

THE QUANTIFICATION OF SOME BIOACTIVE COMPOUNDS IN THE FRUITS OF FOUR BLACKBERRY (*Rubus fruticosus* L.) CULTIVARS, PROPAGATED BY TISSUE CULTURE

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Abstract: The goal of the research was the quantitative determination of biochemical compounds (ascorbic acid, reducing sugars, tannins and titratable acidity) in *Rubus fruticosus* L. fruits – blackberries ('Melana', 'Triple Crown', 'Arapaho' and 'Thornfree'), propagated by tissue culture and cultivated under the ecological conditions of the Republic of Moldova. The spectrophotometric determination of ascorbic acid revealed that the highest content of this phytochemical was quantified in the 'Arapaho' blackberries, 48.28 mg/100 g, followed by the 'Thornfree' cultivar (41.69 mg/100 g), which is an amount about twice as high as in the 'Melana' blackberries (23.8 mg/100 g). The high amount of vitamin C in the researched blackberries makes them comparable to raspberries and gooseberries. The content of reducing sugars varied between 4.72% and 7.26%. The 'Triple Crown' cultivar was characterized by the maximum amounts of these biochemical compounds, and the lowest amounts were found in the fruits of the 'Thornfree' blackberry cultivar. The evaluation of the titratable acidity showed that the highest index of this parameter was recorded in the 'Melana' blackberries (0.74% / 0.77% as compared with malic / citric acid). The quantification of the tannins revealed that the fruits of the 'Triple Crown' cultivar have the lowest tannin content (1.97%), the amount being more than twice lower than the maximum amount (4.16%), recorded in the blackberries of the 'Thornfree' cultivar. The results of the study led to the conclusion that the fruits of *Rubus fruticosus* L., obtained from plants which had been micropropagated in the Embryology and Biotechnology Laboratory (NBGI) and grown under the conditions of the Republic of Moldova are a good source of biologically active substances and can be proposed for commercial production as a promising organic food product.

Keywords: 'Arapaho', ascorbic acid, biochemical parameters, blackberry, 'Melana', tannins, 'Thornfree', titratable acidity, 'Triple Crown', reducing sugars.

Introduction

Rubus fruticosus L. is a perennial shrub, cultivated for its fruits, which are appreciated due to their nutritional value. The popularity of blackberries among consumers is determined not only by their pleasant appearance and delicious taste, but also by the compositional diversity of nutraceuticals they contain. Thus, the fruits of this promising crop are currently appreciated and widely used for nutritional, medicinal and cosmetic purposes.

The need to provide the market with valuable blackberries has led to the expansion of plantations, blackberry being cultivated mainly in Europe and North America. Serbia and Hungary are among the European countries with the largest areas of blackberry plantations [AFIF CHAOUCHE & al. 2015]. Wild blackberry plants also make a significant contribution to world production [STRIK, 2007].

The health benefits of blackberries are attributed to the diversity of biochemical they contain. The results of several studies [JAMBA & CARABULEA, 2002; CANGI & ISLAM, 2003; GERCEKCIOGLU & al. 2003; KAFKAS & al. 2006; PATRAS & al. 2009; DENEV & al. 2010; ŞAHIN & al. 2010; TÜRK BEN & al. 2010; MURAD & al. 2011; DIMIĆA & al. 2012] have demonstrated that blackberries are an extremely rich source of bioactive substances, such as sugars, dietary fibre (cellulose, hemicellulose, pectin), vitamins (B group, C, E, K, P, PP), minerals (potassium salts, copper and manganese), organic acids (citric, tartaric, malic, salicylic), proteins and various macro- and microelements. In addition, blackberries are very rich in phenolic compounds, such as phenolic acids and anthocyanins, flavonoids, flavonols, ellagitannins, gallotannins and proanthocyanidins, which have shown considerable antioxidant properties [SIRIWOHARN & al. 2004; CHO & al. 2005; REYES-CARMONA & al. 2005; PANTELIDIS & al. 2007; MILIVOJEVIĆ & al. 2011; MILOŠEVIĆ & al. 2012a; GARCIA-SECO & al. 2015; KOLNIAK-OSTEK & al. 2015].

The research on the phytotherapeutic potential of blackberries has allowed attributing appreciable healing qualities to these berries. Among the diversity of healing properties of blackberries, there are the anticancer [SEERAM & al. 2006; BOWEN-FORBES & al. 2010; JIMENEZ GARCIA & al. 2013; PEREIRA & al. 2017], antitumour [KAUME & al. 2012], antimicrobial [SEERAM & al. 2012; YANG & al. 2014; AFIF CHAUCHE & al. 2015; ČETOJEVIĆ-SIMIN & al. 2017], antioxidant [SIRIWOHARN & WROLSTAD, 2004; REYES-CARMONA & al. 2005; CHO & al. 2005; ZAFRA-ROJAS & al. 2018], antidiabetic [JIMENEZ GARCIA & al. 2013], anti-inflammatory [DAI & al. 2007; BOWEN-FORBES & al. 2010; KISS & PIWOWARSKI, 2018] and neuroprotective action [TAVARES & al. 2013], they play a role in the prevention of cardiovascular diseases [SEERAM & al. 2006; BASU & al. 2010; JIMENEZ GARCIA & al. 2013; PARMENTER & al. 2020] and are a remedy for bronchitis and respiratory infections [BLUMENTHAL & BUSSE, 1998], etc. Clinical trials have also shown the benefits of eating blackberries to reduce the risk of obesity, degenerative diseases, etc. [PEREIRA & al. 2017; KISS & PIWOWARSKI, 2018; PARMENTER & al. 2020].

The description of the broad spectrum of health benefits of blackberries has contributed to the appreciation of this crop as a highly sought after and considered healthy nutraceutical food with a significant abundance of bioactive compounds. In the Republic of Moldova, blackberry cultivation has started to be practiced in the last decade, and some varieties, such as 'Arapaho' and 'Triple Crown' have been multiplied by tissue culture in the Embryology and Biotechnology Laboratory and have been used as planting material at the establishment of modern plantations in the districts of Dubăsari, Făleşti, Criuleni and Orhei. In order to evaluate the phytotherapeutic value of blackberries, obtained from plants multiplied by tissue culture and cultivated on the territory of the country and on the experimental lands of the "Alexandru Ciubotaru" National Botanical Garden (Institute) (NBGI), we decided to make a quantitative screening of important phytochemicals (ascorbic acid, reducing sugars, tannins and titratable acidity) from the fruits of different genotypes of *Rubus fruticosus*.

Material and methods

The (frozen) fruits of four genotypes of *Rubus fruticosus* L. ('Melana', 'Triple Crown', 'Arapaho' and 'Thornfree'), which had been obtained by tissue culture in the Embryology and Biotechnology Laboratory of the NBGI, served as biological material. The brief characteristic of the four blackberry cultivars [BALAN & al. 2017] and the general appearance of the fruits are presented in Table 1.

Table 1. General description of the studied blackberry genotypes

The name of the cultivar	General description	The aspect of the fruits
'Triple Crown'	<p>It is a cultivar of American origin, obtained in 1998 (Maryland, USA). It has a semi-erect growth, without spines. It produces shoots about 3 m long. It blooms in May-June, being a semi-early cultivar. The ripening period lasts from early July to mid-August. The shape of the fruit is slightly elongated, the weight – 8-10 g and the yield – about 15-20 t/ha. The fruits are sweet and fragrant. They are recommended to be eaten both fresh and processed. They maintain their integrity during handling and transportation. The cultivar has average frost resistance (can withstand temperatures of -20 °C). It is a very popular cultivar and one of the best blackberry cultivars without thorns.</p>	
'Arapaho'	<p>It is a cultivar of American origin (University of Arkansas, USA). It is moderately vigorous, has no thorns, the habit is erect, with vertical growth. It blooms in May, and the ripening period lasts from early June to the end of July, being an early-ripening cultivar. The fruit has a conical shape and a weight of 6-7 g, with small seeds. Productivity is about 15 t/ha. The fruits have good organoleptic qualities. They are recommended to be eaten both fresh and processed. They maintain their integrity during handling and transportation. Mechanical harvesting is also possible. The cultivar is resistant to frost (can withstand temperatures of -24 °C), diseases, pests and drought.</p>	
'Thornfree'	<p>It is a cultivar of American origin, very vigorous, without thorns, with long branches. It blooms in June, and the ripening period lasts from mid-August to early September. The shape of the fruit is conical-elongated, the weight is 8-9 g and the productivity of the cultivar is high (up to 20 t/ha). The fruits have a pleasant taste, are slightly flavoured. The fruits are recommended to be eaten both fresh and processed. They maintain their integrity during handling and transportation. The cultivar is resistant to frost (can withstand temperatures of -20 °C) and to the main diseases and pests. The cultivar has been homologated in the Republic of Moldova and has been registered in the catalogue of plant cultivars.</p>	

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<p>'Melana'</p>	<p>It is a cultivar obtained in the Embryology and Biotechnology Laboratory of the NBGI, which is now in the process of being approved by the Testing Commission (CSTSP), of medium size, moderately vigorous, without spines, productive. The plant has an erect and compact habit; vertical support is recommended. The fruits are conical in shape, large in size, weighing 7-8 g when ripe, blackish in colour, aromatic, maintain their integrity during handling and transportation, can be preserved well for a long time, having an attractive commercial appearance, the ripening of the berries is uniform. Ripening period – from the middle of June until the beginning of July (21-25 days). It is considered an early cultivar, which is highly resistant to frost (-24 °C), drought, diseases and pests, suitable (adapted) to the conditions of the Republic of Moldova.</p>	
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Biochemical investigations were performed at the Institute of Genetics, Physiology and Plant Protection using different biochemical methods (spectrophotometric and titrimetric).

The quantitative determination of vitamin C. The quantification of ascorbic acid content included spectrophotometric quantification at 680 nm wavelength using potassium hexacyanoferrate. In an acidic medium, ascorbic acid reduces stoichiometrically potassium hexacyanoferrate (Fe^{+3}) $\text{K}_3[\text{Fe}(\text{CN})_6]$ (red salt) in potassium hexacyanoferrate (Fe^{+2}) $\text{K}_4[\text{Fe}(\text{CN})_6]$ (yellow salt), which, in the presence of ferric ions, forms *iron* (III) *hexacyanoferrate* (II) (“Berlin Blue”) $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$.

To determine the concentration of vitamin C in the plant extract, the calibration curve was used and the following formula was applied:

$$K = (49,967 \cdot D \text{ opt}) - 11,938$$

The following formula was used to calculate the ascorbic acid content in a sample:

$$C = \frac{K \cdot V}{m}$$

where: C – ascorbic acid content, $\mu\text{g/g}$ biological material; K – concentration of ascorbic acid in a ml of extract, calculated according to the calibration curve, $\mu\text{g/ml}$; V – total volume of the extract, ml; m – the weight of the biological sample, g.

The quantitative determination of reducing sugars was based on the spectrophotometric quantification of the glucose, fructose and galactose content in the aqueous plant extract. The optical density was determined at a wavelength of 582 nm. The reducing sugar content was calculated using the formula:

$$A = \frac{c \cdot V \cdot 100\%}{m \cdot 1000}$$

where: A – sugar content, %; c – glucose content determined on the basis of the calibration curve; V – the volume of the extract corresponding to the sample of plant product, ml; m – the weight of the plant product taken for analysis, g.

Determination of total titratable acidity. The method is based on titrating the test solution with a standard solution of sodium hydroxide (0.1 N) in the presence of phenolphthalein. Exact amounts of aqueous extract were titrated with sodium hydroxide solution (0.1 N) to which phenolphthalein solution (1%) had previously been added. Titration

with stirring was continued until the solution changed its colour to pink, which did not disappear for 30 seconds.

The concentration of tartaric, malic or citric acid (g/l) was determined according to the formula:

$$X = \frac{V_1 \cdot C \cdot M}{V_0}$$

where: V_0 – the volume of the sample taken for titration; V_1 – the volume of sodium hydroxide solution that was consumed during titration, ml; V_2 – the volume to which the sample was adjusted, ml; C – the exact concentration of sodium hydroxide, (0.1 g/mol); m – the weight of the sample, g; M – molar mass of citric (64.0) / malic (67.0) / tartaric (75.0) acid.

The mass fraction of titrated acids relative to tartaric, malic or citric acid (%) was determined by the formula:

$$X_1 = \frac{V_1 \cdot V_2 \cdot C \cdot M}{m \cdot V_0} \cdot 0,1$$

where: V_0 – the volume of the sample taken for titration; V_1 – the volume of sodium hydroxide solution (0,1 N), which was consumed during titration, ml; V_2 – the volume to which the sample was adjusted, ml; C – the exact concentration of sodium hydroxide (g/mol); m – the weight of the sample, g; M – the molar mass of citric (64.0) / malic (67.0) / tartaric (75.0) acid.

The determination of tannins in the researched biological material consisted in their quantification with potassium permanganate (0.1 N), according to the classical titrimetric method [GOST 19885-74] as a result of the process of oxidation of tannins. The calculation of the percentage of tannin content was done using the formula:

$$C (\%) = \frac{(a - a_1) \cdot 0,004157 \cdot V \cdot 100}{V_1 \cdot m}$$

where, a – the quantity of potassium permanganate consumed to oxidize the tannins in the sample; a_1 – the quantity of potassium permanganate consumed to oxidize the tannins in the control (water and indigo carmine); V – the total volume of the sample; V_1 – the volume of the sample used for quantification; m – the dry mass of the sample, g; 0.004157 – the quantity of tannins oxidized by 1 ml of potassium permanganate (0.1 N), g.

Statistical processing. The search results were analysed using the program Microsoft Excel. The average was calculated for each parameter, and the data were expressed as the average of the replicates.

Results and discussions

Blackberries have a diverse content of biologically active substances, and the quantitative assessment of the nutraceuticals present in blackberries is of particular importance in identifying the phytochemical fingerprint of these berries.

Ascorbic acid. It is an essential water-soluble vitamin with excellent reducing properties, well known for its high antioxidant activity due to the neutralization of free radicals and other reactive oxygen species, produced by cellular metabolism, which are associated with several forms of tissue damage and diseases [SKROVANKOVA & al. 2015]. Although ascorbic acid is an important antioxidant, it still provides a maximum of 10% of the total antioxidant capacity of blackberries [MÄÄTTÄ-RIIHINEN & al. 2004; CHO & al. 2005; LANDETE, 2011; LI & al. 2015]. Vitamin C also helps extending the shelf life of berries, including blackberries [ZIA-UL-HAQ & al. 2014].

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The spectrophotometric quantification of ascorbic acid in the four blackberry genotypes studied by us revealed that the lowest content of this phytochemical was determined in blackberries of the 'Melana' cultivar, namely, 23.8 mg / 100 g (Figure 1). 'Thornfree' and 'Arapaho' cultivars contained amounts of ascorbic acid about twice as high as 'Melana' blackberries (41.69 and 48.28 mg / 100 g, respectively). The fruits of the 'Triple Crown' cultivars indicated values of 29.5 mg / 100 g, which is a higher content (by 24%) than the minimum value and significantly lower (by 39%), in comparison with the maximum value detected. Significant variations, which are even bigger than those recorded by us, have been obtained by other researchers. Thus, PANTELIDIS & al. (2007) mentioned that the content of ascorbic acid determined in different blackberry cultivars was between 14.3 and 103.3 mg/100 g of fresh mass. This considerable variation in the researched genotypes can be conditioned by both intrinsic and extrinsic factors.

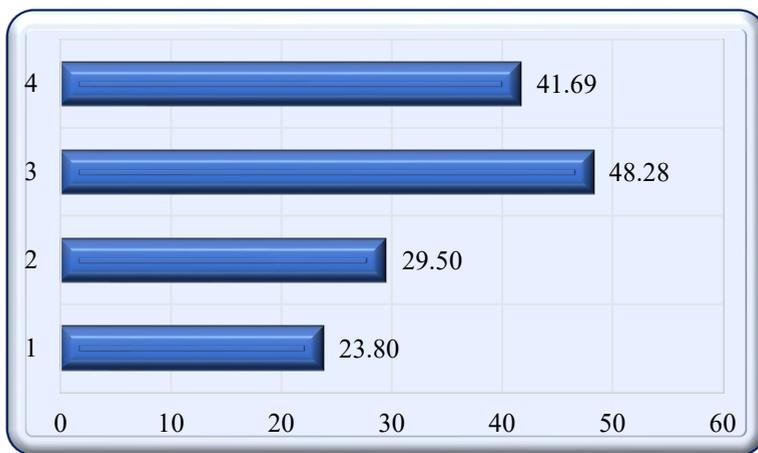


Figure 1. The content of ascorbic acid (mg/100 g) in frozen fruits of *Rubus fruticosus* L. (1 - 'Melana'; 2 - 'Triple Crown'; 3 - 'Arapaho'; 4 - 'Thornfree')

The relatively high content of ascorbic acid in the researched fruits is similar to the results obtained by other researchers. For example, in the blackberry cultivars grown in Serbia the amounts of vitamin C varied between 35.20 mg / 100 g and 44.00 mg / 100 g [MILOŠEVIĆ & al. 2012b]. Moreover, the 'Thornfree' cultivar grown in Serbia reached values of 40.48 mg / 100 g, and the one cultivated in the Republic of Moldova and researched by us contained 41.69 mg / 100 g of vitamin C, the data being very close. The high content of ascorbic acid in the blackberries of the 'Arapaho' and 'Thornfree' cultivars is almost similar to that of raspberries (*R. idaeus*), which according to the data obtained by VELJKOVIĆ & al. (2019) is 46.62 mg / 100 g⁻¹ fresh mass, and according to other authors [ARIFOVA & GORB, 2020] – from 31.7 mg / 100 g to 61.7 mg / 100 g. Also, in terms of vitamin C content, the researched blackberries are comparable to the gooseberry fruits, which according to investigations carried out on nine cultivars of *Ribes grossularia* L., grown under the conditions of the Republic of Moldova, contained between 26.51 mg% and 46.87 mg% ascorbic acid [SAVA, 2015].

The results of a recent study carried out by a group of researchers in Brazil [CROGE & al. 2019] revealed an ascorbic acid content of 20.38 mg / 100 g – 28.07 mg / 100 g, with a

variation of 9.63% depending on the place of cultivation and a variation of 8.47% depending on the cultivar, thus demonstrating a significant correlation between the vitamin C content and both the geographical region and the genotype. KULAITIENĒ & al. (2020) agree with it, they concluded that the concentration of vitamin C in berries and vegetables can be influenced by various factors, such as genotypic differences, climatic conditions before harvest, cultivation practices, maturity, harvesting methods and post-harvest handling procedures.

Reducing sugars. Sugars not only contribute to the nutritional value and the taste of fruits, but also play an important role in redox processes. All monosaccharides are reducing sugars, along with some disaccharides, some oligosaccharides and some polysaccharides [NELSON & COX, 2008], and the most common reducing monosaccharides are galactose, glucose and fructose [CAMPBELL & FARRELL, 2012]. These three monosaccharides were quantified in the fruits of the cultivars under study in order to be able to make conclusions about the reducing sugar potential of these fruits.

The content of dosed reducing sugars varied between 4.72% and 7.26%. The 'Triple Crown' cultivar was characterized by the maximum amount of these biochemical compounds, and the lowest amount was found in the fruits of the 'Thornfree' blackberry cultivar, followed by those of the 'Arapaho' cultivar, the values being close and the difference being only 1.7%. The medium content of reducing sugars was found in 'Melana' blackberries (Figure 2).

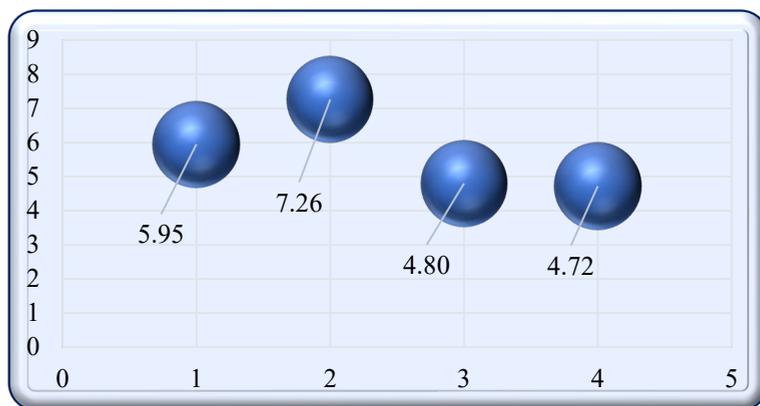


Figure 2. The content of reducing sugars (%) in frozen fruits of *Rubus fruticosus* L. (1 – 'Melana'; 2 – 'Triple Crown'; 3 – 'Arapaho'; 4 – 'Thornfree')

The results obtained by us are comparable to the results of other studies conducted on different cultivars and even species of blackberry. A study on this parameter in the fruits of seven cultivars of blackberries grown in Serbia revealed that the content of reducing sugars varied between 5.65% and 9.08% [MILOŠEVIĆ & al. 2012b].

Another study, conducted by another group of researchers from Serbia [STAJČIĆ & al. 2012], evaluated the content of reducing sugars in the fruits of two blackberry cultivars and established much lower indices (1.32 g / 100 g and 1.46 g / 100 g fresh material). Also, lower indices (0.8 mg/g – 2.0 mg/g fresh material), as compared with our data, were obtained by a group of researchers from the USA [THOMAS & al. 2005]. These significant differences allow us to assume that geographical and pedoclimatic factors are very important for the ability of these plants to synthesize and accumulate reducing sugars.

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Titrateable acidity. It is well known that both sugars and organic acids are the main water-soluble substances in berries and play a crucial role in developing the taste and in the process of ripening of blackberries, as well as being a qualitative index of how they will be appreciated by consumers. Thus, organic acids together with sugars and their properties, along with various secondary and aromatic metabolites determine the taste and organoleptic properties of fruits [MIKULIC-PETKOVSEK & al. 2021]. At the same time, organic acids inhibit the development of bacteria in fruit juices and thus extend the shelf life of the product.

The results obtained during our research showed that the highest amount of titrateable acidity is characteristic of the blackberries of the 'Melana' cultivar (0.74% and 0.77%, as compared with the malic acid and citric acid, respectively), and this index is approx. 30% higher than in the other three cultivars, which recorded similar results (Figure 3).



Figure 3. Titrateable acidity (%; acid malic/citric) determined in the frozen fruits of *Rubus fruticosus* L. (1 – 'Melana'; 2 – 'Triple Crown'; 3 – 'Arapaho'; 4 – 'Thornfree')

In the study conducted by the researchers STAJČIĆ & al. (2012) from Serbia, which was mentioned above with reference to the content of reducing sugars of the fruits of two blackberry cultivars, much higher amounts of titrateable acidity were found (1.36 and 1.39 g / 100 g material fresh).

The biochemical analysis made by YILMAZ & al. (2009) on 16 blackberry genotypes from the spontaneous flora and 9 varieties cultivated in Turkey found titrateable acidity amounts between 0.5% and 1.5%, with higher amounts (about 10%) in the genotypes from the spontaneous flora. The data obtained by MILOŠEVIĆ & al. (2012a) revealed titrateable acidity values in the range of 1.33%-1.89% (2010) and 1.08%-1.64% (2011), concluding that the level of this parameter differs greatly from one cultivar to another, as well as from one year to another and depends very much on the temperature during the fruit ripening period.

In another study carried out in Serbia, by a group of researchers lead by Professor Tomo Milošević, titrateable acidity values between 1.69% and 2.36% were found [MILOŠEVIĆ & al. 2012b], which are much higher as compared with those obtained by us. Significantly lower values as compared with ours were obtained by a team of researchers from the USA [THOMAS & al. 2005], who evaluated the titrateable acidity of the fruits collected from six blackberry cultivars, including 'Arapaho' and 'Triple Crown'. The level of this parameter varied between 0.16% and 0.34%.

The comparison of the results obtained in this study with those obtained by other researchers shows both congruence and differences between our studies and other studies in terms of titratable acidity, which may be due to climatic factors, differences in the harvesting time and total fluctuations in temperature during the growing season. The degree of maturation and ripening of the fruits also proved to be very important. In a study conducted in the USA [SIRIWOHARN & al. 2004] it was established that blackberries (*Rubus* L. hybrids) harvested in different stages of ripening can have a very variable level of titratable acidity, from 0.47 g / 100 g up to 2.38 g / 100 g, and the minimum value was obviously characteristic of overripe fruits. Thus, the multitude of factors that influence the titratable acidity are the reason why significant differences in the level of this biochemical parameter were recorded in various studies.

Tannins. Tannins are polyphenols, which have therapeutic properties and act as antioxidants, thus, they have various pharmacological properties, such as antitoxic, anticancer, antiallergic and anti-inflammatory, anthelmintic, antimicrobial, antiviral etc. [GHOSH, 2015]. They have the ability to combine with proteins, forming impermeable and non-rotting compounds. Simultaneously with the precipitation of proteins, a retraction of the tissue takes place, reducing the surface of action, a property used particularly in the treatment of burn wounds. Due to the action of protein precipitation, tannins also possess antiseptic properties, preventing infection by inhibiting bacterial growth. Naturally, tannins are present in leaves, seeds, bark, roots, fruits and vegetables [HASSANPOUR & al. 2011; GHOSH, 2015].

In our research, it was found that the 'Triple Crown' cultivar had the lowest tannin content (1.97%), the amount being more than twice lower than the maximum amount (4.16%), recorded in the blackberries of the 'Thornfree' cultivar. As in the case of reducing sugars (Figure 2), 'Melana' blackberries showed a moderate tannin content (2.81%) as compared with the other three cultivars (Figure 4).

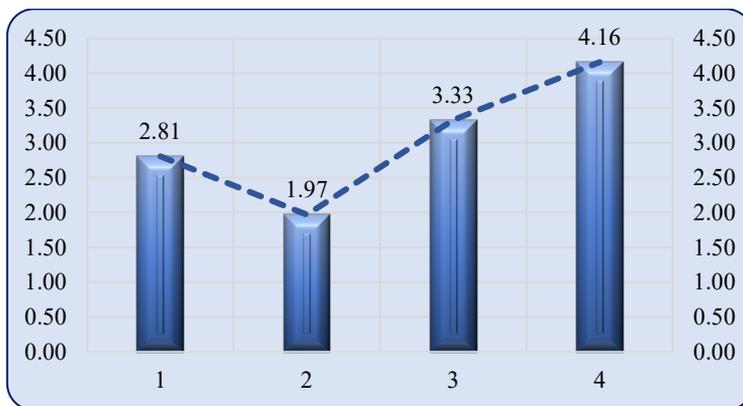


Figure 4. The percentage of tannins in frozen fruits of *Rubus fruticosus* L. (1 – 'Melana'; 2 – 'Triple Crown'; 3 – 'Arapaho'; 4 – 'Thornfree')

A previous study conducted in the laboratory indicated a tannin content of 1.19%-6.16%, quantified in blackberries – *Rubus fruticosus* and *Rubus candicans* [LOZINSCHIL, 2019]. The maximum value was identified in the blackberry fruits of *R. candicans* from the spontaneous flora.

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There is very little data on the content of tannins in blackberries in literature, and the existing ones differ greatly from one study to another and depend primarily on the cultivar and the harvesting time. More research has been done on the content of tannins in blackberry leaves.

The generalization of the obtained results by their comparative analysis and the classification of the cultivars depending on the content of the quantified substances, allowed us to conclude that the four cultivars separated into two groups with relatively similar indices (Figure 5).

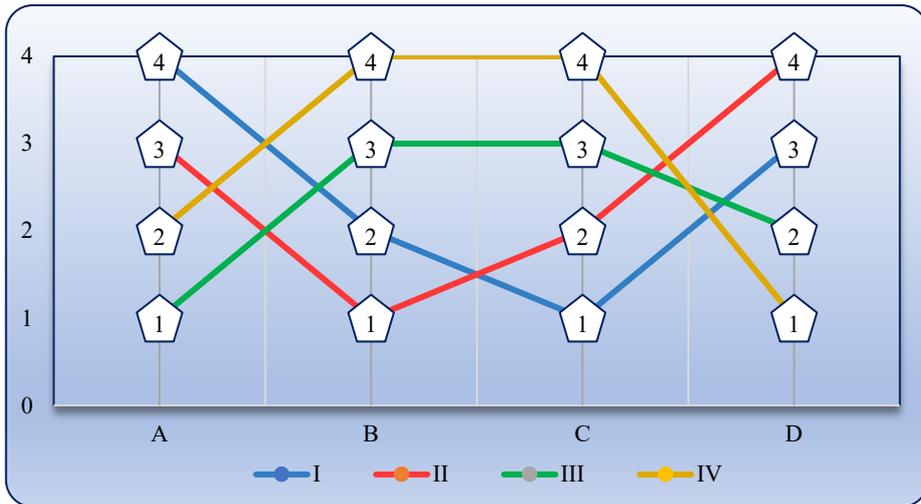


Figure 5. The classification of blackberry genotypes, according to the values of the researched biochemical parameters (1-4 – ranking by content in descending order (1 – 1st place, with maximum value, 4 – 4th place, with minimal value); A-D – biochemical parameters (A – ascorbic acid, B – reducing sugars, C – titratable acidity, D – tannins); (I-IV – blackberry cultivars (I – 'Melana', II – 'Triple Crown', III – 'Arapaho', IV – 'Thornfree'))

The first identified group includes the genotypes 'Melana' and 'Triple Crown' and is characterized by a higher content of ascorbic acid and tannins and lower amounts of reducing sugars and titratable acidity. The second group includes the 'Arapaho' and 'Thornfree' cultivars with a higher level of reducing sugars and titratable acidity and with a lower content of vitamin C and tannins.

Conclusions

The spectrophotometric quantification of ascorbic acid has revealed very different values depending on the genotype, ranging from 23.8 mg / 100 g ('Melana') to 48.28 mg / 100 g ('Arapaho'). The recorded values indicate a considerable amount of vitamin C in blackberries that makes them comparable to raspberries and gooseberries. The quantitative analysis of the reducing sugars revealed a maximum content of 7.26% in the fruits of the 'Triple Crown' cultivar and a minimum one (4.72% and 4.8%) – in the 'Thornfree' and 'Arapaho' cultivars. The mass concentration of titratable acids expressed in malic/citric acid revealed values

between 0.54% and 0.77%. The highest level was determined in the 'Melana' blackberry fruits, and the other three cultivars had similar values, which turned out to be about 30% lower than the maximum. The evaluation of the tannin content revealed maximum values (4.16%) in the 'Thornfree' cultivar, and the minimum tannin content (1.97%) was quantified in the 'Triple Crown' blackberries.

The comparative analysis of the obtained data has resulted a phytochemical outline, in which the four genotypes were separated into two groups with relatively similar indices. The first identified cluster includes the genotypes 'Melana' and 'Triple Crown' and is characterized by a higher content of ascorbic acid and tannins. The second group includes the 'Arapaho' and 'Thornfree' cultivars, with a higher level of reducing sugars and titratable acidity. The results of the study led to the conclusion that the fruits of *Rubus fruticosus* L. ('Melana', 'Triple Crown', 'Arapaho' and 'Thornfree'), obtained from plants which had been micropropagated in the Embryology and Biotechnology Laboratory and grown under the conditions of the Republic of Moldova are a good source of biologically active substances and can be proposed for commercial production as a promising organic food product, with impressive health benefits. At the same time, we would like to mention that the climate of the Republic of Moldova is suitable for the cultivation of *Rubus fruticosus* L., which is a crop that prefers a mild climate.

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