ANTIOXIDATIVE ACTIVITY AND REDUCTION ABILITY OF JUICE FROM ARONIA

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ABSTRACT:

Aronia is a type of berry fruits that possess the nutritional qualities that are desirable in human nutrition. It is one of the leading fruits in the total content of polyphenols, due to which it is attributed strong antioxidant properties. Proanthocyanidins and anthocyanins constitute the largest proportion of total polyphenols and antiradical activity. The biological activity of these compounds is conditioned primarily by their antioxidant capacity, and of great interest for nutritionists and food technologists because of the possibility of using as ingredients of functional foods.
Spectrophotometrically, the antioxidant activity on DPPH radicals and the reduction ability of the Oyaiz method are determined, which is based on monitoring the absorbance at a wavelength of 700 nm, depending on the concentration of the extract. Reduction of Fe^{3+} ions is often used as an indicator of the ability to donate the electrons of the test substances. In the presence of antioxidants, Fe^{3+} reduces to Fe^{2+}. The increase in the measured absorbance indicates an increase in the reductive ability.

1. INTRODUCTION

Aronia is a kind of deciduous shrub from the Rosaceae family, native to North America and most commonly grown in moist forest and wetlands. The most famous and most cultivated is *Aronia melanocarpa* so-called black-headed chokeberry whose bush reaches a height of 3 meters and a length of 2.5 meters. After the development of the oval leaves of the serrated edge, the first white flowers appeared and collected into bunches [1]. The fruits of aronia are round to flattened, covered with an ash coating. There are 5-8 seeds in the fruit, and 15-20 fruits make up the cluster. They ripen from mid-August, and harvest is possible throughout September, as the fruits don't fall off. The flesh of the fruit has an intense red color of sweet to acidic and somewhat aromatic taste, reminiscent of immature blueberries. They are used in the diet because they have amazing nutritional values. The richness of aromas can be mostly due to the presence of amygdala's bitter taste and a mild aroma on almonds and tannins [2], which are rarely used in fresh form and is usually processed into various products such as juices, jams. wines and carbonated drinks [2, 3].
It has been shown that the total antioxidant potential of individual berries depends on the structure of the phenolic compounds present, as well as their total content. Phenolic compounds or polyphenols represent the most abundant and widespread group of biologically active substances in the plant world. So far, more than 8000 different polyphenols have been detected. They are products of secondary metabolism of plants, and their presence in animal tissues is a consequence of consuming plant foods [4]. Aronia fruits are distinguished among berries by their antioxidant activity or polyphenol content. Aronia juice has also been shown to exhibit up to 4 times more antioxidant

activity than other polyphenols-rich drinks, such as blueberry juice, currant juice, and red wine [5, 6]. Determining the antioxidant capacity of fruit juices is significant from the aspect of assessing their positive impact on human health [7]

The proportion of phenolic compounds is influenced by several factors, some of them are climate, harvesting time and aronia type, while the amount of analysis may be influenced by the method of chosen analysis. A mature plant increases the proportion of anthocyanins and reduces the proportion of phenolic acids [8]. Increased anthocyanin content is a major target of black aronia cultivation [9, 10] Due to their high content, anthocyanins contribute most to the overall antioxidant activity of the fruits as well as the juice derived from them. More specifically, anthocyanins contribute to the total antioxidant activity of the fruit at 33 %, while the contribution to the juice is slightly higher and amounts to 42 % of the total antioxidant capacity [11]. As a further factor, the proportion of phenolic components is imposed by processing technology, so lower anthocyanin concentrations in juices can be caused by pasteurization processes [12]. Elevated temperature and the presence of oxygen can lead to the breakdown of anthocyanins and the formation of brown products [13].

Phenolic compounds act as free radical traps (hydroxyl radicals, hydroperoxide radicals, superoxide anion radicals, etc.). They prevent their oxidation, they act antioxidant, protecting against various damage to the cell membrane, enzymes and genetic material in the human body [14, 15]. Reduction capacity is associated with antioxidant activity and can serve as a significant indicator of antioxidant activity [16]. Higher absorbance indicates greater reductive capacity. In this method, the yellow color of the test solution changes to different shades of green or blue, depending on the reducing power of the components present in the test sample. In the presence of reductants (antioxidant components), the Fe^{3+} fercyanide complex is reduced to Fe^{2+} form, and this transformation is monitored at a wavelength of 700 nm by measuring the formation of Perl's Prussian blue [17]. When determining antioxidant activity, it is usually recommended to use more than one method because of the limited and poor selectivity of routine spectrophotometric methods [18].

2. MATERIAL AND METHOD OF WORK

Aronia samples were collected at the end of the season in late August 2018 from the experimental orchards of Biotechnical Faculty in Bihac. The juice is separated from the pulp by pressing, bottling and storing at 4°C. All analyzes were conducted in triplicate. Aronia juice was used in the studies performed. The juice was pasteurized at 80°C for 10 minutes and kept in a 1 L glass bottle in a refrigerator at +4°C until the experiment was carried out.

The total reduction power (RP) of aronia juice samples was determined by spectrophotometric method according to Oyaiz [18], which is based on the absorbance monitoring at a wavelength of 700 nm, depending on the concentration of the sample. The colors of the solution range from the yellow color of the blank, which originates from Fe^{3+}, to the different shades of blue and green (derived from Fe^{2+} ions in solution). The presence of reducing agents, ie antioxidants, leads to the reduction of Fe^{3+} to Fe^{2+} ion. Aronia juice sample and gallic acid vitamin solution in water of different concentrations (0.01; 0.02; 0.05; 0.1; 0.2 and 0.5 mg/ml) were mixed with phosphate buffer (2.5 ml , 0.2 M, pH 6.6) and 2.5 ml of 1% potassiumferricyanide [$K_3Fe(CN)_6$]. The resulting mixture was incubated for 20 minutes at 50°C. After incubation, 2.5 ml of 10 % trichloroacetic acid was added to the mixture and it was centrifuged for 10 minutes at 3.000 rpm. To the resulting supernatant (2.5 ml) was added redistilled water (2.5 ml) and 0.1% $FeCl_3$ (0.5 ml). The absorbance of the sample was measured at a wavelength of 700 nm. by measuring the formation of the Pearl Prussian Blue [17]. The procedure was repeated three times for each sample and for each concentration. Based on the measured absorbance of the samples, the ability to reduce the Fe^{3+} ion to Fe^{2+} was determined from the calibration line of standard gallic acid solution ($A = 0.3021$ [GAE] + 0.0112, $n = 6$, $r^2 = 0.9998$) and the result was expressed as mmol GAE/100ml.

Total phenol content in prepared juices was determined with Folin-Ciocalteu method [20], and the results were calculated from the calibration curve of gallic acid. Total phenol content is expressed as mg/g gallic acid equivalents (GAE)/L of sample. 0.2 mL of sample, 1.8 mL of distilled water, 10 mL of Folin-Ciocalteu reagent and 8 mL of sodium carbonate solution was transferred with a pipette into a test tube. After the sample had been allowed to stand for 2 hours at room temperature, photoLlab 6600 UV-VIS WTW Spectrophotometer was used at 765 nm to measure the absorbance.

A modified colorimetric method with $AlCl_3$ was used to estimate total flavonoid content [21], and standard quercetine solution was used to make the calibration curve. Iodometric titration was used to determine the amount of vitamin C according to Helmenstine Anne Maria [21]. Total anthocyanin concentration was determined by the pH differential method [22], which is based on the anthocyanin structural transformation that occurs with a change in pH. Anthocyanin concentration is calculated thus:

$$\text{Anthocyanin} = \frac{(\text{A x M x FR x } 1000)}{\varepsilon \text{ x l}} \quad \text{mg/kg}$$

A - Absorbance of a sample, which is calculated thus
$A = (A_{513} - A_{700})$ pH 1 - $(A_{513} - A_{700})$ pH 4,5
M - 449,2
FR – dilution factor (DF)
ε - Molar absorptivity, 26 900
l - the length of the cuvette, 1 cm
(M and ε were taken as a dominant type of anthocyanin, namely for cyanidin-3-glucoside)

Antioxidant activity was tested by modified DPPH assay [23]. Standard Trolox solution (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in methanol was used to make the calibration curve. DPPH radical is a stable nitrogen radical with dark purple colour and adding antioxidants induces discolouration because the free radical is reduced into light yellow diphenylpicrylhydrazyl. The decrease in the absorption is measured spectrometrically at 517 nm.
200 µl of sample, 3.8 ml of methanol, 1 ml 0.5 mM of DPPH solution was transferred into a test tube and methanol was used as blank. Test tubes were left in the dark for 20 minutes. Afterwards, absorbance was measured at 517 nm.
30 mM of Trolox was used to make the calibration curve by weighting 0.7509 g of Trolox. The weighted Trolox is dissolved in methanol and methanol is added up to the marking on the volumetric flask. Dissolvents were prepared in volumetric flasks up to 25 mL in concentrations of 12.5; 25; 50; 75; 125; 250; 375, and 500 µg/ml using 30 mM of Trolox solution.

3. RESULTS AND DISCUSSION

The most abundant representatives of phenolic components are flavonoids with the highest amounts found in aronia juice averaging 3180 mg GAE/l [12]. These values are much lower than our results. Aronia is a significant source of flavonoids, with quercetin derivatives being the most prevalent of flavonoid compounds. Earlier studies Jakobek et al. [18] analyzed aronia berry extracts prepared by grinding in hot ethanol acidified with hydrochloric acid and filtered, listing values for total phenol and anthocyanin content of 10637 mg/kg and 4341 mg/kg, respectively. These values are much higher than our results. In our studies, the mean of total phenols and anthocyanins were 6381mg/l and 2521 mg/l, respectively.

Table 1. Total phenol content (TP), flavonoids, anthocyanin, and vitamin C of aronia juice

Sample (n = 6)	TP mg/L	Flavonoids mg Q/L	Anthocya-nin mg/L	Vitamin C mg/100 mL
1	6866	5243	2924	463.5
2	6713	5089	2743	340.1
3	6486	4876	2561	442.7
4	5572	4523	2012	431.8
5	6763	5024	2654	442.9
6	5891	4776	2235	428.1
AV	6381	4921	2521	424.9
SD	528.6	254.5	338.2	43.3

n – number of samples, AV – average value, SD = standard deviation,

The antioxidant effect is also achieved by interactions of flavonoids and other molecules such as vitamins. A synergistic effect was observed in the interaction of vitamin C and quercetin, but also in α-tocopherol and some polyphenolic compounds [24]. Aronia can be involved in defending the body against oxidative stress mainly due to the presence of polyphenolic compounds. As oxidative stress is a risk factor in the development of many diseases, it is recommended to consume foods that are a natural source of antioxidant components [25].

Table 2. Antioxidant activity values using DPPH test and total reductive power (RP) of of aronia juice

Sample (n = 6)	DPPH test mmolTE/100ml	Total reduction power (RP) mmol GAE/100ml
1	1.37	13.82
2	1.13	12.26
3	1.26	11.95
4	1.41	14.48
5	1.29	12.72
6	1.41	15.63
AV	1.31	13.48
SD	0.108	1.424

Benvenuti et al. [26] indicate that berries, aronies, blueberries and black currants have extremely good antioxidant activity. The same authors cited values of total phenols ranging from 140.6 to 888.5 mg/100 g fresh weight (FW), total anthocyanins ranging from 22.0 to 460.5 mg/100 g FW, and reduced ascorbic acid ranged from 12.4 to 153.8 mg/100 g FW. The average EC50 values for *Aronia melanocarpa, Ribes nigrum, Ribes rubrum, Rubus fruticosus* and *Rubus idaeus* were 1.8, 2.8, 5.3, 6.4 and 8.2 mg FW, respectively. Bermúdez-Soto et al. [27] state in their studies that the antioxidant activity of aronia juice produced by DPPH test was better than the antioxidant activity of strawberry and raspberry juice. In our studies, the mean value of the antioxidant activity of chokeberry juice was 1.31 mmolTE/100 ml. In studies Mitic et al. [28], made on samples of cherry (*Prunus cerasus*), raspberry (*Rubus idaeus*), blackberry (*Rubus fructicosus*), thorns (*Prunus spinosa*) and aronia (*Aronia melanocarpa*) were collected in full in Nis, they found that the highest total reductive power of blackberry [(0.1920 ± 0.0042) µmol ascorbic acid Eq/mg dw] and the lowest for raspberry [(0.1180 ± 0.0035) µmol ascorbic acid Eq/mg dw]. In their studies, the total reductive power of aronia was 0.1540 ± 0.0049 (µmol ascorbic acid Eq/mg dw). The total phenols from the frozen fruit were

separated by extraction using ethyl acetate solvent and then evaporated to dryness at 35°C. In our studies, the mean for the total reducing power was 13.48 mmol GAE/100ml.

4. CONCLUSION

Based on the research, it can be concluded that Aronia juice (*Aronia melanocarpa*) is very rich source of biologically active phytochemical compounds whic h are known to have a positive effect on human health. Due to its high content, anthocyanins contribute most to the overall antioxidant activity of aronia juice. Interest in polyphenolic antioxidants has increased significantly in recent decades, due to their increased capacity to remove free radicals associated with various human diseases. The results of this study suggest a further analysis of the chemical composition of the samples of juice and other aronia products in order to identify in more detail the values of the content of the compounds that may be responsible for the antioxidant activity.

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