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# PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF FRUITS OF TWO ARONIA SPECIES GROWN IN BULGARIA

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**Abstract:** Today, the interest in the application of natural phytonutrients in various types of foods products is extremely high. Unconventional sources of biologically active substances are being sought to be used in the prevention of various chronic diseases. With a view to their future application in food products, the aim of this study is a comparative analysis of the chemical composition of fruits of two species of Aronia - *Aronia melanocarpa* (Michx) Elliott (black) and *Aronia arbutifolia* (L.) Pers (red), grown in two regions of the country. Some physical parameters (width, length, weight) and chemical composition (fat content, pectin, tannins, total polyphenols, ascorbic acid, fibers, glucose and reducing sugars, and mineral composition) were determined for the berries. The antioxidant activity was also determined by two methods - DPPH and FRAP. The data indicates that red Aronia berries are larger in size and have a higher weight (1.92 g). In them, the content of fibers (53.20 %), tannins (0.90 %), fat (0.90 %), ascorbic acid (124 g/100 g) and total polyphenols (4269.40 mg GAE·kg$^{-1}$) is higher compared to black chokeberry fruits. However, they contain more pectin (3.50 g·kg$^{-1}$) and sugars (7.50 g/100 g. Black Aronia berries have higher antioxidant activity - DPPH 289.5 µmol TE/100 g and FRAP 152.3 mM TE· g$^{-1}$. The fruits of both Aronia species are a suitable source for obtaining biologically active substances and phytonutrients for use in various food products.

**Keywords:** *antioxidant activity, Aronia melanocarpa (Michx.), chemical composition, Elliott, Aronia arbutifolia (L.) Pers*

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## INTRODUCTION

In recent years, the attention to natural sources of antioxidants is increasing in the prevention of chronic diseases. Various fruits, seeds, flowers, leaves, and other organs of plants are used, which are directly put into food products or from which phytonutrients and other biologically active substances are extracted.
Aronia is a representative of the Rosaceae family and two species are found: black Aronia (*Aronia melanocarpa* (Michx.) Elliott) and red Aronia (*Aronia arbutifolia* (L.) Pers.) [1, 2]. Aronia is a shrub that reaches a height of 180 cm, whose berries ripen at the end of August. They are rich in various biologically active substances - anthocyanins, flavonoids and phenolic acids, proteins, vitamins, organic acids, carbohydrates, polysaccharides, fibers, mineral elements, *etc*. [3].
Aronia berries are a source of phytonutrients and have a high biological value [4]. They are a source of dietary fibers, vitamins (provitamin A, E, B1, B2, B6, P, PP), *β*-carotene, minerals (Mn, Fe, B, Mo, Cu, Mg, J, Ca) and carbohydrates (sorbitol, fructose, glucose, *etc*.), organic acids (malic, quinic, citric, *etc*.) [5].
Denev *et al*. determined the total content of polyphenolic compounds in berries - 411.53 mg/100 g [6]. The authors identified the phenolic acids as gallic (108.37 mg/100 g), chlorogenic (73.47 mg/100 g), ellagic (135.10 mg/100 g), ferulic (9.08 mg/100 g) and caffeic (45.51 mg/100 g), also quercetin (0.52 mg/100g), catechin (154.29 mg/100 g) and epicatechin (530.61 mg/100 g).
According to many studies, anthocyanins are responsible for the antioxidant properties of fruits and the food products and medicinal preparations obtained from them.
Yang *et al*., analyze the content of anthocyanins in Aronia of different varieties and stages of maturity, obtaining the best results for the species *A. melanocarpa* (224.62 mg/100 g FW) and less in *A. arbutifolia* (85.84 mg/100 g FW) [7].
Fresh Aronia berries are not consumed directly due to their astringent taste but are processed to obtain juices, syrups, jams, fruit teas and nutritional supplements [8 – 10]. The difference in the content and amount of biologically active substances in Aronia products in the literature is explained by the origin, type, variety and varieties of the raw material, as well as by the methods used for their identification and determination [11, 12].
Methods have been developed to obtain ethanolic chokeberry extracts that can be used as an antioxidant ingredient in cosmetics and medicine [13].
Aronia berries and the extracts obtained from them can be successfully used in medicine to improve the condition of the liver, to regulate hypertension, to inhibit cancer cells, *etc*. health problems [2, 14, 15].
With a view to their application in food products, the aim of this study is to make a comparative analysis of the chemical composition of fruits of two types of Aronia *A. melanocarpa* and *A. arbutifolia*, grown in different regions of Bulgaria.

## MATERIAL AND METHODS

### Material

In this study, two types of Aronia berries purchased from the commercial network were used: *A. melanocarpa* from the area of the town of Petrich and *A. arbutifolia* from the area of the village of Kuklen, Plovdiv region.
Before analysis, fruits were dried for 5 h at 105 °C and then ground in a laboratory mill (Model PRO 02; 2600 rpm) to sizes up to 0.5-3 mm.
Ground fruits were stored in double paper bags in a wooden cabinet for one year at room temperature (20 ± 2 °C) and away from direct sunlight or other heat sources.

### Methods

***Morphological description of Aronia berries***

The main morphological characteristics of Aronia berries (shape, length, width, shape index and weight) were determined for 100 fresh pseudo fruits for each of both studied species. With a digital caliper (HBM 6212, 0-150 mm), the length (L) is measuring from the handle to the cup. The diameter (D) is determined at the widest part of the pseudo fruit, perpendicular to its length. The shape index is calculated as the ratio of length to width ($L \cdot W^{-1}$). Fruit weight is measured with a Kern weighing scale (Switzerland), model LE011.

***Moisture content in Aronia berries***

It is carried out by drying at a temperature of 105 °C to a constant weight [16].

***Fat content in Aronia berries***

Fat content was analyzed by extracting the sample with an organic solvent (petroleum ether) for 12 h in a Soxhlet extractor [17].

***Pectin content in Aronia berries***

Pectin content was determined by sequentially treating the ground berries with petroleum ether, hexane and 95 % ethanol in a Soxhlet extractor to remove dyes, fats and waxes. The washed raw material is extracted twice, for 30 min at a temperature of 85 °C and periodically stirring with a hot 0.5 % solution of ammonium oxalate. It is filtered through nylon fabric (250 mesh). The filtrate is cooled to room temperature (20 ± 2 °C) and acidified with stirring of the filtrate. The pectin is precipitated with two volumes of 95 % ethanol for 2 h at room temperature (20 ± 2 °C); it is washed several times with 70 % neutral ethanol until no chloride ions are detected, twice with 95 % ethanol, once with acetone and then dried under vacuum at 40 °C. The yield of pectin is determined by weight. The degree of esterification of pectin preparations is determined in % by the neutralization method [18].

***Tannin content in Aronia berries***

The tannin content was determined by exhaustive extraction with hot water and titration of the resulting extract with 0.1 N $KMnO_4$ using indigo carmine as indicator according [16].

***Determination of total polyphenols***

In our following modification, the content of total polyphenols was determined by the Singleton and Rossi method [18]: In a 10 mL measuring tube, 0.1 mL of extract (base solution or fraction), 7 mL of distilled water, 0.5 mL Folin-Ciocalteu reagent and 1.5 mL 7.5 % (w/v) aqueous solution of sodium carbonate are sequentially dosed. After shaking, the test tubes are topped with distilled water up to the mark. After standing at rest for 2 h at room temperature (20 ± 2 °C), the absorbance of the reaction mixture at 750 nm was measured. A blank sample was similarly prepared using distilled water instead of extract. The results obtained are presented as gallic acid equivalents (mg GAE·$kg^{-1}$).

***Ascorbic acid content***

By the 2,6-dichloroindophenol titrimetric method official method for the analysis of vitamin C in juices was applied [19].

***Fiber content***

The amount of total, insoluble and soluble dietary fibers was determined using an enzyme kit K-TDFR-100A (Megazyme, Ireland), according to [20] AACC Method 32-07.01 "Determination of Soluble, Insoluble and Total Dietary Fiber in Foods and Food Products" (Last approved on 10-16-91) as well as the manufacturer's instructions of the enzyme kit.

***Glucose and reducing sugar content***

Glucose content was determined by HPLC analysis, and reducing sugars were estimated by the PAHBAH method described by Petkova and Ognyanov [21].

***Antioxidant activity of Aronia berries***

*DPPH method*

Radical scavenging capacity is determined according to Brand-Williams et al*.,* method with our following modification: 2250 µL of DPPH solution (2.4 mg DPPH in 100 mL of methanol) and 250 µL of extract (main extract or fraction) were sequentially dosed into the cuvette. A blank sample was prepared in a similar manner using methanol instead of extract. After incubation for 15 min at room temperature (20 ± 2 °C) in the dark, the absorbance of the reaction mixture was measured at 515 nm. The results obtained are presented as Trolox equivalents µmol TE/100 g [22].

*FRAP method*

The reagent was prepared by mixing previously prepared 0.3 M acetate buffer with pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3$ x $6H_2O$ in a ratio of 10:1:1. The test extract (0.1 mL) is added to 3 mL of FRAP reagent. The reaction mixture is incubated for 5 min at 37 °C in the dark. The absorbance of the colored substance formed was measured at a wavelength of 593 nm according to Benzie and Strain [23].

***Statistical analysis***

Data from triplicate experiments were processed with MS Office Excel 2010 software, using statistical functions for determination of a standard deviation (±SD). One-way

ANOVA was applied to determine differences between samples and maximum estimation error at significance levels $p < 0.05$.

## RESULTS AND DISCUSSION

### Morphological description of berries

The fruits of both species are round in shape, and information on the main morphological properties is presented in Table 1. It is evident from the data that the fruits of the species *A. arbutifolia* are longer (9.4 ÷ 12.5) compared to those of the species *A. melanocarpa* and their weight varies from 0.85 to 1.92 g.
The results on the size and weight of Aronia berries from the literature vary in different sizes - diameter 8.6 - 13.3 mm, fruit weight 0.5 - 2 g [24, 25]. Other authors, Ochmian *et al*. and Skupien and Oszmianski, found that the fruits of *Aronia melanocarpa* are 12.8 ÷ 16.2 mm in size [26, 27].
These differences in the size and weight of Aronia berries can be explained by biological factors (type and variety, harvesting time, *etc*.) and soil-climatic conditions (features of the regions, method of cultivation, *etc*.).

***Table 1***. *Morphological description of the two Aronia species*

| **Indicators** | ***Aronia melanocarpa*** | ***Aronia arbutifolia*** |
|---|---|---|
| **Width, [mm]** | | |
| max | 12.10 | 12.30 |
| min | 9.60 | 10.60 |
| SD | 0.10 | 0.11 |
| Length, [mm] | | |
| max | 11.3 | 12.5 |
| min | 8.80 | 9. 40 |
| SD | 0.10 | 0.14 |
| **Shape index, [$L \cdot D^{-1}$]** | | |
| max | 0.9 3 | 0.89 |
| min | 0.90 | 0.80 |
| SD | 0.03 | 0.08 |
| **Weight, [g]** | | |
| max | 1.62 | 1.92 |
| min | 0.60 | 0.80 |
| SD | 0.02 | 0.06 |

### The chemical composition of Aronia berries

A detailed analysis was made for the chemical composition of the two types of Aronia; the results are shown in Tables 2 and 3.

***Table 2.*** *Chemical composition of fruits of the two Aronia species*

| **Indicators** | ***Aronia melanocarpa*** | ***Aronia arbutifolia*** |
|---|---|---|
| Humidity, [%] | 22.30 ± 0.17 | 25.20 ± 0.91 |
| Total fibers, [%] | 51.50 ± 0.46 | 53.20 ± 0.50 |
| Glucose, [g/100 g] | 7.50 ± 0.66 | 2.58 ± 0.20 |
| Reducing sugars, [g/100 g] | 3.40 ± 0.30 | 1 4.80 ± 0.90 |
| Tannins, [%] | 0.60 ± 0.0 | 0.90 ± 0.50 |
| Fat, [% ] | 0.12 ± 0.0 | 0.90 ± 0.50 |
| Pectin, [$g \cdot kg^{-1}$] | 3.50 ± 0 .31 | 1.80 ± 0.30 |
| Ascorbic acid, [g/100 g] | 104 ± 0.90 | 124 ± 0.30 |
| Total polyphenols, [mg $GAE \cdot kg^{-1}$] | 1484.50 ± 0.62 | 4269.40 ± 0.60 |

***Table 3.*** *Mineral composition of fruits of the two Aronia species*

| **Indicators** | ***Aronia melanocarpa*** | ***Aronia arbutifolia*** |
|---|---|---|
| Macro elements, [$mg \cdot kg^{-1}$] | | |
| Calcium (Ca) | 138.20 ± 0.20 | 119 ± 0.80 |
| Sodium (Na) | 5.6 0 ± 0.20 | 4.27 ± 0.80 |
| Phosphorus (P) | 168 ± 0.60 | 239 ± 0.50 |
| Potassium (K) | 2850 ± 0.30 | 2780 ± 0.80 |
| Magnesium (Mg) | 165 ± 0.40 | 345 ± 0.80 |
| Microelements, [$mg \cdot kg^{-1}$] | | |
| Nickel (Ni) | 0.21 ± 0.10 | 0.85 ± 0.30 |
| Zinc (Zn) | 2.60 ± 0.70 | 4.09 ± 0.8 |
| Iron (Fe) | 25.20 ± 0.20 | 23.60 ± 0.80 |
| Toxic elements, [$mg \cdot kg^{-1}$] | | |
| Lead (Pb) | 0.06 ± 0.2 | 0.04 ± 0.20 |

The data from Table 2 show that a large part of the analyzed indicators have higher values in *A. arbutifolia* species, such as total polyphenols, reducing sugars, tannins, ascorbic acid.

In a study by Denev, various organic acids were identified, as ascorbic acid is 81.6 mg/100 g. In the researched fruits of black and red *Aronia berries*, the content of ascorbic acid is higher - 104 mg/100 g in black Aronia and 124 mg/100 g in red Aronia [28].

A number of authors define fibers as biologically active substances. In Aronia berries, their amount varies in different limits from 8.1 to 57.24 %. The obtained data on the fiber content in the studied berries of the two types of Aronia do not differ from the data in the literature - 51.5 % for black Aronia and 53.2 % for red Aronia [29].

Black Aronia berries are distinguished by higher pectin content (3.5 $g \cdot kg^{-1}$ against 1.08 $g \cdot kg^{-1}$ for red Aronia). The fruits of both types of Aronia contain almost the same amount of fiber (66.5 and 68.2 %).

Polyphenols are common constituents of plant foods such as fruits, vegetables, whole grains, tea, chocolate, *etc*. Content of polyphenols in high concentrations is found in the berries of red Aronia (4269.4 mg $GAE \cdot kg^{-1}$) compared to those of black Aronia (1484.5 mg $GAE \cdot kg^{-1}$). According to Kahkonen *et al*., and Jakobek *et al*., the total content of polyphenolic compounds in berries can reach up to 10637.20 $mg \cdot kg^{-1}$ [30, 31].

Wang and Lin reported that the phenolic content of berries was affected by maturity stage at yield, genetic differences, environmental conditions at yield, post yield storage conditions and processing [32].

It is obvious from the data presented in Table 3 that there are differences in the content of mineral elements in both types of Aronia. Studies show a complex mineral content in Aronia berries, with the highest potassium content in both species - 2850 $mg \cdot kg^{-1}$ for species *A. melanocarpa* and 2780 $mg \cdot kg^{-1}$ for the species *A. arbutifolia*. The fruits of the species *A. arbutifolia* are richer in P and Mg (239 $mg \cdot kg^{-1}$ and 345 $mg \cdot kg^{-1}$) compared to those of the species *A. melanocarpa.*

The analyses show that Aronia berries have great potential to provide sufficient amounts of Calcium, Magnesium and Phosphorus in the healthy diet of people. The content of the toxic Lead is in very low concentrations. Similar results prove Pop *et al.* [33].

**Antioxidant activity**

Testing the antioxidant activity of natural products and the beneficial effect on human health has gained popularity in recent years. The use of more than one method to assess total antioxidant activity is recommended [28].

It was found that in both methods higher antioxidant activity was observed in *A. arbutifolia* species (Figure 1).

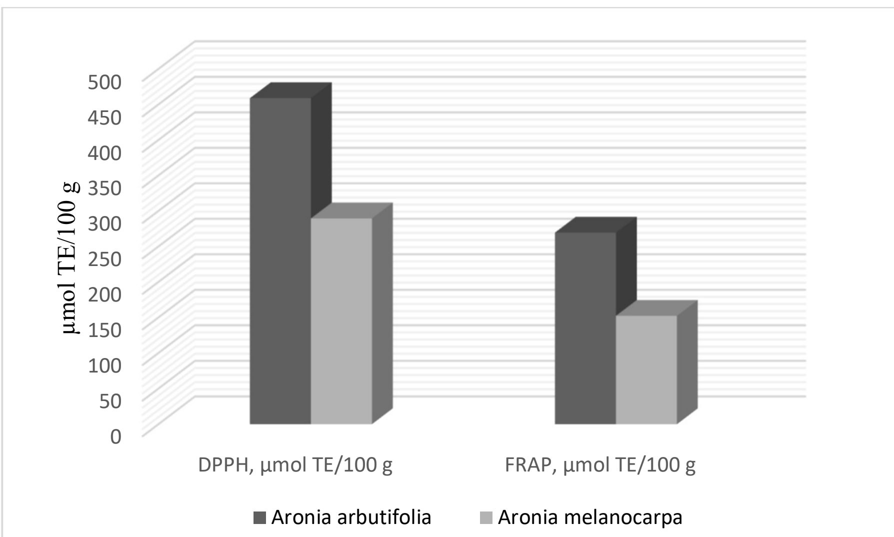


***Figure 1.*** *Antioxidant activity of Aronia berries*

The difference in antioxidant activity between the two Aronia species was significant in both determination methods. In the species *A. arbutifolia*, the antioxidant activity according to DPPH is 458.9 µmol TE/100 g, and according to FRAP - 269.5 mM $TE \cdot g^{-1}$ dry sample, while in the *A. melanocarpa* species the values are respectively: 289.5 µmol TE/100 g (DPPH) and 152.3 mM $TE \cdot g^{-1}$ dry mass per g sample (FRAP). This is probably due to the greater amount of substances with antioxidant potential presented in Table 2. These results are consistent with previous studies conducted on Aronia berries, which show that they have strong antioxidant activity, mainly due to ascorbic acid (vitamin C), polyphenols, anthocyanins, phenolic acids, flavanols, flavonols and tannins [34, 35].

## CONCLUSION

This study presents data on the chemical composition of two Aronia species grown in Bulgaria. Aronia samples differed significantly both in content and composition of mineral elements, polyphenols and antioxidant activity.

Aronia berries can be good sources of bioactive substances and can be successfully used in the food industry. The addition of plant raw materials to various food products allows the latter to be functionalized according to the legislation of the European Union. The higher antioxidant activity in combination with the rich chemical composition of Aronia berries can be successfully used to obtain functional food products, which are a subject of further studies.

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