THE ANTIOXIDANT POTENTIAL OF THE CHOKEBERRY (*ARONIA MELANOCARPA L.)* JUICES FROM BOSNIA AND HERZEGOVINA

PROFESSIONAL PAPER

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ABSTRACT:
In addition to their undeniable importance for industry, natural plant products are widely used in the production of functional food, which, in addition to satisfying nutritional properties, also exhibits certain pharmacological and physiological effects on human health. Chokeberry fruit and products are considered to be excellent sources of polyphenolic compounds. A large part of polyphenolic compounds from aronia berries is found in the juice, but the flesh of the berry that remains behind in the juice production process is also rich in these bioactive compounds. The aim of the conducted research was to examine the antioxidant potential of the chokeberry juices from Bosnia and Herzegovina. The antioxidant potential of chokeberry juices in this study was tested by the ferric reducing antioxidant power (FRAP) and total phenol content (FC). Tests were carried out in other juices for the same parameters in order to compare the obtained values. The research results show that the values of FRAP and total phenols in chokeberry juices vary (20-63 mmol Fe II/L; 850-4930 mg GAE/L), but are significantly higher than in other analyzed juices (7-25 mmol Fe II/L; 220–1265 mg GAE/L). By comparing the content of total phenols and FRAP values in pasteurized and unpasteurized chokeberry juice samples, it can be concluded that they are higher in the sample prepared by the pasteurization process. Statistical parameters show that the linear correlation between the total phenols and the FRAP values of chokeberry juices ($r=0,964$; $p <0,001$) and other analysed juices ($r=0,960$;$p <0,001$) is statistically significant.

KEYWORDS: chokeberry (*Aronia melanocarpa L.)* juice, antioxidant potential, FC, FRAP

INTRODUCTION

Nowadays, we struggle day by day with diseases related to lifestyle, unhealthy diet and insufficient physical activity. Nature has given us hundreds of different antioxidants that protect our body from the oxidation process, which we can find in fruits, vegetables, grains, etc. In the last few years, the world's scientific community has focused on the research of natural biomolecules. Among the many plant species rich in antioxidants, berries stand out.

Aronia is a genus of deciduous shrubs native to the eastern parts of North America. Indians are also mentioned as the first users, who noticed the therapeutic properties of this plant and allegedly used it to make teas. Its application has spread to the territory of Russia and European countries. Thanks to the medicinal effect of its chemical components, aronia is increasingly used in the food industry in the production of: juices, teas, jams, wine and as a natural food colouring. Researching the chemical composition and biological effects of aronia has become popular in recent years. Numerous studies have shown that moderate consumption of aronia products has a beneficial effect on human health. The chemical composition of chokeberry fruit is directly related to its biological activity and potential use in the therapy of many diseases[1]. These effects are mainly attributed to chokeberry phenolic compounds.

In a study where 143 different plant species were analysed for polyphenol content, the highest proportion was found in chokeberry. The authors associate the content of anthocyanins and organic acids with the type and maturity of the fruit. The authors point out that the anthocyanin content can increase between mid-August and mid-September, which means that the proportion of polyphenolic compounds is influenced by: climate, harvest time and chokeberry type [2]. When determining the concentration of polyphenolic compounds, the chosen method of analysis can also affect it.

In addition to polyphenols, chokeberry fruits also contain other bioactive compounds: vitamins, minerals, carotenoids, pectins, organic acids, proteins and carbohydrates. Compared to other berries, chokeberry is one of the richest sources of carotenoids. Among these ingredients, there are vitamins with antioxidant activity, vitamins C and E. Aronia berries have a higher content of polyphenols (especially anthocyanins) than most other fruits. The high content of polyphenols is associated with a very high antioxidant activity. Arginine, tyrosine, histidine, lysine, cysteine, α-alanine, asparagine, serine, glutamic acid and threonine are some of the amino acids in chokeberry. Most amino acids, including essential ones, are found in pomace. Chokeberry pomace is a rich source of dietary fiber, which makes up about 70% of the dry matter [3]. Aronia fruits and products are potentially rich sources of: K, Ca, P, Mg, Na, Fe and Zn [4].

Thanks to the presence of numerous bioactive compounds, primarily polyphenolic compounds, the following properties of chokeberry have been proven and confirmed: antioxidant, antimutagenic, anti-inflammatory, antimicrobial, cardioprotective [5-11].

EXPERIMENTAL

MATERIALS

In order to test the antioxidant activity of chokeberry juice, 13 samples from different geographical origins were collected. One sample of juice from the following locations was analysed: Bijeljina, Srebrenica, Tuzla, Livno, Brčko, Gornja Tuzla, Novi Travnik (pasteurized), Novi Travnik (unpasteurized), Sanski Most, Kalesija, Tešanj, Bosanska Gradiška and Dokanj.

In order to compare the total phenols and FRAP values of chokeberry juices with other juices, the following samples were taken for analysis: blackcurrant, blueberry, blackberry, beetroot, tomato and plum. All juices are produced in Bosnia and Herzegovina.

METHODS

DETERMINATION OF TOTAL PHENOLS

To determine the concentration of total phenols in chokeberry juice samples, 20μL of sample solution (10% v/v), 1580μL of distilled water and 100μL of Folin-Ciocalteu reagent were pipetted. After one minute, 300μL of sodium carbonate solution (200g/L) was added to this mixture, and the mixture was then incubated for 2h at room temperature. Absorption was measured spectrophotometrically (Cecil CE 2021 UV-VIS Spectrophotometer) at a wavelength of 765 nm [12].

The results were recalculated according to the calibration curve for Gallic acid:

$$y=0.00189x–0.00929$$

where y is the absorbance at 765 nm and x is the concentration of Gallic acid expressed in mg GAE/L; R^2=0,98646.

DETERMINATION OF FRAP

To determine the reducing antioxidant power of the tested juices, the method of the reducing property of iron was used [13]. For analysis, 200 μL of sample (10% v/v) and 1.8 mL of FRAP reagent were pipetted out. The FRAP reagent was prepared from 6 mL of a solution of TPTZ (10 mmol/L) in hydrochloric acid (40 mmol/L) and 6 mL of a solution of $FeCl_3 \times 6H_2O$ (20 mmol/L) mixed with 60 mL of acetate buffer (300 mmol/L; pH=3.6). The mixture was then incubated for 10 min at 37°C. As a result of the reaction of the antioxidants present in the sample and the FRAP reagent, coloured solutions are obtained. After that, the absorption is measured spectrophotometrically at a wavelength of 593 nm.

FRAP values were calculated according to the calibration curve for $FeSO_4 \times 7H_2O$:

$$y=0.47268x-0.04447$$

where y is the absorbance at 593 nm and x is the concentration of $FeSO_4 \times 7H_2O$ expressed in mmol FeII/L; R^2=0,98939.

STATISTICAL ANALYSIS

The direction and magnitude of corelation between variables was done using analysis of variance (Minitab relase 13,32; 2000; statistical software) and quantified by the correlation factor „r“. The p-values less than 0,001 were considered statistically significant.

RESULTS AND DISCUSSION

The highest concentration of total polyphenols was measured in a sample of chokeberry juice from the Dokanj and is 4930.105mg/L GAE. The following are the concentrations measured in samples from Brčko > Tuzla > Bosanska Gradiška > Sanski Most > Srebrenica > Kalesija > Livno > Bijeljina > Novi Travnik (pasteurized) > Gornja Tuzla > Novi Travnik (unpasteurized), while the lowest concentration of total polyphenols was measured in a sample of juice in Tešanj (850.211mgGA/L) (Table 1).

Table 1. Total phenols concentration of chokeberry juice samples

	Geographical origin of the chokeberry sample	Concentration (mg GAE/L)
1.	Bijeljina	2203.121
2.	Srebrenica	3476.137
3.	Tuzla	4087.248
4.	Livno	2614.232
5.	Brčko	4421.111
6.	Gornja Tuzla	1853.386
7.	Novi Travnik (pasteurized)	2054.440
8.	Novi Travnik (unpasteurized)	1484.603
9.	Sanski Most	3520.052
10.	Kalesija	2909.470
11.	Tešanj	850.211
12.	Bosanska Gradiška	3769.259
13.	Dokanj	4930.105

Table 2. Total phenols concentration of other analysed juices

	Sample	Concentration (mg GAE/L)
1.	Black currant	1265.555
2.	Blueberry	1171.904
3.	Blackberry	757.089
4.	Beet	974.550
5.	Tomato	268.730
6.	Plum	227.989

Of all the other analysed juices, the highest concentration of total phenols was measured in black currant juice, followed by: blueberry > beet > blackberry > tomato and plum (Table 2). In an analysis that included different chokeberry varieties from two growing seasons, a wide range of total phenol values was observed, ranging from 8563.8 mg/kg GAE to 12557.7 mg/kg GAE [14]. Some studies also cover different growing seasons and report total phenol values ranging from 8834mg GAE/L to 11093mg GAE/L [15].

The range of different values of the content of total phenols in the literature may be a consequence of differences in sample preparation methods, applied analytical procedures, different storage, but also different chokeberry varieties [16]. It should also be borne in mind that in the case of determining total phenols, different ways and conditions of growing plants must be taken into account. The content of total phenols and individual polyphenolic compounds of different types of berries depends on many factors, such as: growing conditions (location, growing techniques, ripening stage), processing and storage. Large variations and discrepancies in the total content of phenols during different growing seasons are the result of different air temperatures, sunlight and rainfall intensity [17]. By comparing the content of phenols in pasteurized and unpasteurized juice samples, it can be concluded that it is higher in the sample prepared by the pasteurization process.

Heat treatment is the most commonly used conservation technique. Pasteurization is one of the methods of heat treatment and takes place at a temperature of 70-80°C. Although thermal treatments cause some harmful effects, such as the loss of some nutrients (vitamin C), it also exhibits many beneficial effects, such as improving the extraction of total phenols and extending the shelf life. The results of research carried out by Kim et al. show that thermal processing can help preserve nutritional values and slow down changes in the quality of fruits and vegetables related to phenols [18]. Also, some other examples involving the improvement of the overall phenolic composition were published earlier in research [19][20]. According to the research of the mentioned authors, the content of total phenols in the samples increases after pasteurization, which is in accordance with this research. Based on the obtained results, it can be concluded that the growth of bioactive compounds in chokeberry juice occurs due to accelerated extraction by the pasteurization process. An increase in temperature leads to the degradation of cell walls, which accelerates the release of cell contents. By comparing the content of total phenols in chokeberry juice samples with the content of total phenols in other analysed juices, it can be concluded that the content of phenols is significantly higher in favour of chokeberry (Table 2). Data presented by other authors show that chokeberry has a higher concentration of phenols compared to other berries, such as blackcurrants, blackberries and raspberries, which is in accordance with the research conducted [21].

This research included the determination of the ferring reducing antioxidant power (FRAP) of chokeberry juice as well as other juices. The results of the measured values are presented in tables 3 and 4.

The highest FRAP values was measured in a sample of chokeberry juice from the Dokanj and is 63.4947 mmolFeII/L. A slightly lower concentration was shown by the sample from the Brčko (60.1852 mmolFeII/L), while the lowest was measured in the juice sample from Tešanj (20.0721 mmolFeII/L) (Table 3). The differences in the obtained results may be related to the method of cultivation and processing of chokeberry In order to fully explain the obtained results, further research is needed that focuses on different processing methods, including: crushing, thermal treatments and drying.

Table 3. Antioxidant capacity of chokeberry juices

	Geographical origin of the chokeberry sample	Concentration (mmol FeII/L)
1.	Bijeljina	44.5665
2.	Srebrenica	47.9176
3.	Tuzla	57.6853
4.	Livno	46.5467
5.	Brčko	60.1859
6.	Gornja Tuzla	32.2368
7.	Novi Travnik (pasteurized)	41.6956
8.	Novi Travnik (unpasteurized)	28.4499
9.	Sanski Most	52.4471
10.	Kalesija	47.0777
11.	Tešanj	20.0721
12.	Bosanska Gradiška	56.7502
13.	Dokanj	63.4947

According to research by Jakobek et al., the antioxidant activity of chokeberry juice (DPPH) was stronger than the antioxidant activity of black currant, elderberry, red currant, strawberry, red raspberry and cherry samples [22]. In order to compare the reducing antioxidant power of chokeberry juice with other berry juices, the following juices were analysed: blackcurrants, blueberries and blackberries. It was observed that the majority of chokeberry samples show a much higher FRAP values than the mentioned juices. By comparing FRAP values of chokeberry juice with beet, plum and tomato samples, it was also proven that chokeberry shows a much stronger reducing antioxidant power (Table 4).

Table 4. FRAP values of other analysed juices

	Sample	Concentration (mmol FeII/L)
1.	Black currant	25.5473
2.	Blackberry	19.7040
3.	Blueberry	20.4466
4.	Beet	20.0234
5.	Tomato	9.1810
6.	Plum	7.7424

In order to confirm the relationship between ferring reducing antioxidant power of juices and the content of total phenols, a correlation analysis was performed (Figure 1). It is proved a high correlation between the analysed parameters ($r=0.964$; $p<0,001$). The data are consistent with the results of other researchers and confirm the fact that high concentrations of total phenol contribute to higher reducing antioxidant potential [23].

Correlation analysis of blackcurrant, blackberry, blueberry, beetroot, tomato and plum juice samples also proved a high correlation between FRAP and total phenols content ($r=0.960$; $p<0,01$) (Figure 2).

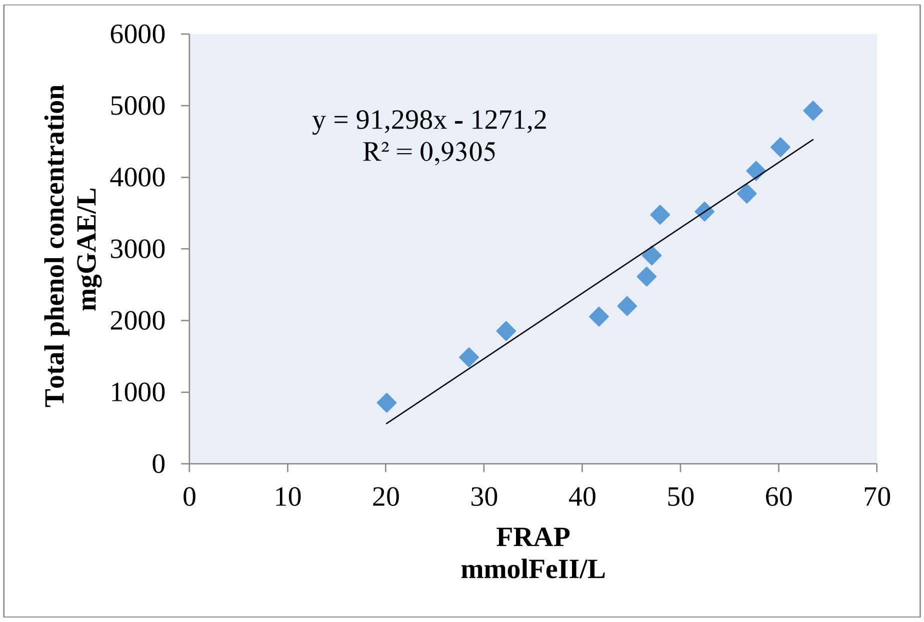

Figure 1. Correlation of FRAP and total phenol concentration of chokeberry juice

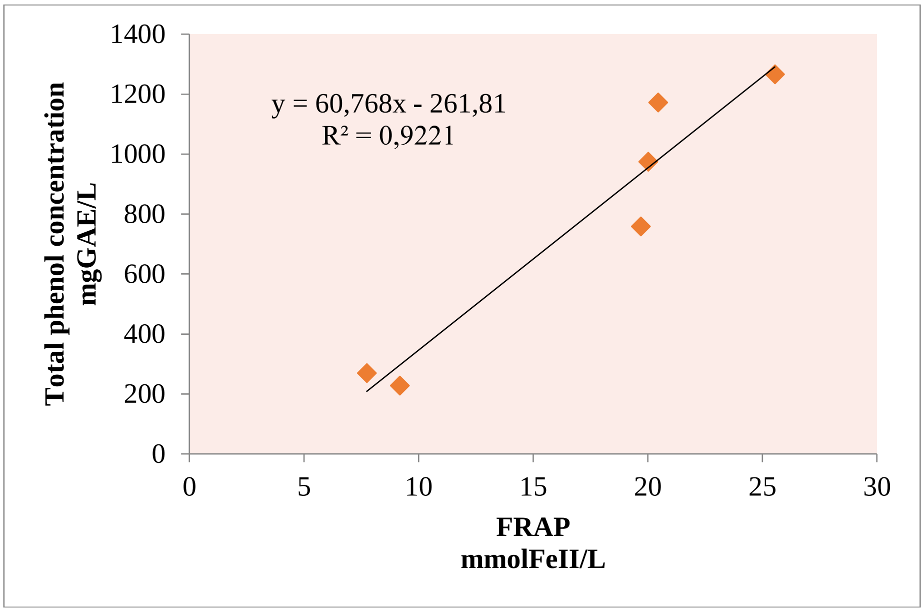

Figure 2. Correlation of FRAP and total phenol concentration of other juices

CONCLUSIONS

The research showed that all chokeberry juices (except for the juice produced in Tešanj) show a much higher antioxidant potential than the other analysed juices (blackcurrant, blueberry, blackberry, beetroot, tomato, plum). No influence of geographical area was observed, but the range of measured concentrations may be due to differences in juice preparation methods and storage methods. In the case of determining total phenols and ferring reducing antioxidant power, different ways and conditions of growing plants, air temperature, sunlight, intensity of precipitation must be taken into account. We are of the opinion that the processing method of chokeberry juice played a crucial role in this research. By comparing the content of total phenols and FRAP values in pasteurized and unpasteurized chokeberry juice, it was observed that the analysed parameters were higher in the sample prepared by the pasteurization process. The growth of bioactive compounds in the juice occurs due to the accelerated extraction of phenols at higher temperatures, which can be explained by the degradation of cell walls, where the increase in temperature accelerates the release of cell contents. There is a highly significant correlation between FRAP values and total phenol content of chokeberry juices and other analysed juices.

The conducted research gives importance to the natural resources of Bosnia and Herzegovina in the area of food quality. Since this kind of research has not been done in chokeberry juice samples from Bosnia and Herzegovina, it undoubtedly represents a contribution to the analysis and promotion of domestic products.

REFERENCES

[1] Borowska S, Brzóska MM. Chokeberries (Aronia melanocarpa) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics. Compr Rev Food Sci Food Saf. 2016,15(6): 982-1017.

[2] Jeppsson N, Johansson R. Changes in fruit quality in black chokeberry (Aronia melanocarpa) during maturation. J Hortic Sci Biotechnol. 2000,75: 34-67.

[3] Nawirska A, Uklańska C. Waste products from fruit and vegetable processing as potential sources for food enrichment in dietary fibre. Acts Sci. Pol., Technol. Aliment. 2008,7(2): 35-42.

[4] Červenka L. Moisture adsorption shcharacteristics of Black currant (Ribes nigrum L.), Black eldeberry (Sambucus nigra L.) and Chokeberry (Aronia melanocarpa, [Minchx.] Ell.) samples at different temperatures. 2011, 34(5): 1419-1434.

[5] Tarko T, Duda-Chodak A, Sroka P, Satora P, Michalik J. Transformations of poliphenolic compounds in an vitro model simulating the human alimentary tract. Food Technol Biotechnol. 2009, 47: 456-463.

[6] Kokotkiewicz A, Jaremicz Z, Luckiewicz M. Aronia Plants: A review of traditional use, biological activities and perspectives for modern medicine. J Med Food, 2010, 13(2): 255-269.

[7] Liepiņa I, Nikolajeva V, Jākobsone I. Antimicrobial activity of extracts from fruits of Aronia melanocarpa and Sorbus aucuparia. Environmental and Experimental Biology.2013, 11: 195-199.

[8] Mitrović MD, Tasić N, Jakovljević VLj, Tasić D, Mitrović NM. Potencijalna uloga polifenolnog standardizovanog ekstrakta aronije (Aronia melanocarpa) na COVID-19 infekciju izazvanu SARS-CoV-2 virusom i njegove

kardiometaboličke komplikacije. PONS Med J. 2021, 18(1): 29-40.

[9] Hensel A, Bauer R, Heinrich M et al. Challenges at the Time of COVID-19: opportunities and innovations in antivirals from nature. Plant Med. 2020,86: 659-64.

[10] Appel K, Meiser P, Millán E, Collado JA, Rose T, Gras CC, Muñoz E. Chokeberry (Aronia melanocarpa (Michx.) Elliot) concentrate inhibits NF-kB and synergizes with selenium to inhibit the release of pro-inflammatory mediators in macrophages. Fitoterapia. 2015,105: 73-82.

[11] Thani NAA, Keshavaez S, Lwaleed BA, Cooper AJ, Roopnai HK. Cytotoxicity of gemcitabine enhanced by polyphenolics from Aronia melanocarpa in pancreatic cancer cell line AsPC-1. 2014, J Clin Pathol. 67: 949-54.

[12] Waterhouse AL. Determination of total phenolics. In: Wrolstad RE, editor. Current protocols in food analytical chemistry. New York, NY, USA: John Wiley and Sons Inc.,2002, pp.I 1.1.1.-1.1.8.

[13] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem. 1996, 239: 70-76.

[14] Jurgoński A, Juśkiewicz J, Zduńczyk. Ingestion of black chokeberry fruit extract leads to intestinal and systematic changes in a rat model of prediabetes and hyperlipidemia. Plant Food Hum Nutr. 2008, 63: 176-82.

[15] Tolić MT, Jurčević IL,Krbavčić IP, Marković K and Vahčić N. Phenolic Content Antioxidant Capacity and Quality of Chokeberry (Aronia melanocarpa) Products. Food Technol Biotechnol. 2015, 53(2): 171-179.

[16] Denev PN, Kratchanov CG, Ciz M, Lojek A and Kratchanova MG. Bioavailability and Antioxidanr Activity of Black Chokeberry (Aronia melanocarpa) Polyphenols: in vitro and in vivio Evidences and Possible Mechanisms of Action: a review. Institute of Food Technologists. 2012, 11

[17] Tolić MT, Krbavčić IP, Vujević P, Milinović B, Jurčević IL, Vahčić N. Effects of Weather Conditions on Phenolic Content and Antioxidant Capacity in Juice of Chokeberries (Aronia melanocarpa L.). 2017, Pol J Food Nutr Sci. 67: 67-74.

[18] Kim N, Kim HJ, Kerr WL, Chou SG. The effect of grinding at various vaccum levels on the color, phenolics and antioxidant properties of apple. Food Chem. 2016, 216: 234-242.

[19] Gil-Izquierdo A, Gil MI, Ferreres F. Effect of processing techniques at industrial scale on orange juice antioxidant and beneficial health compounds. J Agric Food Chem. 2002, 50(18): 5107-14.

[20] Barba FJ, Esteve MH, Frigola A. Asorbic acid is the only bioactive that is better preserved by high hydrostatic pressure than by thermal treatment of a vegetable beverage. J Agric Food Chem. 2010, 58: 10070-10075.

[21] Benvenuti S, Pellati F, Melegari M, Bertelli D. Polyphenols, anthocyanins, ascorbic acid, and radical scavening activity of Rubus, Ribes and Aronia. J Food Sci. 2004, 69(3): 164-169.

[22] Jakobek L, Šeruga M, Medvidović - Kosanović M and Novak I. Antioxidant activity and polyphenols of Aronia in comparison to other berry species. Agric Conspec Sci. 2007,72:301-6.

[23] Kapci B, Neradová E, Čížková H, Voldřich M, Rajchl A, Capanoglu E. Investigating the antioxidant potential of chokeberry (Aronia melanocarpa) products. J Food Nutr Res. 2013, 52: 219-229.