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VITAMIN C AND TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY OF FRESH AND PROCESSED FRUITS OF *ARONIA MELANOCARPA*

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Abstract

There are scientific evidences that a diet rich in fruits and vegetables may reduce the risk to have different chronic diseases. Berries are recommended in a healthy diet as it provides protection against degenerative diseases, cardiovascular diseases and cancer. Fruits of Aronia melanocarpa are rich sources of biologically active compounds, polyphenols (anthocyanins and procyanidins, especially) representing the most important group. Polyphenols are the main substances which give the antioxidant potential of black chokeberry fruits. In this paper are presented results of the performed research for determination of vitamin C and total polyphenol content and antioxidant capacity in case of fresh and processed fruits of Aronia melanocarpa (frozen and dried fruits, juice, jam, compote). Determination of vitamin C was performed by high performance liquid chromatography coupled with high resolution mass spectrometry, using hippuric acid as internal standard. Total polyphenol content was spectrophotometric determined, using Folin-Ciocalteu method, and assessment of the antioxidant capacity was performed using DPPH method. Vitamin C content of the samples taken into study varied in the range 7.25–98.75 mg/100g (minimum value was recorded for jam, and maximum one for fresh, unpasteurized juice). Dried fruits of Aronia registered the highest total polyphenol content (4015.25 mg GAE/100g) and antioxidant capacity (84.45mg Trolox Equivalents/g). Minimum value of antioxidant capacity was recorded for compote of Aronia, 12.25 mg Trolox Equivalents/g, respectively. Taken into consideration that fresh fruits of Aronia are available only a short time period, their processing under diverse forms is of real interest for consumers which can benefit thus of nutritional qualities and antioxidant potential of them.

Key words*: Aronia melanocarpa, fruits, polyphenols, antioxidant capacity.*

INTRODUCTION

Among berries, fruits of *Aronia melanocarpa*, they have gaine recently attention due to the health claims associated with their consumption (Chrubasik et al., 2010; Kokotkiewicz et al., 2010). Black chokeberry (*Aronia melanocarpa* (Michx.) Elliott belongs to the family *Rosaceae* and is native to the North America and Canada, being cultivated in Europe in the early twentieth century (Konić Ristić et al., 2013). Fruits of *Aronia melanocarpa* (Michx.) Elliott are rich sources of biologically active compounds, polyphenols (anthocyanins and procyanidins, especially) representing the most important group. Polyphenols are the main substances which give the antioxidant potential of black chokeberry fruits (Kokotkiewicz et al., 2010). Thus, black chokeberry fruits (*Aronia melanocarpa* (Michx.) Elliott) are an important natural source of cyanidin 3-O-glycoside anthocyanins (cyanidin 3-O-galactoside, cyanidin 3-O-glucoside, cyanidin 3-O-arabinoside, and cyanidin 3-O-xyloside) (González-Molina et al., 2008), quercetin derivatives (Bermúdez-Soto and Tomás-Barberán, 2004), hydroxycinnamic acids (Zheng and Wang, 2003). Total polyphenolic content varies in the range 2-8 mg/100 d.m. and depends on the cultivar, growing conditions and harvesting time (Kähkönen et al., 1999; Hakkinen et al., 1999; Benvenuti et al., 2004; Oszmiański and Wojdyło, 2005; Hudec et al., 2006; Sueiro et al., 2006). Lidija Jakobek et al. (2012) determined polyphenols content in case of three cultivars ('Viking', 'Nero', 'Galicianka') of fruits of chokeberry (*Aronia melanocarpa*) and wild chokeberries, in

Croatia, region Slavonia during two consecutive years (2010 and 2011). Cultivars 'Viking', 'Nero' and wild chokeberries had a similar total polyphenolic content (9,012–10,804 mg kg^{-1} in the first year, 9,361–12,055 mg GAE/ kg FW in the second year).Cultivar 'Galicianka' had a lower total polyphenolic content (8,564 mg GAE/kg FW first year, 8,600 mg GAE/kg FW second year).

Besides polyphenols, fruits of *Aronia melanocarpa* are sources of sugar (10–18%), pectins (0.6–0.7%), the sugar alcohol sorbitol, and parasorboside (Weinges et al.,1998; Niedworok and Brzozowski, 2001; Wolski et al.,2007; Kulling and Rawel, 2008). Also these fruits contain small amounts of fat (0.14% fresh weight), represented especially by linoleic acid glycerides and phosphatidylinositol (Kane et al.,1991; Zlatanov, 1999). Also, Kulling and Rawel (2008) notes that fruits of *Aronia melanocarpa* contain vitamins B (B_1, B_2, B_6, niacin, pantothenic acid), vitamin C (13–270 mg/kg), β-carotene (7.7–16.7 mg/kg), minerals (4.4–5.8 g/kg as ash value), approx. 16–18% of carbohydrates (glucose, fructose, sorbitol), dietary fiber (approx. 55 g/kg) and 1–1.5% of organic acids (malic, quinic, citric).Specific almond flavor of these fruits is given by cyanogenic glycosides – amygdalin (20 mg/ 100 g fresh weight – FW) (Lehmann, 1990; Kullingand Rawel, 2008). Fruits of *Aronia melanocarpa* contain triterpenes (b-sitosterol and campesterol) and over 40 volatile compounds, the most important ones being benzaldehyde cyanohydrine, hydrocyanic acid, and benzaldehyde (Hirviand Honkanen, 1985; Zlatanov, 1999).

Fruits of *Aronia melanocarpa* (black chokeberry) demonstrate antiviral activity against influenza viruses, including an oseltamivir-resistant strain. Ellagic acid and myricetin are two components in fruits of *Aronia*, which give the anti-influenza properties (Parket al., 2013). Also, the polyphenolic-rich *Aronia melanocarpa* juice kills teratocarcinomal cancer stem-like cells, but not their differentiated counterparts (Sharif et al., 2013).

The *in vitro* experiments showed anticoagulant effect of polyphenols-rich extracts from black chokeberry and grape seeds (Bijak et al., 2011). In a pilot study, Maria Handeland et al.(2013) shown that black chokeberry juice (*Aronia melanocarpa*) reduces incidences of urinary tract infection.

Fresh fruits of *Aronia melanocarpa* can be consumed a short period time and thus to benefit by their nutritional qualities and antioxidant potential these fruits are processed under various forms: dried fruits, puree, juice, liqueur, syrup, jam, wine, compote, tea, powder (Chrubasik et al., 2010; Ochmian et al., 2012; Kapci et al., 2013; Šnebergrová et al., 2014). On the other side, fresh fruits of *Aronia melanocarpa*, have sour and astringent taste and therefore consumers prefer juice of *Aronia melanocarpa*, in combination with other fruits, such as, apples, pears and blackcurrant (Lehmann, 1990; Ara, 2002).

Anna Horszwald et al. (2013) studied the influence of drying techniques (spray drying, freeze drying and vacuum drying) on *Aronia* commercial juice, in the temperature range 40-80ºC. It was found that all the obtained powders have a high content in polyphenols, in the range: 27.63±1.38 mg GAE/100 mg DM …34.28±1.77 mg GAE/100 mg DM. Powders obtained by spray drying had the highest content of total flavonoids (5.22±0.32 mg quercetin/100 mg DM), total monomeric anthocyanins (4.80±0.13 mg Cy-3-G/100 mg DM), cyaniding-3-glucoside (21.10±0.63 mg Cy-3-G/100 mg DM) and total proanthocyanidins (59.22±3.69 mg (+)-Catechin/100 mg DM). Also, powders obtained by spray drying had the highest antioxidant capacity (251.34±18.77 µmol Trolox Equivalents/100 mg DM by ABTS; 26.49±2.34 µmol Trolox Equivalents/100 mg DM by TEAC; 248.56±11.06 µmol Trolox Equivalents/100 mg DM by FRAP).

In this paper are presented results of the performed research for determination of vitamin C and total polyphenol content and antioxidant capacity in case of fresh and processed fruits of *Aronia melanocarpa* (frozen and dried fruits, juice, jam, compote).

MATERIALS AND METHODS

Samples

Fresh and dried fruits of *Aronia melanocarpa*, compote, jam and fresh juice of *Aronia* were purchased from private producers. Fresh and

dried fruits of *Aronia melanocarpa* were packed in plastic package. Ingredients of *Aronia* compote, packed in hermetically sealed glass recipients (Twist-off system), 720 mL capacity, were the following: *Aronia* fruits, water, sugar and lemon juice. *Aronia* jam was achieved by concentration of *Aronia* fruits with sugar, with adding of lemon juice and packed in hermetically sealed glass recipients (Twist-off system), 314 mL capacity. Fresh *Aronia* juice was achieved by pressing *Aronia* fresh fruits and packed in hermetically sealed glass recipients (Twist-off system), 330 mL capacity. Frozen fruits of *Aronia melanocarpa* were achieved within the Pilot Experiments Plant for Fruits and Vegetables Processing from fresh fuits purchased from private producer. Thus, fresh fuits of *Aronia melanocarpa* were sorted, washed and frozen in plastic package (net weight 250 g±3%) at – 18°C.

Methods

Vitamin C content

Determination of vitamin C was performed by high performance liquid chromatography (Accela, Thermo Scientific) coupled with high resolution mass spectrometry (LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap Mass Spectrometer, Thermo Scientific) using hippuric acid as internal standard.

LC conditions:

- Column (Hypersil GOLD aQ, 150 x 2.1 mm, 3 µm);
- Column temperature: 40°C;
- Sample temperature: 4°C;
- Mobile phase A: 990 mL water: 10 mL 1M ammonium formate (aq): 1 mL formic acid;
- Mobile phase B: 990 mL methanol: 10 mL 1M ammonium formate (aq): 1 mL formic acid;
- Flow rate: 0.400 mL/min;
- Injection volume: 25 µL;

MS conditions:

Analyzer: FTMS;
Resolution: 60000;
Ionization mode: ESI-;
Specific ions were: *m/z* = 175.02438 (for vitamin C) and *m/z* = 178.05051 (for hippuric acid).

In Figure 1 is presented the calibration curve of vitamin C, achieved in the concentration range 2100-10000 µg/L.

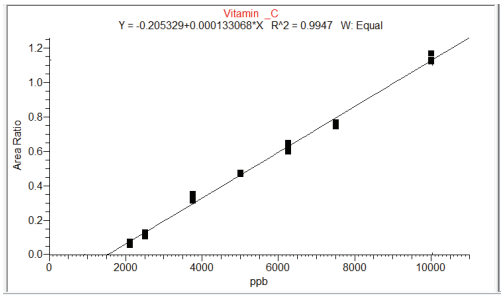

Figure 1.Calibration curve of vitamin C

Total polyphenol content

Total polyphenol content was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0-0.20 mg/mL (Figure 2).

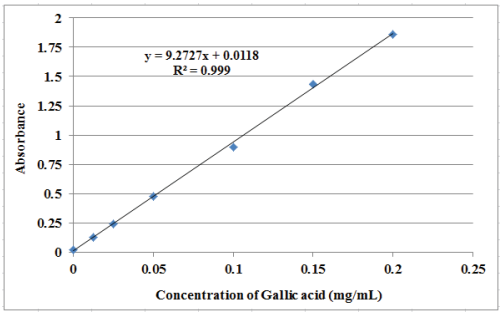

Figure 2. Calibration curve of gallic acid

The extraction of phenolic compounds was performed in methanol:water 50:50, and the absorbance of the extracts was determined at a wavelenght λ = 755 nm. Results were expressed as mg of gallic acid equivalents (GAE) per g product.

Antioxidant capacity

The DPPH scavenging radical assay was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f (Trolox concentration), in the concentration range 0-

0.4375 mmol/L (Figure 3). Results were expressed as mg Trolox Equivalents per g product.

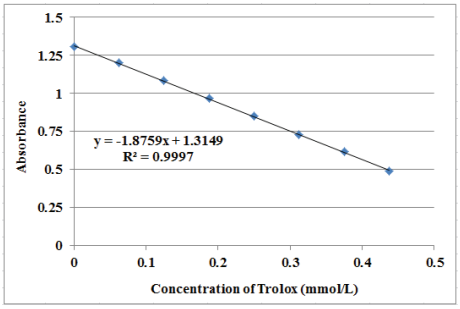

Figure 3. Calibration curve of Trolox

RESULTS AND DISCUSSIONS

Vitamin C content

Vitamin C content of fruits of *Aronia melanocarpa*, fresh and processed (frozen and dried fruits, juice, jam, compote) varied in the range: 7.25–98.75 mg/100 g (Figure 4). Minimum value was recorded for *Aronia* jam, and the maximum one for *Aronia* fresh juice (unpasteurized).

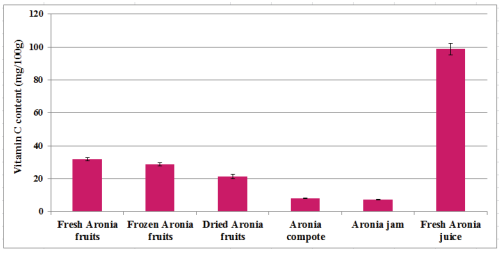

Figure 4. Vitamin C content of fresh and processed fruits of *Aronia melanocarpa*

Vitamin C content of fresh fruits of *Aronia melanocarpa* was 31.85 mg/100 g, and is higher than that mentioned by Kulling and Rawel (2008), 1.3–27 mg/100g and by Karakasova et al. (2014), 17.52 mg/100g, respectively. Also, vitamin C content of frozen fruits of *Aronia melanocarpa* (28.78 mg/100 g) is with 9.64% lower than those of the analyzed fresh fruits in this study, but higher than that mentioned by Karakasova et al. (2014), respectively, 17.15 mg/100 g. Vitamin C content of dried fruits of *Aronia melanocarpa* in this study was 1.4 times higher than that reported by Karakasova et al. (2014), respectively, 15.11 mg/100 g. *Aronia* compote and *Aronia* jam recorded a low vitamin C content (7.96 mg/100 g, respectively, 7.25 mg/100 g), because vitamin C is very sensitive to oxygen and heat treatment. *Aronia* fresh juice analysed in this study is an important source of vitamin C (98.75 mg/100 g). Consumption of about 61 g *Aronia* juice ensure daily requirement of vitamin C for children older than 4 years and adults, respectively (60 mg vitamin C/day). The result obtained for the content of vitamin C of *Aronia* fresh juice is in line with those obtained by Djuricet al.(2015) for *Aronia* juice, obtained from fruits grown on four soil types (91.10-155.20 mg/100 mL). Frei et al. (2012) have shown that dietary supplementation with vitamin C decreased hypertension, endothelial dysfunction, chronic inflammation, and *Helicobacter pylori* infection. At the same time, vitamin C acts as a biological antioxidant that can reduce high levels of oxidative stress and may contribute to chronic disease prevention. Also, based on the performed studies, these authors concluded that 200 mg per day is the optimum dietary intake of vitamin C for the majority of the adult population, to maximize the potential health benefits of this vitamin.

Total polyphenol content

Fruits of *Aronia melanocarpa* are a valuable source of total polyphenols. Total polyphenol content of fresh and processed fruits of *Aronia melanocarpa* is shown in Figure 5. The highest total polyphenol content was recorded for dried fruits of *Aronia* (4015.25 mg GAE/100g), due to the high content of dry matter (89.8%).

The result obtained for the content of bioactive compounds is comparable with that obtained by Kapci et al. (2013) for dried chokeberry (3990±30 mg GAE/100 g, respectively, 5010±40 mg GAE/100 g).

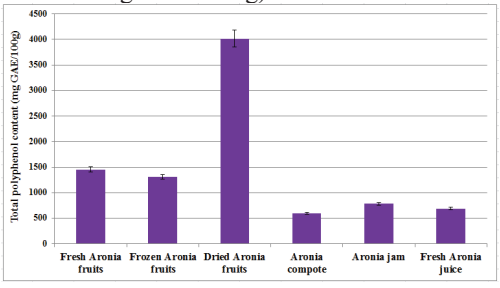

Figure 5. Total polyphenol content of fresh and processed fruits of *Aronia melanocarpa*

Also, total polyphenol content of dried fruits of *Aronia* is about 1.63 times higher than that obtained by Tolić et al. (2015) for chokeberry dried berries (2466±91 mg GAE/100 g of dry matter).

Fresh fruits of *Aronia melanocarpa* in this study had a total polyphenol content (1455.25 mg GAE/100 g) comparable to that reported by Kapci et al. (2013) for fresh chokeberry (1330±3 mg GAE/100 g), but lower than that reported by Ochmian et al. (2012) for four cultivars of chokeberry fruits ('Galicjanka'-2185 mg GAE/100 g; 'Hugin'-2340 mg GAE/100g; 'Nero' - 1950 mg GAE/100 g; 'Viking' - 1845 mg GAE/100 g).

Frozen *Aronia* fruits in this study had a polyphenol content of 1308.75±47.77 mg GAE/100g, comparable to that of fresh fruits.

Aronia compote (containing 55.25% fruits) had the lowest total polyphenol content (590.45±19.18 mg GAE/100 g), lower than that reported by Kapci et al. (2013) for this product (670±3 mg GAE/100 g). Compared to other products, *Aronia* juice had a lower total polyphenol content, this can be explained by the high water content (Shin et al., 2008). Thus, total polyphenol content of *Aronia* fresh juice studied was 688.47±22.75 mg GAE/100 g, higher than that reported by Konić Ristić et al. (2013) in case of commercial chokeberry juice (586±27 mg GAE/100 g), respectively, fresh chokeberry juice (593±33 mg GAE/100 g). Values higher or lower for total polyphenol content of fresh or processed fruits of *Aronia melanocarpa,* reported in the literature, may result by use of various extraction methods and analytical procedures, through application of processing technologies and different conditions, respectively, the differencies between the varieties of these fruits (Denev et al., 2012).

The phenolic compounds are the most important class of bioactive compounds from the fruits of *Aronia melanocarpa,* which are also responsible for many of its medicinal properties (Kulling and Rawel, 2008). Thus, Sikora et al. (2012), in a human study shown that introduction in diet of extract of fruits of *Aronia melanocarpa* had as effects decrease of the lipid levels and significant inhibition of platelet aggregation.

Oprea et al.(2014) have shown that the addition of *Aronia* juice in the normal diet of healthy rats for 10 days, it was correlated with the reduction of values of some markers of oxidative stress and a decrease of blood glucose with 6.85%. Also, administration of *Aronia* juice in case of rats suffering alloxan induced-diabetes resulted in a significant reduction of blood glucose (42.83%).

Recent research undertaken by Daskalova et al. (2015) on animals have shown that treatment with juice of *Aronia melanocarpa* significantly reduced low-density lipoprotein fraction, with pro-aterogenic properties and a decrease of total cholesterol by 16.5%. In case of animals taken into study, dietary supplementation with *Aronia* juice has reduced atherogenic risk and also had a protective effect on the cardiovascular system. However, it was found that *Aronia* juice delay aortic changes that occur with age.

Antioxidant capacity

Antioxidant capacity of fruits of *Aronia melanocarpa*, fresh and processed (frozen and dried fruits, juice, jam, compote) varied in the range:12.25–84.45 mg Trolox Equivalents/g (Figure 6). Minimum value was recorded for *Aronia* compote, and the maximum one for dried *Aronia* fruits. Antioxidant capacity of dried *Aronia* fruits in this study is 2.32 times, respectively, 2.76 times higher than that reported by Kapci et al. (2013) for dried chokeberry (36.3±1.2 mg Trolox Equivalents/g, respectively, 30.5±1.0 mg Trolox Equivalents per g). Antioxidant capacity of frozen *Aronia* fruits (27.39±1.18 mg Trolox Equivalents/g) is with 10.05% lower than that of fresh fruits.

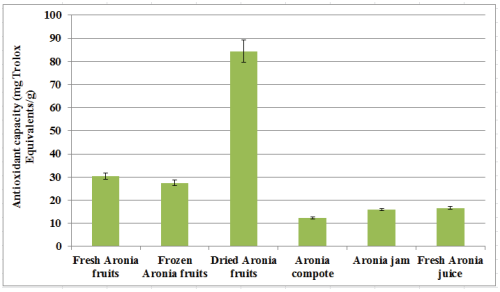

Figure 6. Antioxidant capacity of fresh and processed fruits of *Aronia melanocarpa*

On the other side, antioxidant capacity of fresh *Aronia* fruits is 2.69 times higher than that reported by Kapciet al. (2013) for fresh chokeberry fruit (11.3±0.5 mg Trolox Equivalents/g). Chokeberry fruits have one of the highest *in vitro* antioxidant activities among fruits (Denev et al., 2012).
In this study, *Aronia* jam and fresh *Aronia* juice recorded the following values for antioxidant capacity: 15.88±0.55 mg Trolox Equivalents/g, respectively, 16.55±0.58mg Trolox Equivalents per g. Antioxidant capacity of *Aronia* compote taken into study was 2.55 times higher than that reported by Kapci et al. (2013) for this product (4.8±0.1 mg Trolox Equivalents/g).
Between the total polyphenol content of fruits of *Aronia melanocarpa*, fresh and processed (frozen and dried fruits, juice, jam, compote) in this study and the antioxidant capacity was registered a linear correlation, regression coefficient, R^2, being 0.9988 (Figure 7).

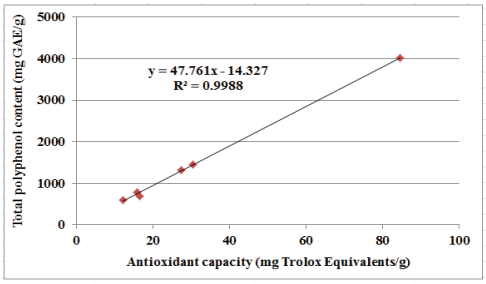

Figure 7. Correlation between total polyphenol content and antioxidant capacity in case of fresh and processed fruits of *Aronia melanocarpa*

Results are in conformity with those of Zheng and Wang (2003), which mentioned a direct correlation between total polyphenol content of fruits of *Aronia* and their antioxidant capacity.

CONCLUSIONS

In this study were evaluated vitamin C content, total polyphenol content and antioxidant capacity (DPPH method) of fruits of *Aronia melanocarpa* fresh and processed (frozen, dried, juice, jam, compote).
Fresh *Aronia* juice recorded the highest vitamin C content (98.75±3,6 mg/100 g). Also, fresh and frozen *Aronia* fruits are valuable sources of vitamin C.
Dried *Aronia* fruits shown the highest total polyphenol content (4015.25±164.63 mg GAE/100 g), followed by the fresh and frozen *Aronia* fruits. Also, fresh *Aronia* juice, *Aronia* jam and compote have an important total polyphenol content.
Dried *Aronia* fruits recorded the highest value of the antioxidant capacity (84.45±4.90 mg Trolox Equivalents/g), followed by fresh and frozen *Aronia* fruits. Fresh *Aronia* juice, *Aronia* jam and *Aronia* compote recorded relatively close values for antioxidant capacity.
Fresh and processed fruits of *Aronia* are valuable because of the content in bioactive compounds and their antioxidant capacity.

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