PHYTOCHEMICAL ANALYSIS OF SEVERAL CULTIVARS OF *ARONIA MELANOCARPA* FROM THE COLLECTION OF THE "ALEXANDRU CIUBOTARU" NATIONAL BOTANICAL GARDEN (INSTITUTE)

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Abstract. The purpose of this research included the quantitative determination of some biochemical compounds (ascorbic acid, reducing sugars, tannins, titratable acidity and phenolic compounds) from the fruits of three cultivars of *Aronia melanocarpa* from the collection of the "Alexandru Ciubotaru" National Botanical Garden (Institute). The quantitative determination of ascorbic acid in the frozen fruits of the three cultivars of black chokeberry demonstrated close values (28.1 and 27.2 mg / 100 g) in the fruits of the cultivars ˈNeroˈ and ˈAlecsandrinaˈ and significantly lower (23.8 mg / 100 g) in those of the cultivar 'Viking'. The minimum and maximum contents of reducing sugars were recorded in the same cultivars – ˈNeroˈ (7.10%) and 'Viking' (6.68%), and those of the cultivar ˈAlecsandrinaˈ showed a value very close to the average (6.83%). The established titratable acidity had values between 0.854% and 0.955% malic acid and between 0.816% and 0.912% citric acid, and the maximum index of this parameter was recorded by the fruits of the cultivar ˈAlecsandrinaˈ. The quantification of tannins highlighted the fruits of the cultivar ˈNeroˈ, which was characterized by the maximum content of tannins (3.118%), exceeding by more than 48% the minimum value (2.079%) detected in the fruits of the cultivar ˈAlecsandrinaˈ. The quantitative biochemical study of phenolic compounds revealed a high content of these biologically active substances in the fruits of all cultivars investigated, and their maximum value (3202.7 mg GAE / 100 g d.m.) was recorded by the fruits collected from the cultivar 'Viking'.

Keywords: *Aronia melanocarpa*, 'Viking', ˈNeroˈ, ˈAlecsandrinaˈ, biochemical parameters, ascorbic acid, reducing sugars, titratable acidity, tannins, phenolic compounds.

INTRODUCTION

Aronia melanocarpa (Michx.) Spach, commonly called black chokeberry, is native to eastern North America, and has been widely cultivated in Europe and Asia. The popularity of black chokeberry is determined by its technological peculiarities, the taste and health benefits of the fruits, as well as the precocity and high productivity, the resistance of plants to environmental conditions, diseases and pests, the healing properties of the leaves and fruits, the technological possibilities for processing etc. (Roșca et al., 2022). Black chokeberry fruit is a very rich source of dietary antioxidants (Oszmiański and Wojdyło, 2005; Kulling and Rawel, 2008). It has been established that as compared with other species, *Aronia melanocarpa* contains more biologically active compounds such as phenolic acids, anthocyanins, flavonoids and proanthocyanidins (Zheng and Wang, 2003; Banjari et al., 2017). These fruits have a higher content of phenolic compounds than most other black fruits (Kähkönen, Hopia and Heinonen, 2001; Wu et al., 2004).

Numerous studies have revealed that chokeberry fruit extracts have anticancer (Gassiorowski et al., 1997; Malik et al., 2003), anti-inflammatory (Ryszawa et al., 2006), antidiabetic (Simeonov et al., 2002; Baum et al., 2016), anti-hypertensive properties (Bell and Gochenaur, 2006) and provide benefits in reducing heavy metal toxicity (Kowalczyk et al., 2003). Also, a recent study (Vahapoglu et al., 2022) mentions the benefits of berries, including chokeberries, for the gastrointestinal, cardiovascular, immune and nervous systems. Various chokeberry extracts are commercially available and are proposed as supplements for the treatment of diabetes, cancer, hypertension and anxiety (Gajica et al., 2020).

The chemical composition of *Aronia melanocarpa* fruits changes significantly during development and ripening. While harvesting fruits as raw material for food products and food supplements, the changes in their chemical composition during ripening should be taken into account. Green, unripe fruits have the highest antioxidant activity due to their high content of procyanidins and flavonoids, despite the absence of anthocyanins. Thus, unripe fruit extracts could be used as a potential food supplement in prophylaxis, as well as a dietary support in the treatment of various conditions. Extracts rich in polyphenols may be especially useful for patients with metabolic syndrome in which severe oxidative stress occurs (Gralec, Wawer and Zawada, 2019).

The biochemical components of chokeberry fruits depend on a number of factors such as cultivar, fertilization, fruit ripening, harvest date or habitat/location (Jeppsson and Johansson, 2000; Skupien and Oszmianski, 2007). The chemical composition of chokeberry fruits or freshly pressed juice differs from other berries also in the high content of sorbitol and polyphenols (Lehmann, 1990; Ara, 2002; Wawer, 2006).

It has been demonstrated that black chokeberry fruits contain dietary fiber in proportion of 5.62 g / 100 g fresh mass (Tanaka and Tanaka, 2001). Besides, relatively low pectin content was detected – between 0.3 and 0.6% (Lehmann, 1990; Strigl, Leitner and Pfannhauser, 1995).

The total organic acid content is relatively low as compared to other berries and is around 1-1.5% of the dry matter (Lehmann, 1990; Tanaka and Tanaka, 2001). The main acids identified were L-malic acid and citric acid (Lehmann, 1990; Tanaka and Tanaka, 2001). In freshly pressed juice prepared under laboratory conditions, from different cultivars from different locations, the total amount of acids was found to be about 5-19 g/L, with L-malic acid being the main component (Kulling and Rawel, 2008).

The amount of reducing sugars recorded in fresh chokeberry fruits was about 16-18% (Strigl, Leitner and Pfannhauser, 1995). According to Seidemann (1993), the sum of glucose and fructose was between 13 and 17.6 g / 100 g fresh mass, and sucrose was not detected (Seidemann, 1993). Glucose (range: 30-60 g/L; average: 41 g/L) and fructose (range: 28-58 g/L, average: 38 g/L) were identified in freshly pressed juice (Ara, 2002). Similarly, the average amount of sorbitol was determined enzymatically to be 80 g/L in freshly pressed juice and 56 g/L in pasteurized juice (Ara, 2002; Wiese, Kruse and Kulling, 2008). Among a number of berries tested, chokeberry was found to contain the highest concentration of sorbitol, suggesting its application as a biomarker for juices mixed with it (for example, in the case of blackcurrant) (Hofsommer and Koswig, 2005). Sorbitol is a sugar substitute often used in healthy diets and is known as a weak, non-stimulant laxative (Kulling and Rawel, 2008).

The total fat content of chokeberry fruits was found to be 0.14 g / 100 g fresh mass (Tanaka and Tanaka, 2001). The lipids from chokeberry seeds were also evaluated to identify their main components (Zlatanov, 1999). The presence of 19.3 g/kg glyceride oil was detected, and linoleic acid has been found to be the main fatty acid. The phospholipid content of the seed oil, represented mainly by phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine, recorded values of 2.8 g/kg. The total amount of sterols was 1.2 g/kg dry matter, the main component being β-sitosterol, followed by campesterol, stigmasterol and Δ (5)-avenasterol (Zlatanov, 1999).

The protein fraction of the fruit was difficultly determined and the amount present was summed to 0.7 g / 100 g fresh mass (Tanaka and Tanaka, 2001). The determination of the amino acid content revealed the predominance of asparagine in the freshly pressed juice (Ara, 2002).

The mineral content of fresh chokeberry fruits was found to be 440 mg/100 g (Tanaka and Tanaka, 2001) and 580 mg/100 g (Lehmann, 1990). Due to the impact of processing, the mineral content of the juices varied between 300 and 640 mg/100 mL (Lehmann, 1990; Kruse and Kulling, 2008). Chokeberry juice had relatively high average amounts of potassium and zinc (Ognik et al., 2006). Also, the following vitamins have been identified in the freshly pressed black chokeberry juice: B_1 (25-90 μg / 100 mL), B_2 (25-110 μg / 100 mL), B_6 (30-85 μg / 100 mL), C (5-100 mg / 100 mL), pantothenic acid (50-380 μg / 100 ml) and niacin (100-550 μg / 100 ml) (Ara, 2002). In addition to these components, β-carotene and β-cryptoxanthin were also found in relatively large quantities (Razungles, Oszmianski and Sapis, 1989; Tanaka and Tanaka, 2001).

However, it is assumed that the most important constituents present in chokeberry, which are also responsible for many of its medicinal properties, are phenolic compounds. Chokeberry fruits have a high content of procyanidins, anthocyanins and phenolic acids (Kulling and Rawel, 2008). Due to the high content of polyphenols in chokeberry fruits and leaves, this species has a higher antioxidant activity as compared to other species of berries or medicinal plants (Shahin et al., 2019).

Therefore, the goal of the present study was to analyze and compare the content of bioactive compounds of three cultivars of *Aronia melanocarpa* ('Viking', ˈNeroˈ and ˈAlecsandrinaˈ) from the collection of “Alexandru Ciubotaru” National Botanical Garden (Institute).

MATERIALS AND METHODS

The (frozen) fruits of three cultivars of *Aronia melanocarpa* ('Viking', ˈNeroˈ and ˈAlecsandrinaˈ) from the collection of “Alexandru Ciubotaru” National Botanical Garden (Institute) (NBGI) served as biological material (Balan et al., 2017; Catalog of plant varieties of the Republic of Moldova, 2023). The plants were grown in the experimental sectors of NBGI. The appearance of the fruits is shown in figure 1.

1 2 3

Fig.1. The appearance of the fruits of the three investigated cultivars of *Aronia melanocarpa* (*1* – 'Viking', *2* – ˈNeroˈ, *3* – ˈAlecsandrinaˈ)

The biochemical investigations were carried out in the Plant Biochemistry laboratory within the Institute of Genetics, Physiology and Plant Protection, by using different classical biochemical methods (spectrophotometric and titrimetric).

Quantitative determination of vitamin C. Determining the ascorbic acid content included spectrophotometric quantification using potassium hexacyanoferrate. In acidic medium, ascorbic acid stoichiometrically reduces potassium hexacyanoferrate (Fe^{+3}) $K_3[Fe(CN)_6]$ (red salt) into potassium hexacyanoferrate (Fe^{+2}) $K_4[Fe(CN)_6]$ (yellow salt), which in the presence of ferric ions forms iron (III) hexacyanoferrate (II) ("Prussian blue") $Fe_4[Fe(CN)_6]_3$.

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

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

To calculate the ascorbic acid content in the sample, the following formula was used:

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

where: *C* – ascorbic acid content, µg / g biological material; *K* – concentration of ascorbic acid per 1 ml de extract, calculated according to the calibration curve, µg/ml; *V* – total volume of the extract, ml; *m* – 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 selected 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 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; 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) acid.

The mass fraction of titrated acids relative to 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) acid.

The determination of tannins in the researched biological material consisted in their quantification with potassium permanganate (0.1 N), according to the classic titrimetric method (ГОСТ 19885-74) as a result of the oxidation process of tannins. The percentage of the tannin content was calculated by applying 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 matter of the sample, g; *0.004157* – the quantity of tannins oxidized by 1 ml of potassium permanganate, g.

The method of determining the phenolic compounds in the investigated biological material (Folin, Ciocalteu, 1927 with modifications proposed by Singleton, Rossi, 1965) was based on the reaction of phenols with the Folin-Ciocalteu reagent, which is reduced in alkaline medium through the interaction with phenolic compounds, thus producing blue complexes. The reaction products were determined spectrophotometrically at a wavelength of 765 nm.

The content of phenolic compounds in one gram of fresh mass was expressed in gallic acid equivalents that provide the same optical density of the reaction (determined based on the calibration curve). To make the calibration curve, gallic acid was used as a standard substance, and to calculate the content of phenolic compounds, the following formula was applied:

$$F = \frac{(C \cdot V)}{m \cdot 1000}$$

where: F – the content of phenolic compounds, mg GAE /100 g d.m.; C – concentration of phenolic compounds determined on the basis of the calibration curve, mg GAE/l; V – total volume of the sample; m – the weight of the sample, g; 1000 – coefficient of converting litre to millilitres.

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 repetitions.

RESULTS AND DISCUSSIONS

Ascorbic acid is an essential water-soluble vitamin with significant reducing properties, well known for its high antioxidant activity due to the neutralization of free radicals and other reactive oxygen species, formed by cellular metabolism, which are associated with several forms of tissue damage and diseases (Skrovankova et al., 2015). Besides, vitamin C contributes to extending the shelf life of berries.

The spectrophotometric quantification of ascorbic acid in the three chokeberry genotypes studied by us revealed that the lowest content of this phytocompound was determined in chokeberry fruits of the cultivar 'Viking', with a value of 23.8 mg / 100 g (fig. 2). The cultivars ˈNeroˈ and ˈ Alecsandrinaˈ showed comparatively higher values, with 18% and 14%, respectively. The fruits of the cultivar ˈNeroˈ contained the maximum amount of ascorbic acid, constituting 28.1 mg / 100 g of frozen fruits. They are followed by the fruits of the cultivar ‘Alecsandrina’, with a vitamin C content of 27.2 mg / 100 g, which represents an insignificantly lower content (by 3.3%) than the maximum detected value. The quantitative determination of ascorbic acid in chokeberry fruits (*Aronia melanocarpa* (Michx.) Elliot) grown in the Republic of Moldova by a group of researchers from the Scientific-Practical Institute of Horticulture and Food Technologies demonstrated values of 17.3-18.74 mg % over the years 2013-2014 (Sava et al., 2015) and 16.72-29.04 mg % – in the period 2015-2017 (Rusnac and Sava, 2018).

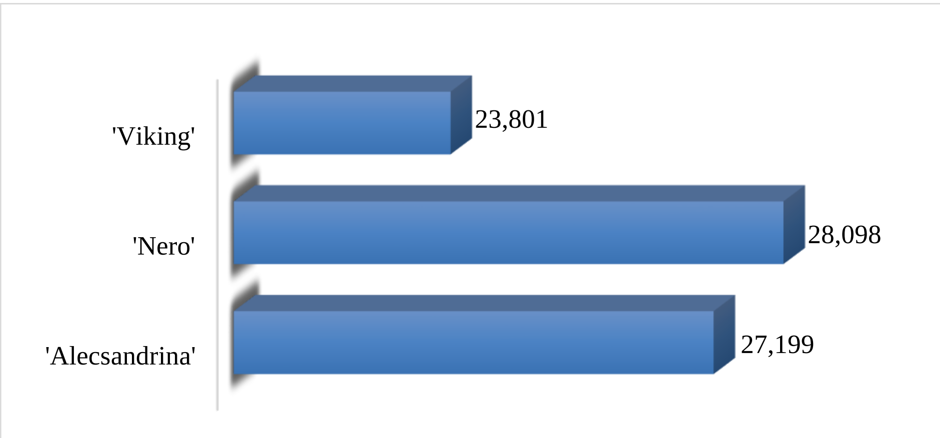

Fig. 2. Ascorbic acid content (mg / 100 g) in frozen fruits of *Aronia melanocarpa*

Significant variations, which have values much higher than those recorded by us, were obtained by Denev et al. (2018) in chokeberry grown in Bulgaria, who presented ascorbic acid values determined in different chokeberry cultivars between 37.3 and 91.8 mg / 100 g of fresh mass – in 2016 and between 39.8 and 91.3 mg / 100 g of fresh mass – in 2017. Other data from the specialized literature have revealed significant variations of this parameter. Thus, values of ascorbic acid in chokeberry fruits of 137 mg/kg fresh mass (Tanaka and Tanaka, 2001) and 13-270 mg/kg fresh mass (Lehmann, 1990) have been reported. This considerable variation within the researched genotypes can be conditioned by intrinsic factors, but even more so by extrinsic ones.

The fruits cultivated in Poland had an ascorbic acid content of 31 mg/100 g of fresh fruits (Ochmian, Oszmiański and Skupień, 2009). These data are similar to those obtained by us, taking into account the fact that our research was conducted on frozen fruits.

Reducing sugars. Sugars, including the reducing ones, not only contribute to the nutritional value and taste of fruits, but also play an important role in redox processes.

The values of the content of reducing sugars measured in the fruits of the three cultivars of *Aronia melanocarpa* varied between 6.68% and 7.11%. The cultivar ˈNeroˈ was characterized by the maximum content of these biochemical compounds, and the minimum amount was detected in the fruits of the chokeberry cultivar ˈVikingˈ, followed by those of the cultivar ˈAlecsandrinaˈ, the values being close, and the difference being only 2.2%. The average content of reducing sugars was recorded in the fruits of the genotype 'Alecsandrina' (fig. 3).

The results obtained by us are comparable with the results of other studies carried out on chokeberry fruits. Thus, Skupień et al. (2008) quantified 6,58 g / 100 g^{-1} reducing sugars, and Ochmian et al. (2012) from Poland identified values of reducing sugars within the limits of 8.83 g/ 100 g^{-1} – 12.48 g / 100 g^{-1}. Moreover, the respective researchers studied 4 cultivars, including ˈNeroˈ and ˈVikingˈ, and the fruits of the cultivar ˈVikingˈ, similar to our research, had the minimum content of reducing sugars, and those of ˈNeroˈ – by 12% more (Ochmian, Grajkowski and Smolik, 2012).

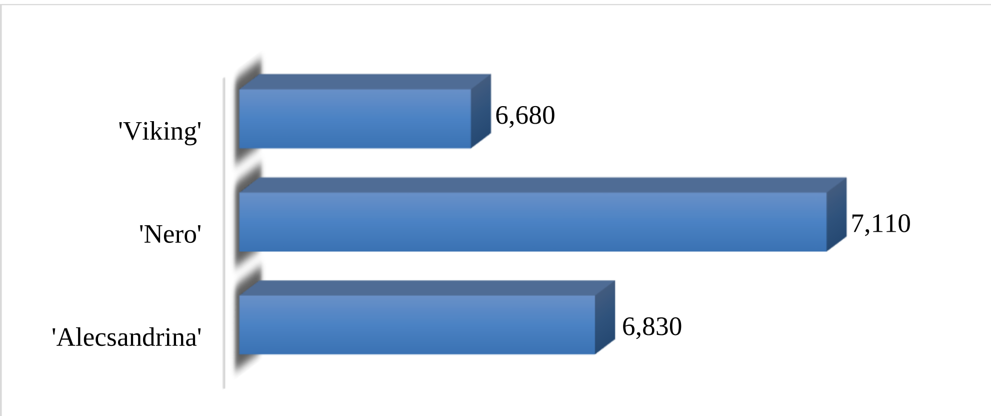

Fig. 3. The content of reducing sugars (%) in frozen fruits of *Aronia melanocarpa*

Another study, carried out by two researchers from Turkey (Poyraz Engin and Mert, 2020), evaluated the content of reducing sugars in the fruits of the same two chokeberry cultivars (ˈNeroˈ and ˈVikingˈ) and established significantly higher values as compared to the data obtained by us (13.04% and 18.61%), demonstrating, at the same time, the strict dependence of the amount of reducing sugars on the fruit harvesting period. Strigl et al., (1995) reported values of 16-18% reducing sugars, quantified in fresh chokeberry fruits. These significant differences allow us to assume that geographical and pedoclimatic factors are very important in the ability of the respective plants to synthesize and accumulate reducing suagrs. Thus, the content of these phytocompounds depends less on the genotype than on the agrotechnical characteristics and microclimate.

Titratable acidity. Organic acids, along with sugars, are the main water-soluble substances in berries and play a key role in providing their taste and maturity, while also representing a qualitative index of how the product will be received by the consumers. Thus, organic acids together with sugars and their respective properties, along with various secondary and aromatic metabolites, determine the taste and organoleptic properties of fruits (Mikulic-Petkovsek et al., 2021). At the same time, organic acids reduce the development of bacteria in fruit juices and thus contribute to extending the shelf life of the product. The results obtained in our research demonstrated that the highest level of titratable acidity is found in 'Alecsandrina' chokeberry fruits (0.955% and 0.912% malic acid and citric acid, respectively), and this value is 11.8% higher as compared with the fruits of the cultivar 'Nero' and 7.5% higher than in those of the cultivar 'Viking' (fig. 4). Various researchers have reported that the predominant organic acids in chokeberry fruits are malic acid and citric acid (Kaack and Kühn, 1952; Jeppsson and Johansson, 2000; Strik et al., 2003; Šnebergrová et al., 2014).

The study carried out by the researchers Ochmian, Oszmiański and Skupień (2009) from Poland, which was also mentioned with reference to the ascorbic acid content of chokeberry fruits, established values of titratable acidity equal to 1.42 g citric acid / 100 g fresh mass. Data comparable to ours were obtained in a study carried out in Poland (Ochmian, Grajkowski and Smolik, 2012), in which the fruits of the cultivar 'Viking' recorded 0.80 g citric acid / 100 g^{-1}, and those of the cultivar 'Nero' – 0.85 g citric acid / 100 g^{-1}.

The biochemical analysis made by Skupień et al. (2008) demonstrated a value of titratable acidity of 1.29 g citric acid / 100 g^{-1}, being by about 40% higher as compared with the data obtained by us. Higher values, as compared with ours, were also obtained by the researchers from the Scientific-Practical Institute of Horticulture and Food Technologies from the Republic of Moldova, who presented the data obtained over three years (2015-2017) and which include titratable acidity values of 2.08-2.52% (Rusnac and Sava, 2018).

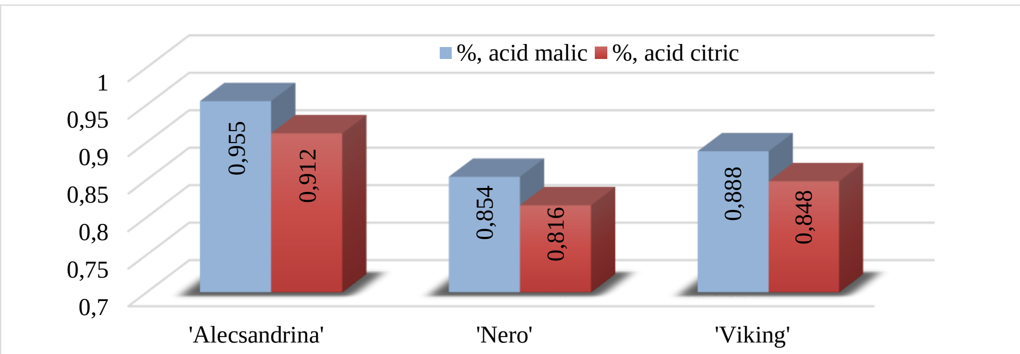

Fig. 4. Titratable acidity (%, malic acid, citric acid) determined in frozen fruits of *Aronia melanocarpa*

The quantification of the acidity of the juice obtained from chokeberry fruits grown in Turkey (Poyraz Engin and Mert, 2020) presented values of 0.60 g malic acid / 100 ml in the cultivar 'Nero' and 0.58 g malic acid / 100 ml for the cultivar ' Viking', demonstrating that the acidity of the juice does not significantly depend on the fruit harvesting period.

The correlation of the results obtained in our study with those obtained by other researchers highlights both similarities and differences in terms of titratable acidity, which may be due to climatic factors, variations in harvest time and total temperature fluctuations during the growing season. Thus, because of the multitude of factors that influence titratable acidity, significant differences in the level of this biochemical parameter are recorded in the framework of different studies.

Tannins. Tannins are polyphenols, which possess therapeutic properties and act as antioxidants, and therefore exhibit various pharmacological properties, such as antitoxic, anticancer, antiallergic and anti-inflammatory, anthelmintic, antimicrobial, antiviral etc. (Ghosh, 2015). In our study, the fruits of the chokeberry cultivar ˈAlecsandrinaˈ had the lowest content of tannins (2.079%), the value being more than 33% lower than the maximum value (3.11%) recorded in the chokeberries of the cultivar ˈNeroˈ. The fruits of chokeberry cultivar 'Viking' were characterized by average values of tannin content (2.494%), which was over 25% lower than the maximum and almost 17% higher than the minimum detected (fig. 5.) .

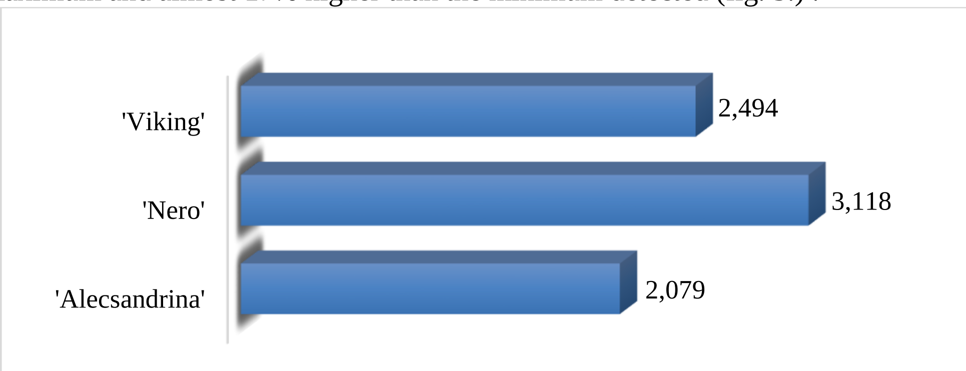

Fig. 5. The content of tannins (as percentage) determined in frozen fruits of *Aronia melanocarpa*

In the specialized literature, there are very few data regarding the content of tannins in chokeberry fruits, and the available ones differ greatly from one study to another and depend primarily on the features of the cultivar and the harvest time.

In the study conducted by Poyraz Engin and Mert from Turkey (2020), the content of soluble tannins was evaluated in the fruits of the chokeberry cultivars ˈNeroˈ and ˈVikingˈ, collected at different periods of fruit ripening, and values comparable to ours were established. Thus, the fruits of the cultivar ˈNeroˈ recorded 3.33 ppm kg^{-1}, and those of ˈVikingˈ – 2.73 ppm kg^{-1}, the difference being 18%. In our research, the same tendency was observed, and this difference was 17%. Pogorzelski et al. (2006) quantified 1.16% of tannins in fruits and 0.85% in the juice obtained from chokeberry grown in Poland, but Atanassova and Bagdassarian (2009) determined the content of tannins in chokeberry juice, and the value of this parameter was 2%.

Phenolic compounds. Phenolic compounds, like other antioxidants, are generally used to reduce the harmful effects of substances with high oxidative potential. The antioxidant properties of polyphenols are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen scavengers (Rice-Evans, Miller and Paganga, 1997).

The determination of total polyphenols by the method of Singleton & Rossi (1965) in the fruits of the three cultivars of *Aronia melanocarpa* revealed maximum values in the cultivar 'Viking' (3202.73 mg gallic acid equivalent / 100 g dry matter) and minimum values – in the cultivar 'Alecsandrina' (2152.97 mg gallic acid equivalent / 100 g dry matter). The cultivar 'Nero' recorded an intermediate value (2429.63 mg gallic acid equivalent / 100 g dry matter), which was 24.1% less than the maximum detected value and 12.9% more than the minimum (fig . 6.).

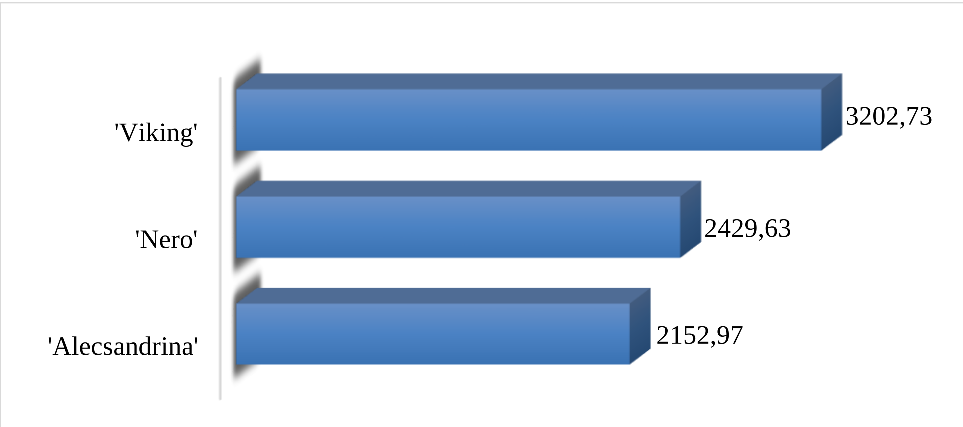

Figure 6. The content of phenolic compounds (mg GAE / 100 g d.m.) in frozen fruits of *Aronia melanocarpa*

The study carried out by the Turkish researchers Poyraz Engin and Mert (2020) reported that ripe fruits collected from the cultivar 'Viking' contained 1957.80 mg gallic acid / 100 g^{-1} fresh mass, and those from the cultivar 'Nero' – 2120.70 mg gallic acid / 100 g^{-1} fresh mass. The same authors concluded that the content of these biochemical compounds with antioxidant properties did not undergo quantitative changes depending on the fruit harvesting period.

Data comparable to ours were obtained by Tolić et al. (2015) from Croatia, who quantified 1954-2466 mg gallic acid / 100 g dried fruits. In chokeberry juices, the quantitative values of phenolic substances constituted 3002-6639 mg gallic acid / liter of juice (Tolić et al., 2015). Ochmian, Grajkowski and Smolik (2012) reported values of the total content of polyphenols determined in four chokeberry cultivars varying between 1845 and 2340 mg gallic acid / 100 g fresh mass, and the cultivars included in our research also recorded a content of 1845 mg gallic acid / 100 g fresh mass in the fruits of the cultivar 'Viking' and 1950 mg gallic acid / 100 g fresh mass – for the fruits of the cultivar 'Nero'.

In other studies, it was established that the total content of phenols in chokeberry fruits recorded values of 3440 mg / 100 g dry matter (Kolesnikov and Gins, 2001), 3760 mg / 100 g dry matter ('Nero') (Hudec et al., 2006), 4010 mg / 100 g dry matter (Kähkönen et al., 1999), 4210 mg / 100 g dry matter ('Viking') (Kähkönen, Hopia and

Heinonen, 2001), up to 7465 mg / 100 g dry matter (Mayer-Miebach, Adamiuk and Behsnilian, 2008).

The extensive research conducted by Denev et al. over two years (2016-2017) on 23 varieties of the cultivar ˈNeroˈ grown in Bulgaria resulted in values of 1022.4-1795.5 mg gallic acid / 100 g of frozen fruits (Denev et al., 2018). Skupień, Ochmian and Grajkowski (2008) presented the research results, indicating a polyphenol content of 1175.6 mg gallic acid / 100 g^{-1} fresh fruits, also concluding that foliar fertilization of plants with Mn fertilizer, 'Alkaline' (N, K and Si) and Mn + ' Alkalin' did not significantly influence the total polyphenol content. Significantly higher values as compared with the data obtained by us were obtained by Oszmianski and Wojdylo (2005), who reported a content of phenolic compounds of 7849 mg / 100 g dry matter in fruits and 3729.07 mg / 100 g dry matter – in juice (Oszmianski and Wojdylo, 2005).

The high content of phenolic compounds determined in the fruits investigated by us, allow us to assume that all three chokeberry cultivars possess an appreciable antioxidant activity, this conclusion also being based on the positive correlations established by other researchers (Denev et al., 2013; Tolić et al., 2015; Poyraz Engin and Mert, 2020). The generalized comparative analysis of the obtained results highlighted the fact that the cultivar 'Viking' recorded the maximum value of phenolic compounds, followed by the fruits of the cultivar 'Nero', which also demonstrated a maximum content of ascorbic acid, reducing sugars and tannins (table 1.). Probably these two cultivars also possess more appreciable properties than the cultivar ˈAlecsandrinaˈ and can be considered superior in terms of nutritional value.

Table 1.

Comparative values of biochemical compounds, quantified in the fruits of three cultivars of *Aronia melanocarpa*

Cultivar	The value of the biochemical parameter				
	ascorbic acid	*reducing sugars*	*titratable acidity*	*tannins*	*phenolic compounds*
ˈVikingˈ	minimal	minimal	average	average	maximal
'Nero'	maximal	maximal	minimal	maximal	average
ˈAlecsandrinaˈ	avrage	average	maximal	minimal	minimal

CONCLUSIONS

The quantitative analysis of ascorbic acid showed close values (28.098 mg/100 g and 27.199 mg/100 g) in the fruits of the cultivars 'Nero' and 'Alecsandrina' and significantly lower (23.801 mg/100 g) in the fruits of the cultivar 'Viking'. Similar to ascorbic acid, the maximum recorded value of reducing sugars (7.11%) was characteristic of the fruits harvested from the cultivar 'Nero'. However, the variation in the values of this parameter is much smaller and is about 6%.

The concentration of titratable acids (%), expressed in malic/citric acid, showed values between 0.813 and 0.955%, and the maximum content is characteristic of the chokeberry fruits harvested from plants of the cultivar 'Alecsandrina'. The fruits of the cultivars 'Nero' and 'Viking' showed values that were about 9% lower, but very close.

The quantification of tannins demonstrated a content of 3.118% in the fruits of the cultivar ‘Nero’, being the maximum amount detected in the investigated cultivars, and the fruits collected from this cultivar are the darkest in color. The large variation of this parameter demonstrates its greater dependence on the genotype than on external factors.

The content of phenolic compounds proved to be very high in all investigated cultivars (2152.97 – 3202.73 mg GAE / 100 g d. m.), and this fact gives them significant antioxidant properties and they are recommended for consumption as fruits with considerable nutritional value.

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