

Content of sugar, titrated acids and biologically active substances in blackberries grown in the forest-steppe of Ukraine

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For the first time was assessed the quality of fruits of four varieties of American blackberries and one of Swiss and Serbian selection, grown in the Forest-Steppe of Ukraine. The limits of variation of fruit mass, content of dry soluble substances, sugars, titrated acids, ascorbic acid, polyphenols and anthocyanins were established. The mass of blackberries varied within a minimum of 6.6 g of Asterina variety and a maximum of 8.2 g – Chester Thornless, the amount of soluble dry substances varied in the range of 9.4 (Cacanska Bestrna) – 15.1% (Heaven Can Wait), and sugars from 7.82 to 12.72% Kiowa and Chester Thornless varieties. The highest content of bioactive substances, in particular ascorbic acid, was accumulated by fruits of Kiowa and Heaven Can Wait varieties, the last of these, among the studied varieties, had the highest amount of polyphenolic substances 845 mg.100 g⁻¹, variability of which was very low, corresponding to 8%. According to the look of the fruit, and in particular its mass, as well as taste, ratio of sugar to acid, there were highlighted varieties that have the prospect of widespread cultivation in industrial plantations, such as Chester Thornless and Chief Joseph. In order to conduct the selection process for the creation of varieties, whose fruits will have excellent marketable, consumer and preventive qualities (apart from the above-mentioned varieties) should be also involved with others, namely Heaven Can Wait and Kiowa.

Keywords: *Rubus fruticosus* L., mass, soluble solids, polyphenolic substances, anthocyanins

1 Introduction

Blackberries are a popular fruit mainly because of their excellent taste and aroma but also because of the rich content of biologically active compounds such as anthocyanins, flavonoids, etc. (Milivojević et al., 2011; Garcia-Seco et al., 2015; Kolniak-Ostek et al., 2015; Žlabur et al., 2021). The demand for blackberries (*Rubus* spp.) has significantly increased in recent years, as its fruits contain large amounts of phenolic compounds and vitamin C, which prevent the development of degenerative diseases (Ali et al., 2011). Due to their valuable nutritional composition and phytochemical content, they are endowed with numerous functional properties, ranging from antioxidant, antimicrobial, anti-inflammatory and antitumor activity (Vergara et al., 2016; Ponder et al., 2017; Zorzi et al., 2020). In addition to their main potent antioxidant activity, anthocyanins have a wide range of

biological functions, mainly associated with intervention at all stages of cancer development, including initiation, advancement, progression, invasion, and metastasis (Kolniak-Ostek et al., 2015; Kiss & Piwowarski, 2018; Parmenter et al., 2020; Briguglio et al., 2020; Cháirez-Ramírez et al., 2021). In addition to these compounds, natural pigments, mainly anthocyanins, are attractive dyes for the production of dairy products, jellies and fruit syrups (Acosta-Montoya et al., 2010).

Blackberries (*Rubus fruticosus* L.) are grown all over the world but the most optimal for its cultivation is a climate with mild winters and long mild summers. The main regions of blackberry production are North America, Europe, Asia, South and Central America, and Africa (Lykins et al., 2021). In Ukraine, the area of this crop, created by varieties of foreign selection, is 300 ha. The basis for expanding the range of plant foods with

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high biological activity is the selection of varieties, including blackberries (*Rubus fruticosus* L.), which are able to form fruits weighing at least 8 g, excellent taste at sugar-acid index 10 and high content of biologically active substances. Unfortunately, the potential for the formation of commodity and consumer indicators of blackberry fruit quality of introduced varieties in the conditions of cultivation of the Forest-Steppe of Ukraine has not been studied. Therefore, the main task of our work was to establish the potential for the formation of commodity and biochemical indicators of quality of blackberries varieties of foreign selection in the Forest-Steppe of Ukraine. Knowledge of the influence of the weather of the Forest-Steppe of Ukraine on the formation of indicators of blackberry fruit quality will allow domestic breeders to correctly select parent pairs for the selection process to create new high-yielding and biologically valuable varieties.

2 Material and methods

Blackberries for the study of biometric and biochemical quality indicators were selected from the research areas of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine (IH NAAS of Ukraine). The location of natural-climatic zone – Forest-Steppe of Ukraine, altitude 187 m (50° 27' 16" N, 30° 131' 25" W), distance to Kyiv 4 km. The fruits for research were collected in 10 days after the advent of the first ripe.

The climate of the region of the Institute of Horticulture of NAAS of Ukraine is temperate-continental. According to long-term data, the average annual air temperature is: +9.7 °C, the maximum: +37 °C and the minimum: -25 °C, and the amount of precipitation: 497 mm.

The weather data for years of research were obtained at the Vantage Pro2 Plus weather station, located on the research area of IH NAAS of Ukraine.

Blackberry plantations were created in 2017, the distance between plants in a row is one meter, between rows – 2 m. The soil of the research area is middle loam alfisol podzolic soil.

The system of keeping the soil in a row – mulching with sawdust, in between rows – turving, the experiment is laid with irrigation, the care for plantations is recommended for the Forest-Steppe zone of Ukraine. The objects of research were the fruits of introduced varieties (Table 1).

Analytical research was performed in the laboratory of postharvest quality of fruit and berry products of the IH NAAS of Ukraine. Blackberry fruits, with the shape and color characteristic of the variety, were selected at the stage of consumer ripeness, the mass of the sample met the requirements of the method "Methodology for assessing the quality of fruit and berry products" (Kondratenko et al., 2008). The average mass was determined by weighing 30 berries on laboratory scales with its precision to the first sign. Crushed analytical samples of fruits to determine the content of nutrients and biologically active substances were prepared using a laboratory homogenizer. The sample was weighed on analytical scales Axis AD 200R with its precision to the second sign.

2.1 Soluble solids

Soluble solids were determined using a portable refractometer ATAGO PAL-1 (Japan). A drop of crushed fruit juice was squeezed through the tissue on the refractometer glass and the temperature error was taken into account when recording the data. Data were expressed as a percentage.

2.2 Titrated acids

To a 250 ml volumetric flask by rinsing with hot distilled water of 150 ml volume, was transferred the crushed sample in the amount of 25 g. The flask was kept in a water bath for 30 minutes at 80 °C and cooled. The contents of the flask were made up to the mark with distilled water and filtered through a filter into a 250 ml conical flask. Pipette 20 ml of the extract into a 250 ml conical flask, added 3–4 drops of phenolphthalein and titrate with 0.1 N sodium hydroxide until a pink color corresponding to pH 7.0 appears. Three parallel measurements were performed and the average value of the indicator was

Table 1 List of varieties whose fruits were studied for physico-chemical quality indicators

Varieties	Ripening group	Country of origin
Kiowa	early	USA
Heaven Can Wait	early	USA
Asterina	early	Switzerland
Cacanska Bestrna	middle	Serbia
Triple Crown	late	USA
Chester Thornless	late	USA
Chief Joseph	remontant	USA

found. The content of titrated acids in the sample was calculated by the formula, using a titer of 0.1 N sodium hydroxide and a conversion factor for malic acid.

2.3 Ascorbic acid

For the extraction of ascorbic acid, the sample was ground in a porcelain mortar with the addition of broken glass and a mixture of 2% oxalic and 1% hydrochloric acid (80 + 20, vol + vol), transferred to a volumetric flask with a capacity of 100 ml. The contents of the flask were adjusted to the mark with a mixture of 2% oxalic and 1% hydrochloric acids (80 + 20, vol + vol) and filtered. The resulting extract was titrated with a solution of 2,6-dichlorophenolindophenol (Tillmans paint). The content of ascorbic acid in the sample was calculated by the formula, using the titer of Tillmans paint (Kondratenko et al., 2008).

2.4 Sugars

Extraction of sugars from apple was performed with hot distilled water. The resulting extract was purified from proteins and pigments by precipitation of the latter with lead acetic acid. Sucrose was hydrolyzed to glucose and fructose by heating in the presence of 10% hydrochloric acid. The hydrolysis products were oxidized with Fehling's solution. The optical density of the obtained solutions was determined on a ULAB 102UV spectrophotometer at a wavelength of 640 nm. The sugar content in the sample was calculated by the formula using the calibration graph. To construct a calibration graph of the dependence of optical density (units of optical density) on the concentration of glucose (mg.ml⁻¹) used standard glucose solutions with different concentrations.

2.5 The sugar-acid index (SAI)

The sugar-acid index (SAI) was defined as the ratio of total sugars to the amount of titrated acids.

2.6 Polyphenolic compounds

To extract the polyphenols, the sample was ground in a porcelain mortar with a small amount of ethyl alcohol and filtered under vacuum on a Büchner funnel through a 'blue ribbon' filter paper into a Bunsen flask. The filter residue is washed with small amounts of ethyl alcohol until the sample is completely discolored. The volume of alcohol used (ml) was recorded. 7.9 ml of distilled water, 0.1 ml of extract, 1 ml of Folin-Denis reagent were poured into a test tube, stirred and after 3 minutes 1 ml of saturated sodium carbonate solution was added and stirred again. The optical density of the tubes was recorded on a ULAB 102UV spectrophotometer at a wavelength of 640 nm for an hour. As a control, the

mixture prepared as follows was used: 8 ml of distilled water, 1 ml of Folin-Denis reagent were poured into a test tube, stirred, 1 ml of saturated sodium carbonate solution was added after 3 minutes and stirred again. Conducted at least 3 parallel measurements and found the average value of the optical density (Kondratenko et al., 2008). The content of polyphenols in the sample was calculated by the formula, using the indicators of the calibration graph. To build a calibration graph of the dependence of optical density (units of optical density) on the concentration of chlorogenic acid (mg.ml⁻¹) used standard solutions of chlorogenic acid with different concentrations.

2.7 Anthocyanins

The content of anthocyanins was determined by the pH difference method (Giusti & Wrolstad, 2001), in which the extracts were dissolved (1 : 150) in two buffer systems: potassium chloride pH 1.0 (0.025 M) and sodium acetate pH 4.5 (0.4 M). Absorption of extracts was measured on a ULAB 102UV spectrophotometer at a wavelength of 520 and 700 nm. The content of anthocyanins was calculated on cyanidin-3-glucoside (molar adsorption – 29,600, molecular weight – 449.2). The results were expressed in mg of anthocyanin per 100 g of fresh fruit.

2.8 Flavonoids

Determination of flavonoid content was performed by spectrophotometric method (Vronska, 2018), which is based on measuring the absorption of a complex of flavonoids with aluminum chloride in ethanol environment (70%). Absorption of extracts was measured on a ULAB 102UV spectrophotometer at a wavelength of 410 nm. Quantitative content was converted into rutin. Simultaneously was measured the absorption of the standard rutin solution (comparison solution). The results were expressed in mg of anthocyanin per 100 g of fresh fruit.

2.9 Statistical analysis

Statistical data processing was performed using the program STATISTICA 13/1 (StatSoft, Inc., USA). The results are presented as means with their standard errors as mean ± standard error ($\bar{x} \pm SE$). Differences between replicates, as well as relative to the average intervarietal value were determined using ANOVA. The research results are presented at the level of reliability at $P < 0.05$. Two-factor analysis of variance of the significance of the genotype of the variety and weather and climatic factors on the content of nutrients and biologically active substances in highbush blueberries and correlation analysis was performed in Excel, tab Data Analysis.

3 Results and discussion

The average daily temperatures in the period from the beginning of the vegetation season of early blackberries to harvest in 2020 were 1.6, and late varieties 1.3 °C higher than in 2021 and 1.3 and 1.0 °C than the average for the last 10 years. There was also more precipitation in 2020 than in 2021, but this amount differed little from the average for the last 10 years. There were 62 mm more of them in the period from the beginning of the growing season to the harvest of fruits of early varieties and 70 mm more – of late varieties in 2020 than in 2021. Under such weather conditions, blackberry fruits had a fruit mass: in 2020 – 6.7–9.0 g, and in 2021 – 6.5–8.0 g, average 7.8 and 7.2 g, respectively. The most dependent on the conditions of the year of cultivation was the mass of blackberries Asterina, a coefficient of variation of 20%, significant stability of this indicator was a variety of Triple Crown, a coefficient of variation of 8%. Blackberries grown in central Slovenia weighed 5.87–8.38 g (Mikulic-Petkovsek et al., 2012), and in Arkansas USA the mass of blackberries ranged from 6.0 to 14.3 g (Threlfall et al., 2016). Comparing the data, it was found that the mass of blackberry varieties grown in Ukraine corresponds to the data obtained by Slovenian colleagues, but the berries of some varieties studied in the United States were larger. Variety Cacanska Bestrna in the conditions of Slovenia, depending on the date of collection gained fruit mass of 5.95–10.08 g (Mikulic-Petkovsek et al., 2012), and in Ukraine, depending on the year of cultivation – 7.7–6.8 g (Table 2), which corresponds to the mass that was in the fruit from Slovenia during the sixth harvest (Mikulic-Petkovsek et al., 2012).

The quality characteristics of blackberry fruits can be significantly influenced by the presence of nutrients which together with sources of biologically active

substances, vitamins, and secondary metabolites are necessary to ensure proper nutrition (Carl, 1999; Callahan, 2003).

The climate of the Federal University of Lavra (UFPA), Lavras, Minas Gerais, is tropical, according to Keppen's classification, characterized by dry winters, rainy summers and mild temperatures (Guedes et al., 2013), which differs significantly from the climate of Ukraine. Under such conditions, the content of dry soluble substances in blackberries grown in this region was 4.87–7.95, the average was 6.97% (Guedes et al., 2013). A slightly wider range of contents (6.6 to 11.5% (Brix °)) of these substances was found in blackberries in the region of the Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig) with an average annual air temperature of about 19.2 °C and an average relative humidity of 75% (Caproni et al., 2016). Their number in the fruits of introduced varieties grown in Ukraine was 10.1–15.1%, the average value was 11.4% (Table 2), which is much more than in berries from the tropical region of Minas Gerais, and the average value is at the level of the maximum, which was obtained by analyzing the berries of varieties studied in Brazil. Heaven Can Wait fruits gained the most soluble solids: 15.4% in 2020 and 14.9% in 2021, coefficient of variation 3% (Table 2).

The main sugars of blackberries, according to Mikulic-Petkovsek et al. (2012) were glucose and fructose. According to their studies, the ratio of these sugars was 1 : 1 and was approximately 90–96% of the total amount of sugars, the rest was sucrose (0.1–3.4 g.kg⁻¹) (Mikulic-Petkovsek et al., 2012). Similar values of sugar content were reported by Veberic et al. (2014). The amount of fructose and glucose in blackberries grown in the Forest-Steppe zone of Ukraine was 6.81–9.32% in 2020 and 4.96–10.47% in 2021, the average – 7.48%,

Table 2 Mass and content of soluble solids in blackberries

Varieties	Mass (g)				Soluble solids (%)			
	2020	2021	average	CV (%)	2020	2021	average	CV (%)
Kiowa	7.8	8.0	7.9 ± 0.9	14	13.6	11.3	12.5 ± 1.1	11
Heaven Can Wait	7.9	6.7	7.3 ± 1.1	19	15.4	14.9	15.1 ± 0.3 ^b	3
Asterina	6.7	6.5	6.6 ± 1.1	20	10.5	11.2	10.9 ± 0.4	4
Cacanska Bestrna	7.7	6.8	7.3 ± 1.1	19	10.1	8.6	9.4 ± 0.7 ^a	10
Triple Crown	7.1	7.2	7.2 ± 0.5	8	11.4	9.6	10.5 ± 0.8	10
Chester Thornless	8.4	8.0	8.2 ± 1.0	15	10.6	12.9	11.7 ± 1.1	12
Chief Joseph	9.0	7.2	8.1 ± 1.2	19	10.4	9.4	9.9 ± 0.6 ^a	7
Average	7.8	7.2	7.5 ± 0.7	12	11.7	11.1	11.4 ± 0.3	3
max	9.0	8.0	8.2 ± 1.0		15.4	14.9	9.4 ± 0.7	
min	6.7	6.5	6.6 ± 1.1		10.1	9.4	15.1 ± 0.3	

which was 66% of the total amount of sugars (Table 3), which is 24–30% less than the indicators obtained by colleagues from Slovenia. According to Milivojević et al. (2011) different values of sugar content are a reflection of different environmental conditions, location of sites and technological measures in blackberry production. Heaven Can Wait of the studied varieties accumulated the biggest amount of glucose and sucrose (8.06% in 2020 and 10.47% in 2021, coefficient of variation 14.5%) which is 99% in 2020 and 94% in 2021 of the total amount of sugars. High stability of fructose and glucose content among the studied varieties was distinguished by varieties Asterina and Chester Thornless, coefficients of variation 7.2 and 5.4%, respectively (Table 3). Their percentage of total sugars is 71% in Asterina and 70% in Chester Thornless.

The amount of titrated acids in the fruits of blackberries of our studied varieties in 2020 varied between a minimum of 0.80% and a maximum of 1.22% and in 2021 from 0.63 to 1.03%, respectively. The high dependence of titrated acids in the fruits of some varieties of blackberries on the conditions of the year of cultivation is evidenced by high

coefficients of variation, in particular 26.6% - Heaven Can Wait and 21.7% - Asterina (Table 3). The content of titrated acids in fruits grown in Brazil was 0.68–2.6% (Guedes et al., 2013) in a slightly smaller range (from 0.73 to 1.55%) their amount was in berries from Serbia (Miodrag et al., 2018) approximately the same number (0.7–1.4%) had fruits studied in Arkansas, USA (Threlfall et al., 2016), which is almost identical to our data. It is important to note that the fruits of all studied varieties gained titrated acids by 0.15% more in 2020 than in 2021.

Organic acids, sugar and their ratios together with various secondary and aromatic compounds play an important role in the taste and organoleptic properties of fruits (Mikulic-Petkovsek et al., 2012). In our studies, the ratio of sugars to titrated acids of blackberry fruits ranged from the lowest 7.3 (Heaven Can Waite) and the highest – 14.0 (Chester Thornless). The last of these varieties had the highest ratio of sugars to titrated acids (15.4) in 2021, the lowest this year it was in the variety Cacanska bestrna (5.9) (Table 3). Similar indicators of sugar-acid index (6.2–16.5) had blackberry fruits studied by American scientists (Threlfall et al., 2016).

Table 3 The content of organic substances in blackberries (%)

Varieties	Fructose + glucose				Sugars			
	2020	2021	average	CV (%)	2020	2021	average	CV (%)
Kiowa	8.27	5.21	6.74 ±1.35	25.0	9.37	6.21	7.82 ±1.38	22.0
Heaven Can Wait	8.06	10.47	9.27 ±1.08	14.5	8.60	11.33	9.97 ±1.22	15.3
Asterina	8.06	7.11	7.59 ±0.44	7.2	11.08	10.30	10.69 ±0.40 ^b	4.7
Cacanska Bestrna	6.81	4.96	5.89 ±0.84	17.9	9.55	6.13	7.84 ±1.56	24.9
Triple Crown	9.32	6.08	7.70 ±1.44	23.4	13.30	7.43	10.36 ±2.59	31.2
Chester Thornless	9.12	8.74	8.93 ±0.38	5.4	13.59	11.84	12.72 ±0.81 ^b	8.0
Chief Joseph	6.16	6.28	6.22 ±0.40	8.1	8.55	8.83	8.69 ±0.34	5.0
Average	7.97	7.03	7.48 ±0.47	7.9	10.58	8.87	9.73 ±0.78	10.0
max	9.32	10.47	9.27 ±1.08		11.08	11.84	12.72 ±0.81	
min	6.81	4.96	5.89 ±0.84		8.55	6.13	7.82 ±1.38	

Varieties	Titrated acids				Sugar-acid index (SAI)	
	2020	2021	average	CV (%)	2020	2021
Kiowa	1.03	0.94	0.99 ±0.06	7.9	9.1	6.6
Heaven Can Wait	1.18	0.77	0.98 ±0.21	26.6	7.3	14.7
Asterina	1.22	0.82	1.02 ±0.18	21.7	9.1	12.5
Cacanska Bestrna	1.00	1.03	1.02 ±0.06	7.9	9.6	5.9
Triple Crown	0.97	0.98	0.97 ±0.06	7.5	13.7	7.6
Chester Thornless	0.97	0.77	0.87 ±0.1	16.4	14.0	15.4
Chief Joseph	0.80	0.63	0.71 ±0.11	18.9	10.7	14.1
Average	1.02	0.85	0.94 ±0.09	11.4	10.5	10.4
max	1.22	1.03	1.02 ±0.18		14.0	14.7
min	0.80	0.63	0.71 ±0.11		7.3	5.9

The amount of vitamin C in blackberries grown in Brazil ranged from 42.69 to 55.7, an average of 46.7 mg.100 g⁻¹ (Guedes et al., 2013), in the fruits studied in the United States it was 7.46–11.43 (Threlfall et al., 2016). A slightly different range of vitamin C content (from 6.45 to 21.36 mg.100 g⁻¹) in blackberries has been established by Serbian researchers (Jazić et al., 2018). They proved that Cacanska Bestrna blackberries grown in Bosnia and Herzegovina are able to gain vitamin C – 10.23–11.25 mg.100 g⁻¹, slightly higher limits of its content were established by our studies (11.1–19.0 mg.100 g⁻¹ WM (Table 4).

The results of studies by Croge et al. (2019) showed that the content of ascorbic acid in blackberries averages 24.5 mg.100 g⁻¹, i.e. 17 percent or less of the daily recommended human intake. In our studies, this figure was lower and was 16.4 mg 100 g⁻¹ WM (Table 4), which corresponds to the level of maximum vitamin C in blackberries from northern Greece (12.3–16.4 mg.100 g⁻¹) (Pantelidis et al., 2007), this is 11% of the daily norm of consumption. Therefore, blackberry fruits (Souza et al., 2014) along with apple and blueberry fruits,

which contain 2.5 and 19.5 mg.100 g⁻¹ of ascorbic acid, respectively (Shevchuk et al., 2021a; Shevchuk et al., 2021b) cannot be recommended as the only dietary source of this vitamin.

Fruits of all the varieties we studied gained more vitamin C in 2020, when the average daily temperature and precipitation of the period of growth and ripening of blackberries was higher than in 2021. Significantly more vitamin C than other studied varieties in both years of research had fruits of the Kiowa variety. (20.5) and Heaven Can Wait (23.4 mg.100 g⁻¹), the variability of this indicator in these varieties was the lowest, the coefficients of variation were 8.6 and 7.3%, respectively (Table 4).

Blackberry fruits that are grown in the temperate climate of Ukraine, contained polyphenolic substances from 528 to 845 mg.100 g⁻¹. Their values, depending on the variety ranged from 546 to 824 mg.100 g⁻¹ in 2020, when the period of fruit growth and development was warmer and wetter than in 2021, the content of polyphenolic were in the range 469–705 mg.100 g⁻¹ (Table 4). Our received data on the content of polyphenols in fruits blackberries

Table 4 The content of biologically active substances in blackberries (mg.100 g⁻¹ WM)

Varieties	Vitamin C				Polyphenols			
	2020	2021	average	CV (%)	2020	2021	average	CV (%)
Kiowa	21.9	19.1	20.5 ±1.4 ^b	8.6	824	705	765 ±67	11
Heaven Can Wait	22.0	24.8	23.4 ±1.4 ^b	7.3	798	892	845 ±57 ^b	8
Asterina	19.2	10.9	15.0 ±3.7	31.0	546	509	528 ±37 ^a	9
Cacanska Bestrna	19.0	11.1	15.0 ±3.5	29.3	602	469	535 ±65 ^a	15
Triple Crown	14.7	9.1	11.9 ±2.5	26.4	648	549	599 ±56	12
Chester Thornless	16.5	9.1	12.8 ±3.3	31.9	682	602	642 ±49	10
Chief Joseph	18.7	13.4	16.0 ±2.4	18.5	791	607	699 ±84	15
Average	18.9	13.9	16.4 ±2.2	16.9	699	619	659 ±39	7
max	22.0	24.8	23.4 ±1.4		824	705	845 ±57	
min	14.7	9.1	11.9 ±2.5		546	469	528 ±37	

Varieties	Flavonoids				Anthocyanins			
	2020	2021	average	CV (%)	2020	2021	average	CV (%)
Kiowa	87.1	205.5	137.8 ±51.1	46.4	72.6	101.0	86.8 ±12.2	17.5
Heaven Can Wait	121.7	109.3	116.4 ±9.5	10.2	65.8	65.3	65.5 ±4.0 ^a	7.5
Asterina	107.6	124.7	114.9 ±10.5	11.4	71.0	125.4	98.2 ±24.2	30.7
Cacanska Bestrna	91.4	64.2	79.7 ±12.0	18.8	44.6	67.8	56.2 ±10.5	23.4
Triple Crown	77.6	142.5	105.4 ±28.6	33.9	74.5	78.6	76.6 ±3.3	5.4
Chester Thornless	118.7	138.9	127.4 ±12.9	12.6	90.5	91.2	90.8 ±2.8	3.9
Chief Joseph	66.9	130.5	94.1 ±27.7	36.8	53.3	89.6	71.5 ±16.4	28.6
Average	95.8	130.8	110.8 ±15.3	17.2	67.6	88.3	77.9 ±9.4	15.0
max	121.7	142.5	137.8 ±51.1		90.5	125.4	98.2 ±24.2	
min	66.9	64.2	79.7 ±12.0		44.6	65.3	56.2 ±10.5	

were higher than in berries from Oregon state of USA and Brazil, where the climate is much warmer than in Ukraine. Cechinel-Filho (2012) reports that from air temperature of the period of growth and formation of blackberry fruits, significantly depends on the intensity of synthesis of polyphenolic compounds. However, a direct positive correlation was found between the duration of cool day and their polyphenol content. Thus, the temperate climate of the Forest-Steppe of Ukraine with more severe winters than in Oregon in the USA and Brazil had a positive effect on the synthesis of polyphenols in the fruits of the studied blackberry varieties.

The amount of flavonoids in blackberries that we studied varied both by years of research and varieties, from 95.8 in 2020 to 130.8 mg.100 g⁻¹ in 2021, an average of 110.8 mg.100 g⁻¹. The content of flavonoids in blackberry fruits did not differ much which was studied by Brazilian colleagues – 102.7–136.07 mg.100 g⁻¹ (Guedes et al., 2014).

Most of the varieties we studied had Kiowa fruit 2021 (205.5 mg.100 g⁻¹), in 2020 this variety accumulated much less (87.1 mg.100 g⁻¹), the variability of the content was 46.4%. The most stable according to the content of flavonoids by years of research were varieties of Heaven Can Wait, Asterina and Chester Thornless, coefficients of variation 10.2; 11.4 and 12.6%, according with (Table 4).

Anthocyanins are considered as important antioxidants in berries (Moyer et al., 2002). Their content in blackberries grown in the southeastern United States was 292.2–446.9 mg.100 g⁻¹ (Cho et al., 2005), in Brazil they were 58.6 mg.100 g⁻¹ (Souza et al., 2014), and in fruits that were studied in Japan – 75–110 mg.100 g⁻¹ (Toshima et al., 2021). The amount of anthocyanins in blackberry fruits that we studied ranged both by years of research and by variety. In 2020, the content of these substances was 44.6–90.5, average 67.6 mg.100 g⁻¹, and in 2021 65.3–125.5, average 88.3 mg.100 g⁻¹ (Table 4). From the studied varieties, anthocyanins more than 100.0 mg.100 g⁻¹ were accumulated in varieties of Kiowa (101.0) and Asterina (125.4 mg.100 g⁻¹) in 2021. The most variable was the content of these substances in the fruits of the last of these varieties, the coefficient of variation of 30.7% (Table 4). Our data are comparable to those obtained by Brazilian and Japanese scientists, but the number of anthocyanins in blackberries studied by American scientists was much higher.

4 Conclusions

As a result of research, it was established that the fruits of blackberries of American, Swiss, and Serbian selection in the conditions of cultivation in the Forest-Steppe of Ukraine are able to form fruits of high marketable and

consumer qualities. Chester Thornless and Chief Joseph are the most suitable for industrial cultivation in terms of a set of physical and consumer quality indicators. Heaven Can Wait and Kiowa varieties should be used as parental forms in the selection work to create varieties with higher content of bioactive substances.

References

- Acosta-Montoya, Ó., Vaillant, F., Cozzano, S., Mertz, C., Pérez, A. M., & Castro, M. V. (2010). Phenolic content and antioxidant capacity of tropical highland blackberry (*Rubus adenotrichus* Schltdl.) during three edible maturity stages. *Food Chemistry*, 119(4), 1497–1501. <https://doi.org/10.1016/J.FOODCHEM.2009.09.032>
- Ali, L., Svensson, B., Alsanius, B. W., & Olsson, M. E. (2011). Late season harvest and storage of *Rubus* berries – Major antioxidant and sugar levels. *Scientia Horticulturae*, 129(3), 376–381. <https://doi.org/10.1016/J.SCIEN.2011.03.047>
- Briguglio, G., Costa, C., Pollicino, M., Giambò, F., Catania, S., & Fenga, C. (2020). Polyphenols in cancer prevention: New insights (Review). *International Journal of Functional Nutrition*, 1(2), 1–1. <https://doi.org/10.3892/IJFN.2020.9>
- Callahan, A. M. (2003). Breeding for fruit quality. *Acta Horticulturae*, 622, 295–302. <https://doi.org/10.17660/ACTAHORTIC.2003.622.27>
- Caproni, C. M., Curi, P. N., Moura, P. H. A., Pio, R., Gonçalves, E. D., & Pasqual, M. (2016). Blackberry and redberry production in crop and intercrop in Pouso Alegre, southern Minas Gerais, Brazil. *Ciência Rural*, 46(10), 1723–1728. <https://doi.org/10.1590/0103-8478CR20150623>
- Carl, E. S. (1999). Preharvest factors affecting postharvest texture. *Postharvest Biology and Technology*, 15(3), 249–254. [https://doi.org/10.1016/S0925-5214\(98\)00098-2](https://doi.org/10.1016/S0925-5214(98)00098-2)
- Cechinel-Filho, V. (2012). *Plant bioactives and drug discovery: principles, practice, and perspectives*. John Wiley & Sons.
- Cháirez-Ramírez, M. H., de la Cruz-López, K. G., & García-Carrancá, A. (2021). Polyphenols as antitumor agents targeting key players in cancer-driving signaling pathways. *Frontiers in Pharmacology*, 12, 710304. <https://doi.org/10.3389/FPHAR.2021.710304>
- Cho, M. J., Howard, L. R., Prior, R. L., & Clark, J. R. (2005). Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture*, 85, 2149–2158. <https://doi.org/10.1002/JSFA.2209>
- Croge, C. P., Cuquel, F. L., Pintro, P. T. M., Biasi, L. A., & de Bona, C. M. (2019). Antioxidant Capacity and Polyphenolic Compounds of Blackberries Produced in Different Climates. *HortScience*, 54(12), 2209–2213. <https://doi.org/10.21273/HORTSCI14377-19>
- García-Seco, D., Zhang, Y., Gutierrez-Mañero, F. J., Martín, C., & Ramos-Solano, B. (2015). Application of *Pseudomonas fluorescens* to Blackberry under Field Conditions Improves Fruit Quality by Modifying Flavonoid Metabolism. *Plos One*, 10(11), e0142639. <https://doi.org/10.1371/JOURNAL.PONE.0142639>
- Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV-visible

- spectroscopy. *Current protocols in food analytical chemistry*, (1), F1–2. <https://doi.org/10.1002/0471142913.FAF0102S00>
- Guedes, M. N. S., de Abreu, C. M. P., Maro, L. A. C., Pio, R., de Abreu, J. R., & de Oliveira, J. O. (2013). Chemical characterization and mineral levels in the fruits of blackberry cultivars grown in a tropical climate at an elevation. *Acta Scientiarum Agronomy*, 35(2), 191–196. <https://doi.org/10.4025/actasciagron.v35i2.16630>
- Guedes, M. N. S., Maro, L. A. C., Abreu, C. M. P., Pio, R. & Patto, L. S. (2014). Chemical composition, bioactive compounds and genetic dissimilarity among cultivars blackberry (*Rubus* spp.) cultivated in South Minas Gerais. *Revista Brasileira de Fruticultura*, 36, 206–213. <https://doi.org/10.1590/0100-2945-230/13>
- Jazić, M. R., Vulić, J. J., Kukrić, Z. Z., Topalić-Trivunović, L. N., & Savić, A. v. (2018). Chemical composition, biological potentials and antimicrobial activity of wild and cultivated blackberries. *Acta Periodica Technologica*, (49), 65–79. <https://doi.org/10.2298/APT1849065J>
- Kiss, A. K., & Piwowarski, J. P. (2018). Ellagitannins, Gallotannins and their Metabolites-The Contribution to the Anti-Inflammatory Effect of Food Products and Medicinal Plants. *Current Medicinal Chemistry*, 25(37), 4946–4967.
- Kolnaci-Ostek, J., Kucharska, A. Z., Sokół-Łętowska, A., & Fecka, I. (2015). Characterization of Phenolic Compounds of Thorny and Thornless Blackberries. *Journal of Agricultural and Food Chemistry*, 63(11), 3012–3021. <https://doi.org/10.1021/JF5039794>
- Kondratenko, P. V., Shevchuk, L. M., Levchuk, L. M. (2008). *Methods for assessing the quality of fruit and berry products*. Kyiv: SPD Zhyteliev S.l., 79.
- Lykins, S., Scammon, K., Lawrence, B. T., & Melgar, J. C. (2021). Photosynthetic Light Response of Floricane Leaves of Erect Blackberry Cultivars from Fruit Development into the Postharvest Period. *HortScience*, 56(3), 347–351. <https://doi.org/10.21273/HORTSCI15571-20>
- Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Stampar, F., & Veberic, R. (2012). Composition of Sugars, Organic Acids, and Total Phenolics in 25 Wild or Cultivated Berry Species. *Journal of Food Science*, 77(10), 1064–1070. <https://doi.org/10.1111/J.1750-3841.2012.02896.X>
- Milivojević, J., Maksimović, V., Nikolić, M., Bogdanović, J., Maletić, R., & Milatović, D. (2011). Chemical and antioxidant properties of cultivated and wild fragaria and rubus berries. *Journal of Food Quality*, 34(1), 1–9. <https://doi.org/10.1111/J.1745-4557.2010.00360.X>
- Moyer, R. A., Hummer, K. E., Finn, C. E., Frei, B., & Wrolstad, R. E. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus*, and *Ribes*. *Journal of Agricultural and Food Chemistry*, 50(3), 519–525. <https://doi.org/10.1021/JF011062R>
- Mullen, W., McGinn, J., Lean, M. E. J., MacLean, M. R., Gardner, P., Duthie, G. G., Yokota, T., & Crozier, A. (2002). Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. *Journal of Agricultural and Food Chemistry*, 50(18), 5191–5196. <https://doi.org/10.1021/JF020140N>
- Pantelidis, G. E., Vasilakakis, M., Manganaris, G. A., & Diamantidis, G. (2007). Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and *Cornelian cherries*. *Food Chemistry*, 102(3), 777–783. <https://doi.org/10.1016/J.FOODCHEM.2006.06.021>
- Parmenter, B. H., Croft, K. D., Hodgson, J. M., Dalgaard, F., Bondonno, C. P., Lewis, J. R., Cassidy, A., Scalbert, A., & Bondonno, N. P. (2020). An overview and update on the epidemiology of flavonoid intake and cardiovascular disease risk. *Food & Function*, 11(8), 6777–6806. <https://doi.org/10.1039/D0FO01118E>
- Ponder, A., Świetlikowska, K., Hallmann, E. (2017). The qualitative evaluation of the fruit of individual cultivars *Rubus* taking into account their usefulness to organic farming. *Journal of Research and Applications in Agricultural Engineering*, 62(4), 99–102.
- Shevchuk, L. M., Grynyk, I. v, Levchuk, L. M., Yareshchenko, O. M., Tereshchenko, Y. Y., & Babenko, S. M. (2021a). Biochemical contents of highbush blueberry fruits grown in the Western Forest-Steppe of Ukraine. *Agronomy Research*, 19(1), 232–249. <https://doi.org/10.15159/AR.21.012>
- Shevchuk, L., Grynyk, I., Levchuk, L., Babenko, S., Podpriatov, H., & Kondratenko, P. (2021b). Fruit Quality Indicators of Apple (*Malus domestica* Borkh.) Cultivars Bred in Ukraine. *Journal of Horticultural Research*, 29(2), 95–106. <https://doi.org/10.2478/JOHR-2021-0019>
- Souza, V. R., Pereira, P. A. P., da Silva, T. L. T., de Oliveira Lima, L. C., Pio, R., & Queiroz, F. (2014). Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chemistry*, 156, 362–368. <https://doi.org/10.1016/J.FOODCHEM.2014.01.125>
- Threlfall, R. T., Hines, O. S., Clark, J. R., Howard, L. R., Brownmiller, C. R., Segantini, D. M., & Lawless, L. J. R. (2016). Physiochemical and sensory attributes of fresh blackberries grown in the southeastern United States. *HortScience*, 51(11), 1351–1362. <https://doi.org/10.21273/HORTSCI10678-16>
- Toshima, S., Hirano, T., & Kunitake, H. (2021). Comparison of anthocyanins, polyphenols, and antioxidant capacities among raspberry, blackberry, and Japanese wild *Rubus* species. *Scientia Horticulturae*, 285, 110204. <https://doi.org/10.1016/J.SCIENTA.2021.110204>
- Veberic, R., Stampar, F., Schmitzer, V., Cunja, V., Zupan, A., Koron, D., & Mikulic-Petkovsek, M. (2014). Changes in the contents of anthocyanins and other compounds in blackberry fruits due to freezing and long-term frozen storage. *Journal of Agricultural and Food Chemistry*, 62(29), 6926–6935. <https://doi.org/10.1021/JF405143W>
- Vergara, M. F., Vargas, J., & Acuña, J. F. (2016). Características físico-químicas de frutos de mora de Castilla (*Rubus glaucus* Benth.) provenientes de cuatro zonas productoras de Cundinamarca, Colombia. *Agronomia Colombiana*, 34(3), 336–345. <https://doi.org/10.15446/AGRON.COLOMB.V34N3.62755>
- Vronska, L. V. (2018) Development of spectrophotometric method of flavonoids determination in bilberry shoots. *Pharmaceutical Review*, (4), 49–56. <https://doi.org/10.11603/2312-0967.2018.4.9703>
- Zorzi, M., Gai, F., Medana, C., Aigotti, R., Morello, S., & Peiretti, P. G. (2020). Bioactive Compounds and Antioxidant Capacity of Small Berries. *Foods*, 9(5), 623. <https://doi.org/10.3390/FOODS9050623>
- Žlabur, J. Š., Mikulec, N., Doždor, L., Duralija, B., Galić, A., & Voća, S. (2021). Preservation of Biologically Active Compounds and Nutritional Potential of Quick-Frozen Berry Fruits of the Genus *Rubus*. *Processes*, 9(11), 1940. <https://doi.org/10.3390/PR9111940>

