' (25.5%) can be considered unstable (Table 3).

The phytochemical composition of blueberries, according to many researchers, has a significant positive effect on the health of the human body (Stevenson & Scalzo, 2012). Turkish scientists claim that the content of polyphenols in highbush blueberries is a genetically fixed indicator, as much as the number of varieties grown under the same conditions differed significantly (Celik et al., 2018). The total polyphenol content in *V. corymbosum* L. fruits grown in the United States ranged from 48 (Ehlenfeldt & Prior, 2001) to 304 mg 100 g^{-1} by mass (Moyer et al., 2002) and was strictly dependent on the variety (Taruscio et al., 2004) was adjusted growing conditions and the state of their maturity (Zadernowski et al., 2005; Castrejón et al., 2008). The fruits of highbush blueberries, which were analyzed by Baltic scientists, accumulated these substances at the level of 228.63 mg 100 g^{-1} (Ozola & Dūma, 2020). Using two-factor analysis of variance, we confirmed the results of Turkish and American colleagues. We found a significant relationship on the amount of polyphenolic substances and anthocyanins on the genotype of the variety at the level of 98.4 and 92.9%, respectively. The content of chalcones was more adjusted by weather and climatic conditions of the growing year (65.5%), and the influence of other factors was higher by 2.55 than varietal peculiarities (Fig. 3).

Table 3. Content of the biologically active substances in the *V. corymbosum* L. fruits (2017–2019) ($n = 9$)

		2017	2018	2019	Average for 3 years	Coefficient variation, %
Ascorbic acid,	‘Reca’	20.00 ± 1.13	15.70 ± 0.55	16.20 ± 0.57	17.30 ± 2.41	12.3
mg 100 g^{-1} WM	‘Elizabeth’	27.00 ± 1.33	16.70 ± 0.40	16.80 ± 0.45	20.17 ± 5.82	25.5
	‘Bluegold’	22.50 ± 5.00	19.60 ± 0.62	20.60 ± 0.68	20.90 ± 1.65	7.0
SE					2.39	
x					19.46	
Polyphenolic	‘Reca’	375.33 ± 12.46	348.00 ± 18.11	350.67 ± 33.95	358.00 ± 25.04	6.2
substances,	‘Elizabeth’	297.33 ± 14.15	268.00 ± 12.45	276.00 ± 27.16	280.44 ± 22.23	7.0
mg 100 g^{-1} WM	‘Bluegold’	504.67 ± 33.95	492.67 ± 31.69	485.33 ± 40.17	494.22 ± 32.16^{a}	5.8
SE					61.58	
x					377.33	
Anthocyanins,	‘Reca’	39.00 ± 2.26	30.13 ± 1.41	35.33 ± 1.73	34.82 ± 4.86^{b}	11.8
mg 100 g^{-1} WM	‘Elizabeth’	55.00 ± 3.39	48.00 ± 0.45	44.10 ± 2.38	47.37 ± 3.60	6.7
	‘Bluegold’	71.00 ± 3.39	64.00 ± 5.66	68.20 ± 4.70	67.73 ± 5.32^{a}	6.9
SE					9.73	
x					50.50	
Chalcones,	‘Reca’ (c)	12.03 ± 0.62	11.80 ± 0.23	10.21 ± 0.24	11.35 ± 1.04	8.1
mg 100 g^{-1} WM	‘Elizabeth’	15.10 ± 1.24	10.00 ± 0.23	10.07 ± 0.07	11.72 ± 2.93	22.1
	‘Bluegold’	16.00 ± 1.13	112.60 ± 0.11	11.13 ± 0.17	13.24 ± 2.51	16.8
SE					1.42	
x					12.10	

Note: $^{a;\ b}$ is the notion of the indices which differ substantially from the average one (x) for the studied group of varieties ($p < 0.05$).

The amount of polyphenolic substances in highbush blueberry fruits of the studied cultivars on average in variety 'Bluegold' was 494 mg 100 g^{-1} with a minimum of 485 and a maximum of 504 mg 100 g^{-1} of raw mass, 358 - 'Reca' (min - 348, max - 376 mg 100 g^{-1}) and 280 - 'Elizabeth' (min - 268, max - 297 mg 100 g^{-1}). In the fruits of such varieties as 'Bluecrop' and 'Duke' from Romania, the amount of polyphenolic substances was 424.84–952.27 mg 100 g^{-1} (Bunea et al., 2011), which is significantly higher than our data. In fruits cultivated in Turkey, the amount of polyphenolic substances ranged from 111.60 to 438.9 mg 100 g^{-1} (Gündoğdu, 2016), this data is comparable with the data obtained by us and confirm the results regarding the significance of the genotype of the variety. The coefficient of variation from 5.8 ('Reca') to 7.0% ('Elizabeth') proves the low correlation among the amount of polyphenols in the fruits of the studied varieties and weather and climatic conditions of the year of cultivation (Table 3).

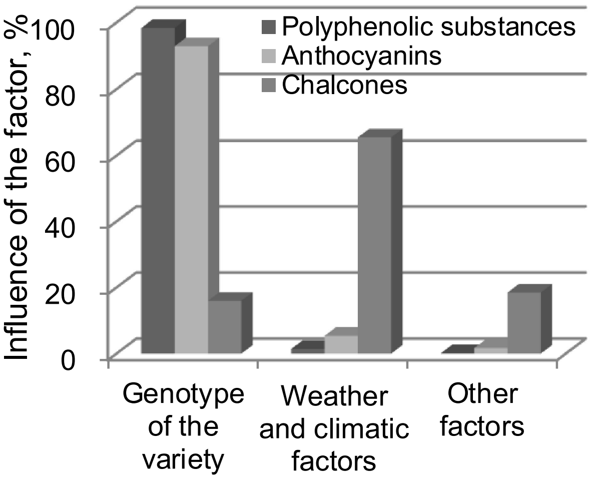

Figure 3. Effect of the factors established the amount of the polyphenolic substances, anthocyanins and chalcones in the highbush blueberries.

The content of anthocyanins in blueberry fruits according to Mazza & Miniati (1993) varies from 25 to 495 mg 100 g^{-1} of raw weight, and it depends on the variety, fruit size, stage of ripening, as well as climatic and weather conditions of the growing region. The fruits of highbush blueberry of the studied varieties contained from 35 to 68 mg 100 g^{-1} of anthocyanins. In our research, the biggest content of the mentioned substances was accumulated in fruits of Bluegold from 64 to 71 mg 100 g^{-1}, in Elizabeth berries there were a little less (44–55 mg 100 g^{-1}) and 'Reca' (30–39 mg 100 g^{-1}) had the least amount. The variability of this indicator over the years of research in all varieties was insignificant, the coefficients of variation was at the level and less than 11.8%. The most favourable year for the accumulation of anthocyanins in the fruits of all studied varieties of the highbush blueberries was 2017, when the HTC was 0.3–0.4 (Table 3).

The chalcone component in fruits of the studied highbush blueberry varieties was insignificant - 11–13 mg 100 g^{-1}. Their biggest content was accumulated in fruits of all studied varieties in 2017, in particular 'Reca' - 12 mg 100 g^{-1}, 'Elizabeth' - 12 and 'Bluegold' - 16 mg 100 g^{-1}; the average content for the years of research was 11, 12 and 13 mg 100 g^{-1}, respectively. The content of chalcones was stable in terms of variation in the variety 'Reca' (V = 9%), the average value of variability was in the varieties 'Bluegold' (V = 19%) and 'Elizabeth' (V = 25%) (Table 3).

By correlation analysis, we established the relationship between the berry mass and biochemical parameters of the fruits of highbush blueberries. It was determined, the increase in fruit mass of 'Reca' has a strong indirect effect on the content of dry matter, titrated acids and anthocyanins, (r = -0.822; -0.995 and -0.911 respectively) and on that of the soluble solids, sugars, vitamin C and polyphenols (r = -0.442; -0.424; -0.575

respectively). The amount of dry matter and soluble solids, which was contained in 'Reca' fruits are strongly positively correlated with most of the studied substances, the level of correlation is medium and strong. However, the correlation of DM with chalcones and sugar-acid index was not detected (*r* = 0.197 and 0.082). The content of glucose and fructose in the highbush blueberry fruits significantly depends on the amount of total sugars (*r* = 0.996), and also determines the content of polyphenols and anthocyanins (*r* = 0.998 and 0.830 respectively). The latest two mentioned substances had positive substantial direct correlation with sugars (*r* = 0.985 and 0.759 respectively). The correlation coefficient at a level of 0.948 proved the considerable connection of the titrated acids and anthocyanins contained in the 'Reca' fruits. A strong and medium correlation was established between the content of active substances of Reca, a correlation coefficient above 0.508; however, no correlation of the content of anthocyanins with the number of chalcones was detected (Table 4).

The content of monosaccharides (glucose and fructose) in berries of the 'Elizabeth' variety has a significant indirect correlation with fruit weight (the correlation coefficient is - 0.783). The correlation of medium strength was established between the weight of the fruit and the amount of anthocyanins (*r* = 0.555). There existed a close direct dependence between the last of the mentioned substances and soluble solids (*r* = 0.917). The positive connection between dry matter and SS, as well as the amount of sugars, titrated acids, ascorbic acid, polyphenols and chalcones was determined at the level of correlation coefficients *r* = 0.859; 0.999; 0.998; 0.976; 0.917 and 0.995, respectively. The soluble solids have a strong positive connection with most of the studied substances, medium – with glucose and fructose (*r* = 0.475) and indirect with a taste index (SAI) (*r* = 0.976). Titrated acids strongly correlated with the content of ascorbic acid, polyphenols and chalcones (*r* = 0.854, 0958 and 0.856 respectively) and had reverse correlation with the sugar-acid index (r = -0.959). SAI of 'Elizabeth' fruits indirectly, but significantly depends on the amount of vitamin C, polyphenolic substances, anthocyanins and chalcones (*r* = -0.966; -0.995; -0.808; -0.967 respectively). The biologically active substances of 'Elizabeth' berries were in a close direct correlation connection, with coefficients above 0.808 (Table 5).

The content of soluble solids, titrated acids, ascorbic acid and anthocyanins of the 'Bluegold' variety had an indirect strong correlation with the weight of fruits (the coefficients *r* = -0.727; -0.939; 0.764 and -0.918 respectively). The medium-strength connection was established between fruit weight and the amount of dry matter, glucose and fructose (*r* = -0.594; 0.692 respectively). The increase of the content of sugars, titrated acids and biologically active substances significantly depended on the increase of the amount of dry matter and soluble solids. There was no established dependence between the content of DM and SS with the amount of glucose and fructose (*r* = -0.170; 0.008 respectively). The correlation of glucose and fructose content with the sugar-acid index was positive (*r* = 0.729). However, the total amount of sugars had a reverse connection with the taste indicator (SAI) of the highbush blueberry variety 'Bluegold' (*r* = -0.754). At the same time a direct significant correlation was established with the content of the titrated acids, ascorbic acid, polyphenolic substances, anthocyanins, chalcones, the corresponding coefficient: *r* = 0.871; 0.987; 0.843; 0.898; 0.887.

Table 4. Dependence of the consumption quality indicators of *V. corymbosum* L. fruits ‘Reca’ variety

	Average mass	Dry matter	Soluble solids	Glucose and fructose	Sugar	Titrated acids	Sugar-acid index	Ascorbic acid	Poly-phenolic substances	Antho-cyanins	Chalcones
Average mass	1										
Dry matter	-0.822	1									
Soluble solids	-0.422	0.863	1								
Glucose and fructose	-0.527	0.917	0.993	1							
Tonal sugar	-0.424	0.864	0.998	0.993	1						
Titrated acids	-0.995	0.876	0.512	0.610	0.513	1					
Sugar-acid index	0.500	0.082	0.574	0.473	0.573	-0.410	1				
Ascorbic acid	-0.589	0.944	0.981	0.997	0.982	0.668	0.405	1			
Polyphenolic	-0.575	0.938	0.984	0.998	0.985	0.655	0.421	0.998	1		
Anthocyanins	-0.911	0.984	0.758	0.830	0.759	0.948	-0.099	0.870	0.861	1	
Chalcones	0.396	0.197	0.665	0.572	0.664	-0.301	0.993	0.508	0.524	0.017	1

Table 5. Correlation dependence of consumption quality indicators of *V. corymbosum* L. fruits ‘Elizabeth’ variety

	Average mass	Dry matter	Soluble solids	Glucose and fructose	Sugar	Titrated acids	Sugar-acid index	Ascorbic acid	Poly-phenolic substances	Antho-cyanins	Chalcones
Average mass	1										
Dry matter	-0.351	1									
Soluble solids	0.177	0.859	1								
Glucose and fructose	-0.783	0.858	0.475	1							
Tonal sugar	0.159	0.868	0.999	0.490	1						
Titrated acids	-0.323	0.998	0.874	0.842	0.883	1					
Sugar-acid index	0.043	-0.950	-0.976	-0.656	-0.980	-0.959	1				
Ascorbic acid	0.217	0.838	0.998	0.438	0.998	0.854	-0.966	1			
Polyphenolic	-0.040	0.950	0.976	0.653	0.980	0.958	-0.995	0.967	1		
Anthocyanins	0.555	0.584	0.917	0.084	0.910	0.608	-0.808	0.933	0.809	1	
Chalcones	0.213	0.840	0.995	0.441	0.998	0.856	-0.967	0.996	0.968	0.931	1

Table 6. Correlation dependence of indicators of consumption quality of *V. corymbosum* L. fruits ‘Bluegold’ variety

	Average mass	Dry matter	Soluble solids	Glucose and fructose	Sugar	Titrated acids	Sugar-acid index	Ascorbic acid	Poly-phenolic substances	Antho-cyanins	Chalcones
Average mass	1										
Dry matter	-0.594	1									
Soluble solids	-0.727	0.984	1								
Glucose and fructose	-0.692	-0.170	0.008	1							
Tonal sugar	-0.649	0.998	0.994	-0.101	1						
Titrated acids	-0.939	0.835	0.919	0.401	0.871	1					
Sugar-acid index	-0.011	-0.798	-0.678	0.729	-0.754	-0.335	1				
Ascorbic acid	-0.764	0.973	0.998	0.063	0.987	0.940	-0.637	1			
Polyphenolic	-0.138	0.879	0.780	-0.620	0.843	0.471	-0.989	0.744	1		
Anthocyanins	-0.918	0.864	0.940	0.348	0.898	0.998	-0.387	0.958	0.520	1	
Chalcones	-0.223	0.917	0.831	-0.549	0.887	0.546	-0.972	0.799	0.996	0.592	1

The titrated acids correlated quite strongly with the content of ascorbic acid and anthocyanins, the corresponding coefficients were 0.940 and 0.998. The amount of ascorbic acid significantly depends on the increase of the content of both polyphenols and anthocyanins and chalcones ($r = 0.744$; 0.958; 0.799, respectively). The total amount of polyphenolic substances had a significant direct connection with content of chalcones ($r = 0.996$) and the middle-strength of the anthocyanins ($r = 0.520$). The established correlation between the last two mentioned substances was on the same level ($r = 0.592$) (Table 6).

The research of different scientists (Mazza & Miniati, 1993; Prior et al., 1998; Starast et al., 2007; Correia et al., 2016) revealed the dependence of physical and biochemical quality indicators of highbush blueberries fruits, not only on the genotype of the variety, but also on weather conditions. Thus, the accumulation of sugars in berries was influenced considerably by the climatic conditions (Correia et al., 2016), and especially the light intensity, as fruit metabolism depends on photosynthesis (Mikulic-Petkovsek et al., 2014).

Using mathematical analysis, namely data approximation, we detected significant effect of one of the weather factors (precipitation during fruit development) on the content of dry matter and soluble solids in highbush blueberries (approximation coefficients - 0.565 and 0.586, respectively) (Fig. 4).

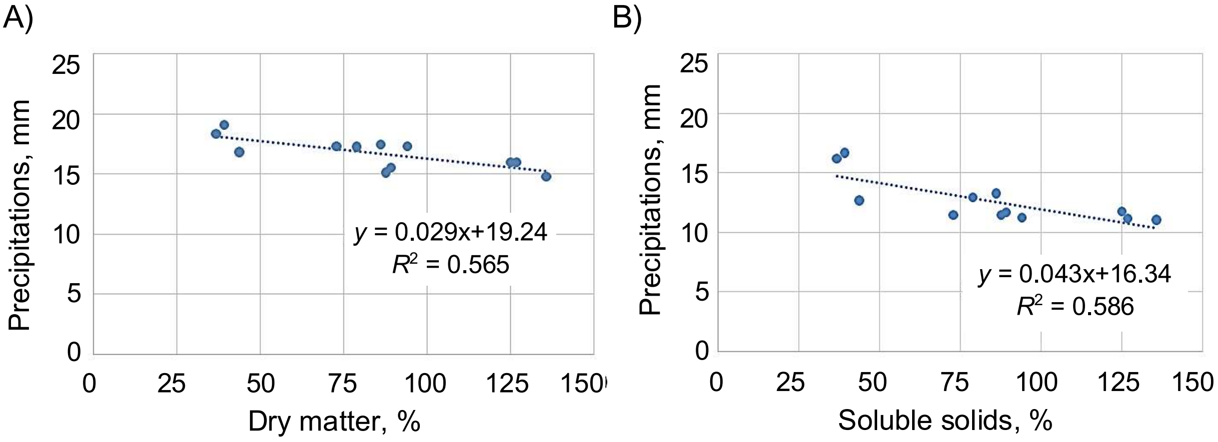

Figure 4. Correlation dependence of dry matter (A) and soluble solids (B) related to the precipitation amount.

CONCLUSIONS

It was established that the content variation of organic substances in highbush blueberry fruits of the studied varieties such as Reca, Bluegold, Elizabeth is an evidence of their genetic diversity from one side and the influence of weather conditions from the other side. Fruits of Bluegold variety according to coefficient of variation have a high stability of dry matter soluble solids, titrated acids and biochemically active substances, which is important for the breeding process.

We found a significant dependence of the content of titrated acids, polyphenols and anthocyanins on the genetic characteristics of the variety. A high correlation was found among the biochemical parameters of highbush blueberries. There was established a significant indirect effect of increasing fruit weight on the content of nutrients in fruits of Reca variety.

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