

ORIGINAL SCIENTIFIC PAPER

Polyphenolic profile of fresh chokeberry and chokeberry products

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Abstract

Chokeberry (*Aronia melanocarpa*) fruits and its products are one of the richest sources of polyphenols which may play important role in human health as for example in regulation of blood pressure levels, reducing concentrations of triglycerides, low density and total cholesterol. The objective of this study was to identify and quantify the content of polyphenols, specifically phenolic acids (gallic, caffeic, *p*-coumaric, ellagic, chlorogenic) and flavonols (quercetin and kaempferol) using high performance liquid chromatography (HPLC) in two varieties of fresh chokeberry fruit (type Nero and Viking) and in different types of chokeberry products available from Croatian market: eleven samples of juices, three samples of powders, two samples of both capsules and dried berries and four samples of teas.

The results showed that there is a difference in amount of examined polyphenols between different product categories, and significant difference was also observed within the same product group ($p \leq 0.05$). In all analysed samples the highest phenolic acid quantified was *p*-coumaric acid, and gallic acid was the lowest one. Berry juices from fresh chokeberry fruits had higher amount of *p*-coumaric acid ($284.55 \pm 6.34 \text{ mgL}^{-1}$) compared to commercially available juices ($195.48 \pm 3.05 \text{ mgL}^{-1}$). The same phenolic composition was observed within the rest of the products where capsules have the higher amount of phenolic acids, followed by powders, dried berries and teas (eg. *p*-coumaric 81.36; 79.76; 36.28; 28.81 mg/100 g dry matter, respectively). According to flavonol amount, all observed products had higher values of quercetin compared to kaempferol. Obtained results represents a valuable set of data for novel and under-examined plant source of health promoting bioactive compounds.

Keywords: *Aronia melanocarpa*; High Performance Liquid Chromatography; Polyphenols; Phenolic acids; Flavonols

Sažetak

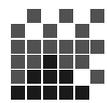
Aronija (*Aronia melanocarpa*) i njeni proizvodi smatraju se jednim od najbogatijih izvora polifenola, nutrijenata koji imaju važnu ulogu u fiziološkim funkcijama povezanim s ljudskim zdravljem, kao što je npr. utjecaj na regulaciju krvnog tlaka te smanjivanja koncentracije triglicerida, ukupnog i LDL kolesterola. Cilj ovog rada bio je primjenom tekućinske kromatografije visoke djelotvornosti (HPLC) identificirati i kvantificirati sadržaj polifenola, posebice fenolnih kiselina (galna, kafeinska, *p*-kumarinska, elaginska, klorogenska) i flavonola (kvercetin i kamferol) u dvije sorte svježe aronije (tipovi Nero i Viking) te u različitim vrstama proizvoda na bazi aronije (sokovi - 11 uzoraka, prahovi - 3 uzorka, kapsule - 2 uzorka, sušene bobice - 2 uzorka i čajevi - 4 uzorka) koji su dostupni na hrvatskom tržištu. Dobiveni rezultati ukazuju na postojanje razlika u udjelu polifenola između različitih skupina proizvoda, ali i u postojanju razlika u sadržaju polifenola unutar iste skupine proizvoda. S obzirom na udio fenolnih kiselina, u svim analiziranim proizvodima najveći udio je *p*-kumarinske dok je najmanje utvrđeno galne kiseline. Sokovi dobiveni iz svježih plodova imali su veći udio *p*-kumarinske kiseline ($284,55 \pm 6,34 \text{ mg/L}$) u usporedbi s komercijalno dostupnim sokovima ($195,48 \pm 3,05 \text{ mg/L}$). Fenolni sastav sličan sokovima utvrđen je i u ostalim analiziranim proizvodima, gdje su kapsule imale najviši udio fenolnih kiselina, a slijede ih praškovi, sušene bobice i čajevi (na primjeru *p*-kumarinske kiseline: 81,36; 79,76; 36,28; 28,81 mg/100 g suhe tvar). Gledano prema udjelu flavonola, udio kvercetina bio je viši u usporedbi s udjelom kamferola u svim analiziranim uzorcima. Rezultati dobiveni ovim istraživanjem predstavljaju važan set podataka za biljnu vrstu velikog potencijala koja se pokazala kao izuzetno dobar izvor za ljudsko zdravlje blagotvornih bioaktivnih komponenata, a istovremeno je još uvijek relativno nova i atraktivna te nedovoljno istražena.

Ključne riječi: *Aronia melanocarpa*; tekućinska kromatografija visoke djelotvornosti; polifenoli; fenolne kiseline; flavonoli

Introduction

Polyphenols are considered to be the most important antioxidants in human diet. Various berries are rich sources of polyphenols and it is assumed that chokeberry (*Aronia me-*

lanocarpa) fruits are one of the richest sources. *Aronia melanocarpa* is a species of shrubs native to eastern parts of North America and it was introduced to Europe in 20th century (Kulling and Rawel, 2008). The fruit is dark purple, almost black with bitter taste and is good source of polyphenols. It



was traditionally used as the cure for different health conditions among members of the tribes in North America (Kokotkiewicz et al., 2010). Nowadays is mostly used for production of natural food colorants, some dietary supplements and different processed products (juices, jams, jellies etc.) (Jurikova et al., 2017). There is small but increasing number of plantations in Croatia (Nikolić and Milivojević, 2010) as well as growing interest among consumers.

Chemical composition of chokeberry fruit depends on numerous conditions such as type, growing conditions, climate, weather conditions, maturity of fruit etc. (Jeppsson and Johansson, 2000) as well as time of the harvest (Bolling et al., 2015), and it significantly differ from other fruits according to higher amount of polyphenols (Wawer et al., 2006). Polyphenols have protective function for plant and are carriers of characteristic flavour, smell, colour, nutritive value and antioxidative activity (Robards et al., 1999). Same as chemical composition, its amount in fruit depends on various conditions (Oszmianski and Wojdylo, 2005; Kokotkiewicz et al., 2010). The most common are phenolic acids (especially caffeic, chlorogenic, *p*-coumaric, gallic and ellagic acids) and flavonoids (quercetin and kaempferol as representatives) (Manach et al., 2004; Mattila et al., 2006; Szajdek and Borowska, 2008; Kokotkiewicz et al., 2010). Those polyphenolic compounds are the most important for beneficial impact that chokeberry has for human health. Up today it was conducted only few clinical researches for establishment that impact with results suggesting beneficial effect in treatment of metabolic syndrome, hypertension and diabetes type 2 (Banjari et al., 2017).

The objective of this study was evaluate, identify and quantify the content of polyphenols using high performance liquid chromatography (HPLC), specifically phenolic acids and flavonols present in two varieties of fresh chokeberry fruit and in different types of chokeberry products such as juices, powders, capsules, dried berries and teas available on Croatian market.

Materials and methods

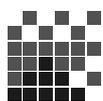
Fresh chokeberry samples were collected at maturity stage at the end of harvest season during August 2012, 2013, and 2014 from experimental orchard of the Institute of Pomology of the Croatian Centre for Agriculture, Food and Rural Affairs in Donja Zelina, Croatia (type *Nero*) and from family farm Medić, Veliko Vukovje, Croatia (type *Viking*). Berries were inspected and all damaged or over-matured chokeberries were excluded from the sample. Within the 4 h after harvesting berries were frozen and stored at -20 °C until analysis. The day before analysis chokeberries were thawed at room temperature.

Commercially available chokeberry products (table 1) were purchased on Croatian market during February 2014. Only requirement was that they had to contain only chokeberry, without added sugar or other fruits. In total 22 commercially available products, from which was eleven samples of juices (S1 – S11), three samples of powders (S12 – S14), two samples of capsules (S15 and S16) two samples of dried berries (S17, S18) and four samples of teas (S19-S22), were analysed. All collected products were stored at 4 °C until analysis.

All analyses were carried out in triplicate.

Table 1. *Producer, country of origin, fruit content, composition and code of fresh chokeberry and chokeberry products*

Sample code	Producer	Country of origin	Composition
Juices			
S1	Aronia Original Naturprodukte GmbH	Germany	Chokeberry fruit
S2	Aronija Live d.o.o.	Croatia	Chokeberry fruit
S3	Vitanea LTD	Bulgaria	Chokeberry fruit
S4	Alnavit GmbH	Germany	Chokeberry fruit
S5	Biotta AG	Germany	Chokeberry fruit
S6	Not available, imported in Croatia by Biovega d.o.o.	Poland	Chokeberry fruit
S7	Bobica d.o.o.	Croatia	Chokeberry fruit
S8	Armedina d.o.o.	Serbia	Chokeberry fruit
S9	Aronija Vita d.o.o.	Serbia	Chokeberry fruit
S10	Voelkel GmbH	Germany	Chokeberry fruit
S11	Medicura Naturprodukte AG	Germany	Chokeberry fruit
Juices from fresh berries			
SN	Institute of Pomology, Croatian Centre for Agriculture, Food and Rural Affairs, Donja Zelina	Croatia	Chokeberry fruit
SV	Family farm Medić, Veliko Vukovje	Croatia	Chokeberry fruit
Powders			
S12	Aronia Original Naturprodukte GmbH	Germany	Chokeberry pulp
S13	Bobica d.o.o.	Croatia	Chokeberry fruit
S14	Aronija Vita d.o.o.	Serbia	Chokeberry pomace
Capsules			
S15	Darvitalis d.o.o.	Serbia	Chokeberry extract



S16	Bobica d.o.o.	Croatia	Chokeberry extract
Dried berries			
S17	Aronia Original Naturprodukte GmbH	Germany	Chokeberry fruit
S18	Bobica d.o.o.	Croatia	Chokeberry fruit
Fruit teas			
S19	Aronija Live d.o.o.	Croatia	Chokeberry pomace
S20	Darvitalis d.o.o.	Serbia	Chokeberry pomace
S21	Bobica d.o.o.	Croatia	Chokeberry pomace; Chokeberry leaves
S22	Vitanea LTD	Bulgaria	Chokeberry pomace

Total solid content of the fresh chokeberry juices and commercial juices and products was determined using a gravimetric method. A mass of (2±0.0001) g of chokeberry sample was mixed with about 5 g of sea sand and dried at 105 °C until constant mass. Samples pH was determined at room temperature using an MA 5740 pH meter (ISKRA, Kranj, Slovenia).

For HPLC analysis three replicates of chokeberry extract were prepared and analysed the same day. Method for extraction and hydrolysis of flavonols was developed by Hertog et al. (1992) and optimized by Häkkinen et al. (1998). Phenolic compounds were identified and quantified using Shimadzu LC 10AD system (USA) using SPD-M10A detector (diode array detector) and Zorbax Eclipse XDB-C18 column (4,6 x 250 mm inner diameter, 5 µm, Agilent Technologies). The flavonols and phenolic acids were separated using 1 % water solution of acetic acid as mobile phase A and 100 % HPLC grade methanol as mobile phase B. The flow rate was 0.8 mL min⁻¹ and the gradient programme was as follows: 0–30 min from 5 to 80 % B; 30–33 min 80 % B; 33–35 min from 80 to 5 % B. The column temperature was set to 20 °C and the injection volume was 20 mL. All standards were obtained from Sigma-Aldrich (St. Louis, MO).

Flavonols and phenolic acids were identified and quantified using calibration curves.

The data were analysed by one-way analysis of variance (ANOVA) using *Statistica v.22* (Statsoft Inc, Tulsa, OK, USA). Values were expressed as means (N=3) ± standard deviation (SD). Differences were considered significant at p≤0.05.

Results and discussion

Chokeberry fruits and its products gained considerable popularity lately and are included in human diet because they are assumed to be good sources of polyphenols. As data on phenolic content of chokeberry have been reported in several studies (Oszmianski and Wojdylo, 2005; Jakobek et al., 2007;

Kulling et al., 2008) this study contributes to existing knowledge by providing new data of different types of chokeberry products using HPLC method.

HPLC was used for the separation of phenolic compounds in chokeberry samples because such analyses are rapid and enable systematic profiling of the complex plant samples and specifically focuses on their identification and consistent evaluation of the identified compounds.

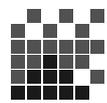
For analysis of fresh juices cultivars type *Nero* and type *Viking* were chosen because in literature is shown that those two cultivars are the most significant for the chokeberry fruit production (Jurikova et al., 2017).

Physicochemical properties, pH and total dry matter (table 2) were determined as one of basic parameters of sample quality. Total dry matter in commercial juices in this study was similar to results obtained in earlier study (Daskalova et al., 2015) where 18.1 % of dry matter was obtained. Also in accordance to literature data (Sójka et al., 2013) are data obtained in this study for total dry matter of capsules, teas and powders. Regarding on pH value it is obvious that juices from chokeberry are sour. From health point of the view the consumption of acidic juices can contribute to erosion of tooth structure. Reddy et al. (2016) tried to determine the erosive potential of commercially available beverages by measuring of the pH and they proposed three zones of the chemical erosive potential of beverages. According to that potential chokeberry juices fall into erosive (pH from 3.00 to 3.99).

Additionally, low pH compared with astringency and low sweetens is probably reason why chokeberry juices were not widely accepted among consumers (Duffy et al., 2016)

Table 2. *Physicochemical properties (pH and total dry matter) in analysed chokeberry samples*

Code	pH	Total dry matter (%)
Juices		
S1	3.90±0.02	14.70±0.04
S2	3.90±0.02	21.54±0.03
S3	3.86±0.02	18.98±0.14
S4	3.71±0.03	15.75±0.11
S5	3.80±0.03	15.79±0.15
S6	3.68±0.02	14.50±0.16
S7	3.74±0.02	14.61±0.22
S8	3.75±0.02	17.01±0.21
S9	3.92±0.01	14.32±0.11
S10	3.54±0.01	13.42±0.05
S11	3.89±0.02	14.27±0.01
Juices from fresh berries		
<i>SN</i>	3.89±0.02	22.55±0,04
<i>SV</i>	3.98±0.02	7.96±0.01



Powders		
S12	4.10±0.02	94.80±0.01
S13	4.02±0.01	91.82±0.32
S14	4.13±0.01	90.44±0.15
Capsules		
S15	3.31±0.02	93.96±0.27
S16	4.10±0.01	93.60±0.26
Dried berries		
S17	4.28±0.02	84.61±0.92
S18	4.01±0.02	82.00±0.55
Fruit teas		
S19	4.13±0.02	91.90±0.26
S20	4.01±0.01	88.32±0.27
S21	4.01±0.02	89.74±0.19
S22	4.04±0.02	96.01±0.14

*SN – type Nero; SV – type Viking

Content of phenolic acids and flavonols identified and quantified in analysed samples using HPLC (expressed as mgL⁻¹ or mg per 100 g dry matter) are shown in tables 3. and 4.

Fresh berries and berry extracts such as black chokeberries, blueberries, cranberries and grapes, have high concentrations of phenolic compounds (e.g. phenolic acids, flavonoids) (Szajdek and Borowska, 2008; Nile and Park, 2014; Olas, 2017).

Earlier studies showed that chokeberry fruits have low content of flavonols, around 1.30 % of total polyphenols, and most of it was quercetin while kaempferol was not detected (Wu et al., 2004) or was detected in low amount (Maatta-Riihinen et al., 2004), what corresponds with our results (table 3).

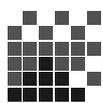
Table 3. Content of flavonols in analysed chokeberry samples

Code	Flavonol	
	quercetin	kaempferol
Juices mgL ⁻¹		
S1	22.76±0.18	ND
S2	27.93±1.06	ND
S3	16.86±2.76	ND
S4	12.35±0.24	ND
S5	20.32±0.24	ND
S6	14.76±6.18	ND
S7	12.55±1.50	ND

S8	46.89±0.05	ND
S9	13.97±0.05	ND
S10	11.80±2.31	0.90±0.01
S11	18.54±1.27	ND
Juices from fresh berries mgL ⁻¹		
SN	73.60±3,46	ND
SV	18.89±0.52	ND
Powders mg/100 g dm		
S12	47.19±4.87	1.24±0.75
S13	79.79±6.07	2.45±0.88
S14	29.40±0.97	0.12±0.03
Capsules mg/100 g dm		
S15	56.31±3.06	0.47±0.02
S16	86.75±2.48	1.39±1.90
Dried berries mg/100 g dm		
S17	10.29±1.54	ND
S18	12.42±0.34	ND
Fruit teas mg/100 g dm		
S19	18.40±2.43	ND
S20	3.05±0.13	ND
S21	4.56±0.27	ND
S22	16.89±0.86	ND

*SN – type Nero; SV – type Viking; ND – not detected; dm – total dry matter

Phenolic acids are the most abundant polyphenols in chokeberry fruit. From all five identified phenolic acids gallic acid was quantified in lowest concentration in all observed juice samples what is similar to results of earlier studies (Jakobek et al., 2008; Kapci et al., 2013), and was also not detected in lot of analysed samples, especially in powders, teas and dry berries samples. Chlorogenic acid was quantified in highest concentration in fresh juice type Nero (111.19±5.14 mgL⁻¹) what is less than was detected in earlier researches where was found 200 – 400 mgL⁻¹ of chlorogenic acid in chokeberry juices (Mayer-Miebach et al., 2012; Daskalova et al., 2015). Caffeic acid is according to previous researches one of most abundant hydroxycinnamic acid in fresh chokeberry fruit (Jakobek et al. 2007; Jakobek et al., 2008). In this study the highest amount of caffeic acid was detected in form of capsules (25.78 mg/100 g dm). Earlier studies found that amount of *p*-coumaric acid in fresh aronia berries was 69.00 mgkg⁻¹ (Kapci et al., 2013) what differ from this study where average value was 132.97 mg/100 g dm. Ellagic acid is the most abundant derivative of hydroxybenzoic acid in average amount of 6.7 mg/kg in fresh fruits (Jakobek et al., 2007). In analysed samples the highest amount was detected in fresh juice type Nero (23.03±0.95 mgL⁻¹) and in commercial sample S6 (27.05±2.19 mgL⁻¹), from



other products the best source of ellagic acid was determinate in capsules. In earlier researches was shown that the contents of phenolic acids in beverages widely varies, ranging from 0 to 97 mg/100 g, depends on beverage type (Mattila et al., 2006).

Table 4. Content of phenolic acids in analysed chokeberry samples

Code	Phenolic acid				
	gallic	chlorogenic	caffeic	p-coumaric	ellagic
Juices					
mgL ⁻¹					
S1	5.51±0.73	96.73±3.97	3.57±0.41	204.55±1.60	19.58±1.00
S2	0.52±0.04	74.61±3.17	2.12±0.26	189.81±2.08	18.93±0.61
S3	0.82±0.11	75.55±4.03	1.74±0.29	178.31±5.45	11.63±0.92
S4	ND	69.15±6.23	1.59±0.16	187.41±1.37	8.17±0.26
S5	2.86±0.17	75.53±1.38	1.88±0.43	185.65±0.25	19.96±0.39
S6	ND	79.49±7.63	0.99±0.12	281.92±5.09	27.05±2.19
S7	ND	46.11±1.47	1.28±0.06	99.28±3.55	9.70±1.21
S8	2.37±0.24	72.78±4.67	2.65±0.23	232.81±3.66	15.63±0.41
S9	ND	75.62±3.99	0.89±0.10	107.55±1.75	10.54±2.52
S10	ND	71.21±8.53	2.03±0.53	256.01±5.29	18.57±0.75
S11	2.89±0.06	84.67±2.56	2.54±0.64	226.96±3.49	8.70±1.17
Juices from fresh berries					
mgL ⁻¹					
SN	ND	111.19±5.14	1.78±0.31	284.55±6.34	23.03±0.95
SV	0.60±0.06	69.35±2.02	1.72±0.08	181.57±1.50	5.71±0.05
Powders					
mg/100 g dm					
S12	ND	23.94±1.10	12.91±0.42	51.13±4.18	16.19±1.19
S13	ND	24.40±1.36	13.00±0.53	87.33±3.68	16.95±0.35
S14	ND	31.69±6.84	13.37±1.84	100.82±4.43	18.47±3.03
Capsules					
mg/100 g dm					
S15	1.47±0.06	24.79±0.69	16.51±0.68	61.13±3.40	25.90±1.50
S16	ND	30.53±1.77	35.05±2.18	101.58±4.33	55.21±5.36
Dried berries					
mg/100 g dm					
S17	ND	9.01±0.06	3.11±0.08	36.63±0.76	1.51±0.00
S18	ND	9.14±0.57	2.77±0.00	35.87±0.38	1.48±0.24
Fruit teas					
mg/100 g dm					
S19	ND	13.68±1.59	19.64±1.54	34.51±1.51	14.92±0.29
S20	ND	16.67±1.24	20.47±2.18	53.18±3.41	18.27±1.18
S21	ND	9.73±0.58	17.92±2.04	14.62±0.82	8.17±0.27
S22	ND	8.83±0.80	11.92±1.00	12.93±1.97	7.93±1.06

*SN – type Nero; SV – type Viking; ND – not detected; dm – total dry matter

Chokeberry polyphenols are in focus because of potential beneficial impact on human health. Results of the so far published studies indicate that consumption of berries may play a role in the prevention of cardiovascular disorders, but the

development of well-controlled clinical studies with berries is encouraged. (Olas, 2017). Research conducted on rats showed that chokeberry juice is an extremely rich source of polyphenols with very high antioxidant activity and treatment with



this bioactive product had impact in reduction of atherogenic risk and has potential cardioprotective effect (Daskalova et al., 2015),

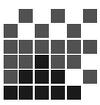
Recent researches on humans showed that chokeberry polyphenols have impact in reduction of total and LDL cholesterol, but not blood pressure and markers of oxidative stress in former smokers (Xie, et al., 2017). Other researches points out relationship between chokeberry intake and improved platelet aggregation, reduction of total and LDL cholesterol, reduction of blood pressure as well as total risk for metabolic syndrome among healthy and subjects with risk factors for developing cardiovascular diseases (Olas, 2017). Also, researchers are trying to establish relationship between chokeberry juice intake with improving of oxidative stress markers (Garcia-Flores, 2018) or fatty acid profiles and lipid peroxidation (Petrovic et al, 2016) among athletes due to its polyphenolic profile.

Conclusions

Chokeberry have high amount of polyphenols. The main phenolic acid in all products is *p*-coumaric acid and main flavonol is quercetin. Fresh chokeberry juice samples type Nero have significantly higher phenolic content compared to other fresh and commercial ones. Significant amount of kaempferol was quantified in powders and capsules samples. Obtained results represents a valuable set of data for novel and still under-examined plant source of health promoting bioactive compounds which gained great popularity among consumers recently.

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