Scientific Papers. Series B, Horticulture. Vol. LXVII, No. 1, 2023
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CONTENT OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY IN CHOKEBERRIES JUICE (*ARONIA MELANOCARPA*)

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Abstract

Aronia fruits (Aronia melanocarpa) are very rarely used in the Romanian diet, although they have the highest antioxidant properties of all fruits. Therefore, the objectives of this study were to evaluate the bioactive compounds and antioxidant activity of fresh aronia juice and dried fruit residues. The antioxidant activity of the samples was determined by DPPH assay. Total antioxidant capacity was evaluated by the phosphomolybdate method. The content of polyphenols, flavonoids and anthocyanins in the aronia samples was also investigated. The results showed that fresh aronia juice contains the highest levels of phenols, flavonoids and antioxidant activity. A high level of anthocyanins was found especially in the dried fruit residues. These results demonstrate the potential of Aronia melanocarpa as a healthy and nutritionally rich dietary food with many functionalities and benefits.

Key words*: Aronia melanocarpa, phenols, flavonoids, anthocyanins, antioxidant activity.*

INTRODUCTION

Aronia (*Aronia melanocarpa*), commonly called black chokeberry, belongs to the *Rosaceae* family and is a shrub native to North America whose fruits are highly sought after and valued for their wonderful therapeutic properties. Aronia was introduced in Europe about a century ago (Kulling & Rawel, 2008; Sainova et al., 2012; Kim et al., 2013). Aronia culture, relatively new in our country, is conquering more and more farmers, especially those with not very large plots of land. Thus, they may have the opportunity to come to the market with some fruits quoted at a better price and recognized for their extraordinary therapeutic properties. The components of the plant contain several useful bioactive compounds such as polyphenols, flavanols, and anthocyanins (Kulling & Rawel, 2008; Sharif et al., 2013; Malinowska et al., 2013) with a positive effect on the human health. Previous studies on aronia have reported that the leaves of several Aronia species are used in traditional medicine because of their anti-inflammatory, antiviral, antimicrobial, and antiproliferative activities against cancer cells (Ljubuncic et al., 2005; Martini et al., 2009). Therefore, aronia leaves might contain bioactive compounds and have biological effects resulting from the polyphenols, flavonoids, and chlorophylls that they contain. Other studies focused on the juice of the aronia fruit (Sainova et al., 2012; Kim et al., 2013; Sharif et al., 2013), aronia leaves (Nhuan Do Thi & Eun-Sun Hwang, 2014) and on Aronia wastes obtained after juice extraction because these products contain many phenolic compounds, including anthocyanins (D'Alessandro et al., 2013). Anthocyanins are phenolic compounds which are water-soluble pigments. They are responsible for imparting a variety of colors to the plants like orange, red, pink, blue and purple. It is well known that anthocyanins possess antioxidant activity and have different other pharmacological properties. Anthocyanins can be used industrially as natural colors and can be used for a wide variety of foods, cosmetics, and drugs. As a natural product, anthocyanins are good for health due to their antioxidant properties and may have a role in immunity by boosting our immune system (Wegdan et al., 2020). Data on the antioxidant activity and

phenolic content of chokeberry have been reported in several studies (Oszmianski & Wojdylo, 2005; Jakobek et al., 2007; Denev et al., 2012).
In this context, the aim of this study was to evaluate the content of total phenolics, flavonoids, and anthocyanins as well as antioxidant properties of chokeberry fresh juice and dried residue material.

MATERIALS AND METHODS

Plant material. The fresh chokeberry fruits of the 'Melrom' variety were purchased from the Research and Development Station for Fruit Tree Growing Băneasa, Bucharest (Figure 1).

Figure 1. Fresh fruits of *Aronia melanocarpa* "Melrom" variety

Samples preparation. 60 g of fruits were weighed and blended to obtain juice. The aronia residue was dried at 100°C until 1 g of dry substance was obtained. Then 1 ml of juice and 1 g of dry material were each mixed with 10 ml of methanol acidified with 2% HCl. The obtained fresh juice and the acidified methanol extracts were used for the determination of the total phenolic content (TPC), total flavonoids (TFC), total anthocyanins (TA), as well as for the determination of the antioxidant activity.
Determination of total phenolics. For determination of TPC, a method with Folin-Ciocalteu reagent (Sigma-Aldrich) (Singleton, 1999) was used. An aliquot (20 µL) of diluted chokeberry sample or standard solutions of gallic acid (25-500 mg/L) was mixed with 1580 µL of distilled water and 100 µL of Folin-Ciocalteu reagent. A volume of 300 µL of sodium carbonate solution was added to the mixture. After incubation at room temperature for 2 h, the resulting absorbance was measured by the spectrophotometer (Eppendorf UV-Vis) at the wavelength of 765 nm against the blank sample, which was used as reference. The results were calculated according to the calibration curve for gallic acid as follows: y = 0,0012x + 0,0153; R = 0,9994 (Figure 2), where: y is the absorbance at 765 nm and x is the concentration of gallic acid in mg/L; R2=0.9994. Total phenolics were expressed as mg of gallic acid equivalents (GAE) per l of chokeberry juices and as mg of GAE per g of dry matter (dm).

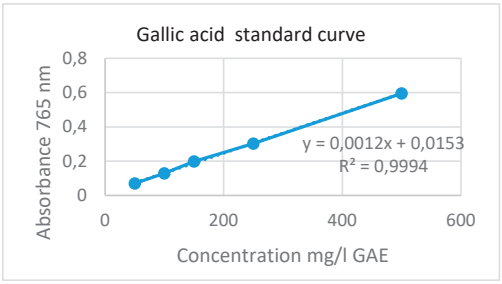

Figure 2. Gallic acid standard curve and the regression equation

Determination of total flavonoids content. The total content of flavonoids in the samples was determined by the colorimetric method with aluminum chloride (Chang et al., 2002). Quercetin was used as a standard agent, and the total flavonoid content was expressed in µg quercetin equivalent/ml. The reaction mixture consisted of: 1 ml sample/standard, 3 ml methanol, 200 µl $AlCl_3$, 200 µl 1 M potassium acetate and 5.6 ml distilled water. The absorbance of this reaction mixture was recorded at 420 nm using a UV spectrophotometer (Eppendorf UV-VIS). The concentration of flavonoids (mg quercetin equivalent/ml) in the samples was determined based on the standard calibration curve (y = 0.009x + 0.0538; R^2 = 0.9913) obtained for different concentrations of quercetin (25, 50, 100, 150 and 200 mg/ml) (Figure 3) and as mg of QE per g of dry matter (dm).

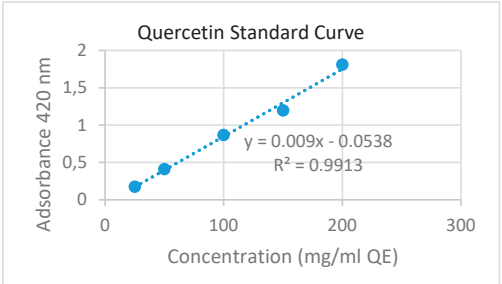

Figure 3. Quercetin standard curve and the regression equation

Determination of total anthocyanins content. Total anthocyanins content (TA) was carried out using the pH differential spectrophotometric method (Giusti & Wrolstad, 2000). Each sample was diluted 10 times to a final volume of 2 ml in two buffer solutions: potassium chloride buffer 0.025 M (pH 1.0) and sodium acetate buffer 0.4 M (pH 4.5). The diluted portions were filtered using PTFE membrane filters (0.2 mm). After filtration the absorbance of each diluted solution was measured at 520 nm against distilled water as blank and corrected for haze by measuring the absorbance at 700 nm. TA in juice was calculated according to the following formula and expressed as mg **cyanidin-3-glucoside** equivalents (CGE) per ml of chokeberry samples and as mg of CGE per 100 g of dm:

$$\frac{A \; x \; Mw \; x \; DF \; x \; 10^3}{\varepsilon \; x \; l}$$

Where:

$A\ (Abs) = (A_{520\ nm} - A_{700\ nm})pH\ 1.0 - (A_{520\ nm} - A_{700\ nm})\ pH\ 4.5;$

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF (dilution factor) = 10; l (pathlength) in cm; ε (molar extinction coefficient) = 26,900 L × mol^{-1} × cm^{-1} for cyanidin-3-glucoside; and 10^3 = conversion factor for g to mg.

Antioxidant activity

DPPH method. The free radical scavenging activity of the samples taken in the study was determined with 1, 1-diphenyl-2-picrylhydrazyl (DPPH), described by Braca et al. (2001). A volume of 200 µl of each sample of different concentrations (10-100 µg/ml) with 2 ml of 0.004% methanol solution of DPPH (0.1 mM). After 30 minutes of incubation in the dark at room temperature, the color change from dark purple to light yellow was determined at 517 nm against 1 ml methanol (blank) using a UV spectrophotometer (Eppendorf UV-VIS). Different concentrations of ascorbic acid (10-200 µg/ml) were used as a standard agent. The scavenging ability (%) was calculated as follows:

$$\%\ Inhibition = \frac{Standard\ absorbance - Crude\ extract\ absorbance}{Standard\ absorbance}$$

Ascorbic acid was used as positive standard. The antioxidant capacity of the samples was expressed as inhibitory concentration, IC_{50} µg/ml. The lower IC_{50} value indicates the greater overall effectiveness of the antioxidant. ***Total antioxidant capacity***. The total antioxidant capacity of the samples was evaluated by phosphomolybdate method (Prieto et al., 1999) using ascorbic acid as a standard (Garrat, 1964). The reaction mixture consisted of 0.3 mL extract combined with 3 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. After the samples cooled to room temperature, the absorbance of the solution was measured at 695 nm against the blank using a spectrophotometer. Methanol (0.3 ml) was used as control. The results were expressed in ascorbic acid equivalent in µg/ml extract based on the standard calibration curve (y = 0.0067x + 0.0029; R = 0.9982) obtained for different concentrations of ascorbic acid (10-200 µg/ml) (Figure 4). The higher absorbance value indicated higher antioxidant activity.

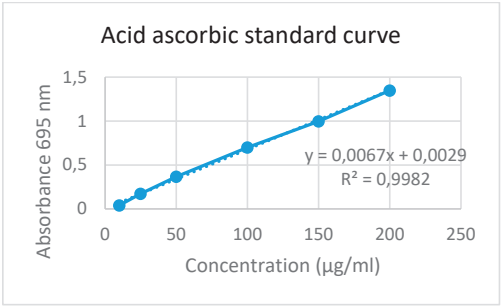

Figure 4. Ascorbic acid standard curve and regression equation

Statistical Analysis. Results were expressed as standard error of the mean (SEM) for triplicate measurements. The graphics were plotted by using Microsoft Office Excel 2010.

RESULTS AND DISCUSSIONS

Total phenolics, flavonoids and anthocyanins

Total phenolic content values were higher in crude juice (P1) (2.69 ± 0.009 mg GAE/l) than in acidified methanolic juice (P2) (0.182 ± 0.002 mg GAE/l) and dried berries residue (P3) (1.02 ± 0.024 mg GAE/g dry matter). Lower or higher values reported in the literature might have resulted from different extraction methods used for analysis, differences in analytical procedures

applied, different processing technologies and storage conditions, or differences in chokeberry cultivars (Denev et al., 2012). Researchers report processing influences the phenolic content of final products reaching consumers (Kobus et al, 2019), it was found that *Aronia melanocarpa* products contain high amounts of polyphenols (Tolić et al., 2015). Tolić et al. (2017) showed how weather conditions, such as temperature and insolation, influenced phenolic content in the juice. It was found that warm and dry climate conditions have a positive impact on the increasing value of total phenolics.

Flavonoid content was predominant, and their amounts varied from 295.92 mg of QE/ml in P1 sample to 57.92 mg of QE/ ml in P2 sample. Average of total flavonoid content in dried berries residue (P3) was 158.8 mg QE/g dry matter. These results suggest that flavonoids were the most abundant in chokeberry fresh juice and in dried berries. In this case, our results are in concordance with other research which showed that the content of total flavonoids was higher in fresh juices and in dried chokeberries (Kapci et al., 2013).

The total flavonoid content was predominant, and their amounts varied from 295.92±6.801 mg of QE / ml in P1 sample to 57.92±0.163 mg of QE/ ml in P2 sample. Average of total flavonoid content in dried berries residue (P3) was 158.8 mg QE/g dry matter. These results suggest that flavonoids were the most abundant in chokeberry fresh juice and in dried berries. In this case, our results are in concordance with other research which showed that the content of total flavonoids was higher in fresh juices and in dried chokeberries (Kapci et al., 2013).

Anthocyanins content

Chokeberries contain relatively higher amounts of ***anthocyanins*** compared to other berries fruits, grape and cherry, which are known as rich sources of anthocyanins (Kulling and Rawel, 2008; Denev et al, 2012). Our results sowed lower amounts of anthocyanins in juice mixed with methanol/2 % HCl (20.15 ± 0.619 mg CGE /ml) and in fresh juice (14.2 ± 1.338 mg CGE /ml) and a higher concentration of anthocyanins accumulated in fruit residue (155.9±5.891 mg CGE/100 g dry matter). Cyanidin-3-galactoside and cyanidin-3-arabinoside are predominant in the berries with a cumulative content >90 % (Denev et al., 2012). As a natural product, anthocyanins are good for health due to their antioxidant properties and may have a role in immunity by boosting our immune system (Wegdan Ali Shehata et al. 2020). The content of total phenolics (TPC), total flavonoids (TFC) and total anthocyanins (TA) in aronia raw juice and in acidified methanolic extracts of juice and dried material are presented in Table 1 and in Table 2.

Table 1. Total content of phenol (TPC), flavonoids (TFC) and anthocyanins (TA) in aronia fresh juice

Sample	TPC mg GAE/l	TFC mg QE/ml	TA mg CGE/ml
P1	2.69±0.009	295.92±6.801	14.2±1.338
P2	0.182±0.002	57.92±0.163	20.15±0.619

The values are presented as mean ± SEM for triplicate measurements. P1 - chokeberry fresh juice, P2 - chokeberry juice mixed with methanol/2% HCl.

Table 2. Total content of phenol (TPC), flavonoids (TFC) and anthocyanins (TA) in aronia residue

Sample	TPC mg GAE/g dm	TFC mg QE/g dm	TA* mg CGE/100 g dm
P3	1.02±0.024	158.8±0.136	155.9±5.891

The values are presented as mean ± SEM for triplicate measurements. P3 - Berries residue with methanol/2% HCl. Contents of TPC and TFC in dried berries residue methanol/2% HCl samples are expressed as mg per g of dry matter (dm) and *TA are expressed as mg of cyanidin-3-glucoside equivalent (CGE) per 100 g of dry matter (dm).

Antioxidant activity

To evaluate the antioxidant activity of *Aronia melanocarpa*, the most commonly used assays include the inhibition of DPPH radicals (2,2-dipheny-l-1-picryl-hydrazyl) (Oszmiański & Wojdylo, 2005). In our experiments, DPPH scavenging ability assay and total antioxidant capacity (TAC) assay were used to evaluate the antioxidant activity of each sample. The results are show in Table 3.

Table 3. Antioxidant Activity by DPPH and TAC assays

Sample	DPPH $IC_{50\%}$ (µg /ml)	TAC (µg/ml)
P1	14.42±0.226	1442.1±1.825
P2	54.21±0.032	61.97±1.770
P3	25.14±0.223	197.8±6.93
AA	12.27±0.294	

Data are mean ± SEM for triplicate measurements.
P1 - chokeberry fresh juice; P2 - chokeberry juice mixed with methanol/2% HCl; P3 - chokeberry dried material with methanol/2% HCl; AA - Ascorbic acid.

In present study, ascorbic acid as a well-known potent antioxidant, was used as positive control for DPPH scavenging activity. Concentration of

samples and ascorbic acid (AA) resulting in 50% inhibition on DPPH ($IC_{50\%}$ value) were calculated. The lower IC_{50} value indicates a higher antioxidant activity. Chokeberry raw juice (P1) showed highest ability in DPPH scavenging activity (14.42 ± 0.226 µg/ml) followed by methanolic chokeberry dried residue (P3) (25.14±0.223 µg/ml) compared to methanolic juice sample (P2) which measured by the lowest IC_{50} value (54.21 ± 0.032 µg/ml), but it has lower antioxidant capacity compared to ascorbic acid (12.27 ± 0.294 µg/ml) (Figure 5).

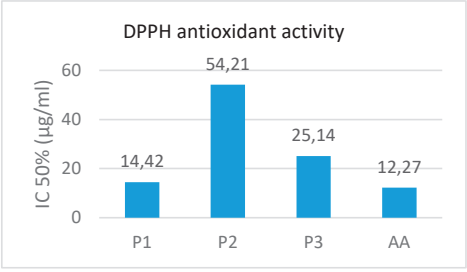

Figure 5. DPPH antioxidant activity in aronia samples

(P1 - chokeberry fresh juice; P2 - chokeberry juice mixed with methanol/2% HCl; P3 - chokeberry dried material with methanol/2% HCl; AA - Ascorbic acid)

The phenolic content in the raw juice and residue samples may contribute to the antioxidative action by hydrogen donating ability. The high content of polyphenols is responsible for the strong antioxidant properties of chokeberries and their products (Tolić et al., 2017).

The phosphomolybdate method has been used routinely to evaluate the total antioxidant capacity of plant extracts (Prieto et al., 1999; Prasad et al, 2009). The total antioxidant capacity (TAC) of different chokeberry samples is shown also in Table 2 and in Figure 6. The higher absorbance value indicated higher antioxidant activity (Prasad et al., 2009). Examined samples showed that the highest total antioxidant activity is rich by chokeberry raw juice (P1) (1442.1 ± 1.825 µg/ml) and methanolic dried material (P3) (197.8±6.93 µg/ml), and the lowest total antioxidant activity was in methanolic/2% HCl chokeberry juice (P2) (61.97 ± 1.770 µg/ml) (Figure 6).

These results are in accordance with other reports in the literature, which showed positive strong correlation between antioxidant activities and total polyphenol contents (Zhao et al, 2008).

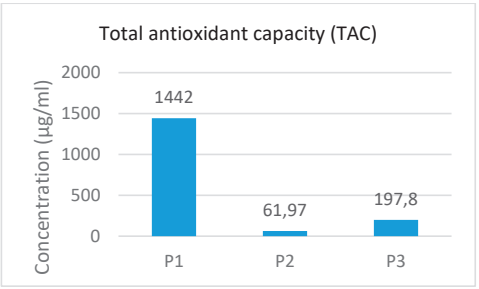

Figure 6. Total antioxidant capacity (TAC) of different chokeberry samples

(P1 - chokeberry fresh juice, P2 - chokeberry juice mixed with methanol/2% HCl; P3 - chokeberry dried material with methanol/2% HCl)

CONCLUSIONS

In this investigation, high contents of phenolic and flavonoid compounds and high values of antioxidant properties were observed in the fresh juice of *Aronia melanocarpa*. The presence of anthocyanins was observed in high amounts, especially in the residual material. The results show that the fresh fruits have significant antioxidant properties. Due to their high content of natural antioxidants, their consumption could bring health benefits. The present study contributes to the existing knowledge by providing new data.

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