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Phenolic Content, Antioxidant Capacity and Quality of Chokeberry (*Aronia melanocarpa*) Products

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Summary

Chokeberries (*Aronia melanocarpa*) are rarely used in diet in Croatia but they have high content of polyphenolic compounds and one of the highest *in vitro* antioxidant activities among fruits. The aim of this study is to compare the quality, phenolic content and antioxidant capacity of different chokeberry products (juices, powders, fruit tea, capsules and dried berries). It can be expected that processing influences antioxidant activity and phenolic content of final products reaching consumers. Characterisation of phenolic compounds was carried out by using spectroscopic methods (Folin–Ciocalteu and pH differential methods). Antioxidant activity of chokeberry products was determined using 2,2-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. The results show that the investigated products contain high amount of phenols (3002 to 6639 mg per L and 1494 to 5292 mg per 100 g of dry matter) and lower amount of total anthocyanins (150 to 1228 mg per L and 141 to 2468 mg per 100 g of dry matter). The examined juices and other chokeberry products possess high antioxidant capacity (12.09 to 40.19 mmol per L or 58.49 to 191.31 mmol per 100 g of dry matter, respectively) and reducing power (38.71 to 79.86 mmol per L or 13.50 to 68.60 mmol per 100 g of dry matter, respectively). On the basis of phenolic content and antioxidant activity, capsules and powders stand out among other products. The study indicates that there are significant differences ($p<0.05$) in the quality, phenolic content and antioxidant capacity among examined products.

Key words: chokeberry products, quality, phenols, anthocyanins, DPPH, FRAP

Introduction

Increased consumption of fruits and vegetables is recommended in dietary guidelines worldwide (*1*). Among different fruit species, berries have attracted great attention for their bioactivity. In addition to nutritive dietary components (vitamins, minerals, sugars, organic acids, dietary fibres and unsaturated fats), berries are also a good source of different classes of phytochemicals such as flavonoids (anthocyanins, flavonols and flavanols), tannins (proanthocyanidins, ellagitannins and gallotannins), stilbenoids (*e.g.* resveratrol), phenolic acids (hydroxybenzoic and hydroxycinnamic acid derivates) and lignans (*2*). Berry fruits are popularly consumed not only in fresh and frozen forms but also as processed and derived products including canned fruits, yogurts, beverages, jams and jellies. In addition, there has been a growing trend in the intake of berry extracts as ingredients in functional foods and dietary supplements, which may or may not be combined with other colourful fruits, vegetables and herbal extracts (*1,2*). In Croatia, berries like red raspberries, blackberries, blueberries and strawberries are commonly used in diet, but black chokeberry is almost unknown fruit (*3*).

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Black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) belongs to the Rosaceae family, subfamily Maloideae, and is a deciduous shrub originating from the eastern part of North America (*4,5*) where it has been used for the treatment of cold by native Americans (Abnakians and Potawatomians). Today, chokeberry is also cultivated in Eastern European countries and Russia (*6*), where it is used for production of homemade or commercial juices, jams, fruit tea, wine and natural food colourants (*5,7*). It shows high resistance to frost, mechanized harvesting, damage during transportation and cold storage. Due to these advantages, popularity of chokeberry has increased recently (*8*). Chokeberries have very high contents of polyphenols, namely phenolic acids, proanthocyanidins, anthocyanins, flavonols and flavanones (*9–12*). In a study where 143 different plant samples were analysed for polyphenols, the highest contents of these compounds were found in chokeberry (*13*). The high content and composition of the phenolic constituents of *Aronia melanocarpa* seem to be responsible for the wide range of the fruit's potential medicinal and therapeutic effects. Chokeberries have one of the highest *in vitro* antioxidant activities among fruits. The mechanisms of the *in vivo* antioxidant activity of their phenolics after absorption spread out far beyond radical scavenging and include suppressing the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), inhibition of prooxidant, and restoration of antioxidant enzymes, and probably also cellular signalling to regulate the level of antioxidant compounds and enzymes (*14*). Although recent studies have pointed out different positive effects of chokeberry juices and extracts (*15–21*), current evidence of effectiveness does not yet meet the accepted standards that would secure chokeberry products an indisputable place in therapy. Promising indications from laboratory and clinical data need to be confirmed in more rigorous studies before putative therapeutic uses can be confidently recommended for chokeberry products (*22*).

There are no studies on compositional and physical properties of *Aronia melanocarpa* products present on the Croatian market. Therefore, in this study 22 chokeberry products are evaluated. The objective of this study is to evaluate the physicochemical properties, the content of phenolics (total phenolics, flavonoids, nonflavonoids and anthocyanins) as well as antioxidant properties of different chokeberry products present on the market.

Materials and Methods

Chokeberry products (Table 1) were purchased on Croatian markets during February 2014. There was only one requirement: they had to contain only chokeberry, without added sugar or other fruits. Products were stored at 4 °C until analysis.

Determination of physicochemical parameters

Total solid content of the chokeberry 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. Soluble solid content was determined with a digital refractometer (Atago PAL-3, Tokyo, Japan) and expressed as °Brix. Sample pH was determined at room temperature using an MA 5740 pH meter (ISKRA, Kranj, Slovenia). Two-point calibration was obtained using buffers at pH=7.0 and 4.0. Titratable acidity was determined by titration of the water solution of chokeberry product with 0.1 M NaOH to end point of neutral pH (8.1). The volume of 0.1 M NaOH required to reach pH=8.1±0.2 was determined. The total titratable acidity was expressed as percentage of citric acid using a conversion factor of 0.070 (*23*).

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

Producer	Country of origin	w(fruit) / %	Composition
Juices			
Aronia Original Naturprodukte GmbH	Germany	100	Chokeberry fruit
Aronija Live d.o.o.	Croatia	100	Chokeberry fruit
Vitanea LTD	Bulgaria	100	Chokeberry fruit
Alnavit GmbH	Germany	100	Chokeberry fruit
Biotta AG	Germany	100	Chokeberry fruit
NA	Poland	100	Chokeberry fruit
Bobica d.o.o.	Croatia	100	Chokeberry fruit
Armedina d.o.o.	Serbia	100	Chokeberry fruit
Aronija Vita d.o.o.	Serbia	100	Chokeberry fruit
Voelkel GmbH	Germany	100	Chokeberry fruit
Medicura Naturprodukte AG	Germany	100	Chokeberry fruit
Powders			
Aronia Original Naturprodukte GmbH	Germany	100	Chokeberry pulp
Bobica d.o.o.	Croatia	100	Chokeberry fruit
Aronija Vita d.o.o.	Serbia	100	Chokeberry pomace
Capsules			
Darvitalis d.o.o.	Serbia	100	Chokeberry extract
Bobica d.o.o.	Croatia	100	Chokeberry extract
Fruit tea*			
Aronija Live d.o.o.	Croatia	100	Chokeberry pomace
Darvitalis d.o.o.	Serbia	100	Chokeberry pomace
Bobica d.o.o.	Croatia	93	Chokeberry pomace; chokeberry leaves
Vitanea LTD	Bulgaria	100	Chokeberry pomace
Dried berries			
Aronia Original Naturprodukte GmbH	Germany	100	Chokeberry fruit
Bobica d.o.o.	Croatia	100	Chokeberry fruit

*Dried and ground chokeberry pomace. The pomace consists of chokeberry skin and pips that are left when chokeberries are processed to become chokeberry juice
NA=data about producer not available, imported by Biovega d.o.o.

Determination of juice colour

The colour of the chokeberry juices was measured in a transmitted mode through Konica Minolta CM-3500d spectrophotometer (Konica Minolta, Inc., Tokyo, Japan). Measurements were conducted in CIE $L^*a^*b^*$ system. L^* is a measure of lightness, where values range from completely opaque (0) to completely transparent (100), a^* is a measure of redness (or $-a^*$ of greenness) and b^* of yellowness (or $-b^*$ of blueness) on the hue circle. The hue angle, $h°$, (Eq. 1) describes the relative amounts of redness and yellowness where 0°/360° is defined for red/magenta, 90° for yellow, 180° for green and 270° for blue colour:

$$h° = \arctan\frac{b^*}{a^*} \qquad /1/$$

Chroma (C^*) gives further information on the saturation or intensity of colour (*24,25*):

$$C^* = \sqrt{a^2 + b^2} \qquad /2/$$

Extraction of phenolics

Phenolics were extracted according to the modified method by Benvenuti *et al.* (*26*). Exactly 6 g of samples were weighed out and mixed with 20 mL of methanol/2 % HCl (95:5, by volume). After 60 min the solution was filtered under vacuum in a 50-mL volumetric flask. Extraction of the residue was repeated using the same conditions. The filtrates were combined and adjusted to 50 mL in a volumetric flask with methanol/2 % HCl (95:5, by volume). The obtained extract was used for determination of total phenolic content (TPC), total nonflavonoids (TN), total anthocyanins (TA) as well as for antioxidant capacity assay by DPPH method and reducing power assay using FRAP method.

Determination of total phenolics

For determination of TPC, a method with Folin-Ciocalteu reagent was used (*27*). An aliquot (20 μL) of diluted chokeberry extract 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 (200 g/L) was added to the mixture which was then shaken. After incubation at room temperature for 2 h, the resulting absorbance was measured by the spectrophotometer Pye Unicam SP6-500 (Pye Ltd., Philips, Cambridge, UK) 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:

$$y = 0.00103x - 0.01128 \qquad /3/$$

where y is the absorbance at 765 nm and x is the concentration of gallic acid in mg/L; R^2=0.9973. Total phenolics were expressed as mg of gallic acid equivalents (GAE) per L of chokeberry juices and as mg of GAE per 100 g of dry matter (dm) of other chokeberry products.

Determination of total flavonoid and nonflavonoid contents

Determination of total flavonoid (TF) content was performed by the indirect method using formaldehyde to precipitate these compounds, as described by Ough and Amerine (*28*). A mixture of 3 mL of chokeberry extract solution, 1.5 mL of aqueous solution of hydrochloric acid (1:4, by volume) and 3 mL of formaldehyde was prepared in a 25-mL flask. In order to remove air, nitrogen gas was injected and the stoppered flask was left in the dark for 24 h at 22 °C. The next day it was filtered and the clear liquid was used in the same procedure (*27*) as the one used to prepare samples for TPC determination. The amount of TF was calculated as the difference between total phenolics and total nonflavonoids (TN). The results were expressed as mg of GAE per L and as mg of GAE per 100 g of dm for chokeberry juices and other products, respectively.

Determination of total anthocyanins

Total anthocyanin (TA) content, calculated as cyanidin-3-glucoside, was determined by the pH differential method of Giusti and Wrolstad (*29*). Two dilutions of each chokeberry extract were prepared, one with potassium chloride buffer (pH=1.0), and the other with sodium acetate buffer (pH=4.5). After 15 min of incubation at room temperature, the absorbance was measured simultaneously at the wavelengths of 510 and 700 nm. The content of TA was calculated using Eqs. 4 and 5 with molar absorption coefficient of cyanidin-3-glucoside of 26 900 L/(mol·cm) and molar mass of 449.2 g/mol:

$$A = \left(A_{510\,\mathrm{nm}} - A_{700\,\mathrm{nm}}\right)_{\mathrm{pH=1.0}} - \left(A_{510\,\mathrm{nm}} - A_{700\,\mathrm{nm}}\right)_{\mathrm{pH=4.5}} \qquad /4/$$

$$\mathrm{TA} = \frac{A}{\varepsilon \cdot L} \cdot M_r \cdot \mathrm{DF} \cdot \frac{V}{m} \cdot 100 \qquad /5/$$

where A is absorbance, ε is molar absorption coefficient of cyanidin-3-glucoside equivalents (CGE) (L/(mol·cm)), L is cell pathlength (1 cm), M_r is molecular mass of CGE, DF is dilution factor, V is final volume (mL), and m is mass of the sample (mg). Results were expressed as mg of CGE per L of chokeberry juices and as mg of CGE per 100 g of dm of other products.

Determination of total antioxidant capacity by DPPH method

The effect of chokeberry products on 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical was determined according to the method of Brand-Williams *et al.* (*30*). The method was based on the reduction of stable DPPH radical in the presence of antioxidants. A volume of 2 mL of diluted chokeberry extract or methanol solution of Trolox (25–200 μmol/L) was mixed with 2 mL of methanol and 1 mL of 0.5 mM DPPH methanolic solution. The mixture was vortexed and kept in the dark for 20 min. After incubation, the absorbance was measured at the wavelength of 517 nm against a blank of methanol without DPPH. The results were calculated according to the calibration curve for Trolox:

$$y = -0.62525x + 1.33117 \qquad /6/$$

where y is the absorbance at 517 nm and x is the concentration of Trolox in μmol/L; R^2=0.9817. DPPH values were expressed as mmol of Trolox equivalents (TE) per L and mmol of TE per 100 g of dm for chokeberry juices and other products, respectively.

Determination of FRAP

The ferric reducing antioxidant power (FRAP) assay was conducted according to Benzie and Strain (*31*). The method is based on the reduction of the Fe^{3+}-2,4,6-tripyridyl-*s*-triazine (TPTZ) complex to the ferrous form at low pH. This reduction is monitored by measuring the absorbance change at 595 nm. The FRAP reagent was prepared from 5 mL of TPTZ solution (10 mmol/L) in hydrochloric acid (40 mmol/L) and 5 mL of $FeCl_3$ solution (20 mmol/L) mixed with 50 mL of acetate buffer (0.3 mol/L, pH=3.6). For the determination of the antioxidant capacity, the FRAP reagent (2.08 mL) was mixed with 240 μL of water and 80 μL of the appropriately diluted sample or standard solution of $FeSO_4 \cdot 7H_2O$ (0.125–2.000 mmol/L). The mixture was allowed to stand for 5 min at 37 °C before the absorbance was measured at 595 nm. FRAP values were calculated according to the calibration curve for $FeSO_4 \cdot 7H_2O$:

$$y=0.72126x–0.06396 \qquad /7/$$

where y is the absorbance at 595 nm and x is the concentration of $FeSO_4 \cdot 7H_2O$ in mmol/L; R^2=0.9987, and they were expressed as mmol of Fe^{2+} equivalents (FE) per L and as mmol of FE per 100 g of dm for chokeberry juices and other products, respectively.

Statistical analysis

The data were analysed using STATISTICA v. 12.0 (Statsoft Inc, Tulsa, OK, USA). Analysis of variance (ANOVA) was used to establish significant differences between and within groups of chokeberry products. Differences were considered significant at $p \leq 0.05$. Values were expressed as means (*N*=3). For comparison of the contents of TPC, TF, TN, TA and DPPH or FRAP assays and also for comparison of colour parameters and TPC, TF, TN or TA contents, the coefficients of correlation were determined for each combination.

Results and Discussion

Chokeberry fruits are not popular as table fruits but they are generally consumed as processed chokeberry products including juice, jam, syrup and nutritional supplements. Data on the phenolic contents of chokeberry have been reported in several studies (*3,6,26,32–35*), and the present study contributes to the existing knowledge by providing new data on different chokeberry products such as powders, capsules, fruit tea and dried berries.

Physicochemical parameters and juice colour

The analysis of chokeberry samples indicated different physicochemical properties among groups of products as well within groups (Table 2). In case of chokeberry juices, the total solid content ranged from 13.42 % in juice sample J10 to 21.54 % in juice sample J2, while in the other samples it was much higher. Chokeberry capsules had the highest total solid content among groups (mean value 93.78 %) followed by chokeberry powders (mean value 92.35 %), fruit tea (mean value 91.49 %) and dried berries (mean value 83.31 %). In research of Mayer-Miebach *et al.* (*36*) the dry matter content of berries ranged from 17.9 to 26.0 %, in juices from 11.1 to 17.4 % and in pomace from 44.6 to 50 %. The mean value of total solid content of chokeberry capsules (93.78 %), fruit tea (2.35 %) and powders (91.49 %) present on the market is very similar to the results of Sójka *et al.* (*37*), who investigated chokeberry pomace obtained in an industrial-scale processing of fruit into juice. The lowest value of soluble solid content (13.70 °Brix) was in juice sample J10, while the highest value characterised capsules, *i.e.* sample C1 (83.71 °Brix). The soluble solid content in chokeberries depends on numerous factors: weather, environmental conditions, crop period and variety, and it amounts to 12.4 or 18.3 % (*5*). Chokeberry products had a mean pH value of 3.90 ranging from 3.54 (sample J10) to 4.28 (sample DB1). The mean total titratable acidity (TTA) of all products was 1.42 (as percentage of citric acid) ranging from 0.29 (sample J2) to

Table 2. Physicochemical properties of chokeberry products

Sample	pH	TTA/%	Total solid content/%	°Brix	°Brix/TTA
Juices					
J1	3.90±0.02	0.67±0.07	14.70±0.04	14.56	24.72
J2	3.90±0.02	0.29±0.07	21.54±0.03	20.99	78.54
J3	3.86±0.02	0.85±0.07	18.98±0.14	18.52	23.98
J4	3.71±0.03	1.13±0.12	15.75±0.11	15.47	15.20
J5	3.80±0.03	1.06±0.07	15.79±0.15	15.27	16.08
J6	3.68±0.02	1.26±0.07	14.50±0.16	14.09	12.52
J7	3.74±0.02	0.87±0.07	14.61±0.22	13.99	18.06
J8	3.75±0.02	1.32±0.07	17.01±0.21	17.34	14.61
J9	3.92±0.01	0.84±0.07	14.32±0.11	13.69	18.28
J10	3.54±0.01	1.30±0.07	13.42±0.05	13.30	11.48
J11	3.89±0.02	0.97±0.07	14.27±0.01	13.79	16.01
Powders					
P1	4.10±0.02	1.67±0.07	94.797±0.001	26.75	16.02
P2	4.02±0.01	2.30±0.07	91.82±0.32	37.53	16.36
P3	4.13±0.01	2.17±0.07	90.44±0.15	37.34	17.24
Capsules					
C1	3.31±0.02	4.66±0.07	93.96±0.27	83.71	17.97
C2	4.10±0.01	2.10±0.07	93.60±0.26	31.91	15.23
Fruit tea					
FT1	4.13±0.02	1.34±0.06	91.90±0.26	38.22	28.52
FT2	4.01±0.01	1.08±0.06	88.32±0.27	12.17	11.24
FT3	4.01±0.02	1.60±0.07	89.74±0.19	33.42	20.86
FT4	4.04±0.02	1.37±0.06	96.01±0.14	25.48	18.63
Dried berries					
DB1	4.28±0.02	(1.13±0.06)^a	(84.61±0.92)^b	25.49	22.62
DB2	4.01±0.02	(1.37±0.11)^a	(82.00±0.55)^b	20.93	15.30

The values are presented as mean±standard deviation (S.D.). The same letter in the superscript in the same column indicates no significant differences ($p>0.05$). TTA=total titratable acidity as citric acid, J1–J11=chokeberry juices, P1–P3=chokeberry powder, C=chokeberry capsules, FT=chokeberry fruit tea, DB=chokeberry dried berries

4.66 % (sample C1). Comparing the groups of products, it is evident that capsules have the highest total titratable acidity and juices the lowest. Ochmian *et al.* (*5*) reported similar values for titratable acidity in the range from 0.75 to 1.05 g of citric acid per 100 g of berries. The °Brix/TTA ratio is a quality attribute used by the fruit industry to indicate the tartness of fruits and fruit juices (*38*). This ratio increases with maturity of the fruit and is used to identify the optimum maturity for harvesting to produce maximum product quality (*39*). The mean °Brix/TTA ratio was 20.42 and ranged from 11.24 in the fruit tea sample FT2 to 78.54 in the juice sample J2 (Table 2). ANOVA showed significant differences of physicochemical properties among juices, powders, fruit tea, capsules and dried berries and also among individual samples within groups, with the exception of total solid content and total titratable acidity of samples of dried berries.

Since the colour of the product, especially juices, is extremely important feature that contributes to the overall quality, one of the aims of this paper was to determine colour parameters of chokeberry juices (Table 3). Values of variable L^* were low in all samples, from 0.52 (juice sample J10) to 15.00 (juice sample J7), which indicates that samples were very dark since the variable L^* varies from 0 representing black to 100 representing white. Similar values of the parameter L^* of chokeberry juices were observed by Ochmian *et al.* (*5*). The a^* value, providing information of the position in the colour gamut between green and red, measured on the juice surface ranged from 3.74 (juice sample J10) to 46.42 (juice sample J7). The juice surface colour defined by the b^* parameter, indicating the location on the axis between yellow and blue colours, ranged from 0.88 (juice sample J10) to 25.84 (juice sample J7), which means that yellow colour is present. Positive a^* values were also observed in chokeberry juices, pulp and fruit by Ochmian *et al.* (*5*) and in chokeberry powders by Horszwald *et al.* (*40*). In a research of Horszwald *et al.* (*40*) yellow colour was present in chokeberry powders, while in the work of Ochmian *et al.* (*5*) b^* values were negative, which indicates the presence of blue colour. Parameters L^*, C^* and $h°$ are related to the physiological attributes of visual response (*41*). Hue describes the visible colour and chroma describes the brightness or intensity of the hue. Indices of L^*, C^* and $h°$ are usually useful for tracking colour changes (*42*). The decrease in chroma means an increase in the tonality of the fruit colour (*43*).

Table 4 shows the correlation coefficients between the colour parameters and TPC, TN, TF and TA, from which a negative correlation of colour parameters with the content of total nonflavonoids and of colour parameters with the content of total anthocyanins is evident.

Table 4. Correlation coefficients (R) between phenolics and colour parameters of chokeberry juices

Colour parameters	TPC	TN	TF	TA
L^*	–0.21	–0.61[a]	–0.10	–0.76[b]
a^*	–0.02	–0.52[a]	–0.08	–0.81[b]
b^*	–0.21	–0.61[a]	–0.10	–0.76[b]
$h°$	–0.28[a]	–0.59[a]	–0.17	–0.70[b]
C^*	–0.06	–0.54[a]	–0.04	–0.81[b]

[a,b]significant at p≤0.05 and p≤0.001, respectively
Contents of total phenolics (TPC), total nonflavonoids (TN), total flavonoids (TF) and total anthocyanins (TA) are expressed as mg per L. TPC, TN and TF are expressed as mg of gallic acid equivalents (GAE), while TA is expressed as mg of cyanidin-3-glucoside equivalents (CGE)

Total phenolics, flavonoids, nonflavonoids and anthocyanins

The content of total phenolics (TPC), total flavonoids (TF) and total nonflavonoids (TN) in twenty-two chokeberry products is given in Table 5. TPC ranged from 1494 mg of GAE per 100 g of dm in fruit tea sample FT3 to 5292 mg of GAE per 100 g of dm in capsule sample C2. Comparing the results of our research with the results of other authors, the mass fraction of TPC in chokeberry juices was lower than in the findings of others (*3,14,35,43*). Some authors noticed higher values of phenolics in black chokeberry fruit in comparison with our results (*14,26,32–34*), while Jurgoński *et al.* (*44*) reported much higher values of total phenolics in commercial chokeberry extract. Different cultivars of chokeberries were analysed and total phenolic values ranged from 8563.8 to 12055.7 mg of GAE per kg of fresh mass (fm) (*34*). 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 (*14*). It was demonstrated that the total phenolics in hot-air-dried tomatoes increased up to 29 % compared to the corresponding levels in fresh tomatoes (*45*). In comparison with other products, chokeberry juices had lower phenolic content, which might be related to the differences in their moisture content (*46*). In total phenolic content, flavonoids were predominant, and their amounts varied from 867 mg of GAE per 100 g of dm in DB1 sample to 3317 mg of GAE per 100 g of dm in P2 sample. Average total flavonoid content in chokeberry juices was 3180 mg of GAE per L. It was calculated that percentages of TF in

Table 3. Colour parameters of chokeberry juice samples (J1–J11)

Colour parameters	Sample										
	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11
L^*	1.20	5.93	8.87	4.27	13.85	2.38	15.00	5.91	9.75	0.52	5.31
a^*	8.14	33.95	39.29	27.76	44.92	16.64	46.42	33.51	39.44	3.74	31.58
b^*	2.04	10.19	15.21	7.35	23.79	4.08	25.84	10.15	16.79	0.88	9.12
$h°$	14.1	16.7	21.2	14.8	27.9	13.8	29.1	16.9	23.1	13.2	16.1
C^*	8.4	35.4	42.1	28.7	50.8	17.1	53.1	35.0	42.9	3.8	32.9

Table 5. Total phenolics (TPC), total nonflavonoids (TN), total flavonoids (TF) and total anthocyanins (TA) in chokeberry products

Sample	TPC	TN	TF	TA
		Juices		
J1	5202±252	1383±124	3819±160	526±20
J2	5448±479	1064±92	4384±571	592±24
J3	3908±682	1088±240	2819±451	216±10
J4	3358±702	1090±161	2267±550	434±13
J5	4672±644	1156±259	3515±384	154±6
J6	4083±490	1415±174	2667±330	504±16
J7	3002±388	808±52	2193±386	150±4
J8	6639±455	1368±83	5271±527	541±29
J9	3759±692	1370±238	2389±618	235±11
J10	3500±338	1320±213	2180±519	1228±5
J11	5002±572	1527±417	3474±587	303±19
		Powders		
P1	4434±153	1602±124	2831±189	1641±24
P2	4951±230	1634±67	3317±240	1576±74
P3	4233±234	1906±139	2327±373	1165±10
		Capsules		
C1	4511±184	(2051±184)[a]	2459±31	2468±102
C2	5292±243	(2300±231)[a]	2992±265	1997±138
		Fruit tea		
FT1	3436±242	1113±86	2322±168	675±17
FT2	2435±75	1557±52	878±124	459±34
FT3	1494±179	574±55	919±125	282±11
FT4	1504±90	479±22	1024±110	353±16
		Dried berries		
DB1	1954±54	(1086±74)[b]	867±109	(141±9)[c]
DB2	2466±91	(1072±84)[b]	1394±20	(147±17)[c]

The values are presented as mean±standard deviation (S.D.). The same letter in the superscript in the same column indicates no significant differences ($p>0.05$). J1–J11=chokeberry juices, P1–P3=chokeberry powders, C=chokeberry capsules, FT=chokeberry fruit tea, DB=chokeberry dried berries. Contents of TPC, TN, TF and TA are expressed as mg per 100 g of dry matter (dm) in powder, capsule, fruit tea and dried berry samples. Contents of TPC, TN, TF and TA in juice samples are expressed as mg per L. TPC, TN and TF are expressed as mg of gallic acid equivalent (GAE), while TA are expressed as mg of cyanidin-3-glucoside equivalents (CGE)

TPC varied between 36.06 and 80.46 %. The obtained results suggest that flavonoids were the most abundant phenolics in chokeberry products. Chokeberries are a rich source of anthocyanins, proanthocyanidins and hydroxycinnamic acids (*14*). Oszmianski and Wojdylo (*35*) showed that polymeric proanthocyanins are the major class of polyphenolic compounds in chokeberry and represent 66 % of polyphenols in fruits. Their content ranged between 1578.79 mg per 100 g of dm of chokeberry juice up to 8191.58 mg per 100 g of pomace. In a research of Kapci *et al.* (*47*) the content of total flavonoids was higher in chokeberry juices and in dried chokeberries. According to the literature, the main contributor of total flavonoid content is quercetin. Quercetin and several quercetin glycosides (quercetin-3-galactoside, quercetin-3-glucoside and quercetin-3-rutinoside) were also detected in chokeberries but in relatively low mass fractions of about 71 mg per 100 g of fm (*14*).

All samples had lower content of TN (808 to 1527 mg of GAE per L and 479 to 2300 mg of GAE per 100 g of dm) and TA (150 to 1228 mg of CGE per L and 141 to 2468 mg of CGE per 100 g of dm). Chlorogenic and neochlorogenic acids are the major non-flavonoid polyphenolic compounds in chokeberries, and according to Oszmianski and Wojdylo (*35*) they represent about 7.5 % of chokeberry fruit polyphenols. The hydroxycinnamic acids are represented by significant amounts of chlorogenic (61 to 193 mg per 100 g of fm) and neochlorogenic acids (85 to 123 mg per 100 g of fm) (*14*). Higher contents of TA in chokeberry juice were reported by Jakobek *et al.* (*34*) and others (*26,47*), while Horszwald *et al.* (*40*) reported higher content of TA in chokeberry powders. Results of all chokeberry samples were found to be lower, which can be explained by using pH differential method instead of HPLC method. Anthocyanins represented significant fraction of total phenolics in powder and capsule samples (from 27.53 in P3 to 54.72 % in C1 sample). Chokeberries contain relatively higher amounts of anthocyanins compared to other fruits including blueberry, blackberry, raspberry, grape and cherry, which are known as rich sources of anthocyanins (*14*). In research of Jakobek *et al.* (*3*) the fraction of anthocyanins in chokeberry was 41 %, which was much higher compared to the fraction in red raspberry (19 %) and strawberry (23 %). Similar to total phenolic content, Jurgoński *et al.* (*44*) reported considerably higher concentration of anthocyanins. Compared to other berries, the aronia anthocyanin profile is very simple, consisting almost exclusively of cyanidin glycosides, namely cyanidin-3-arabinoside, cyanidin-3-galactoside, cyanidin--3-glucoside and cyanidin-3-xyloside. Cyanidin-3-galactoside and cyanidin-3-arabinoside are predominant in the berries with a cumulative content >90 % (*14*). Lower levels of total anthocyanins in chokeberry products can be the result of factors such as pH, chemical composition, temperature, light and oxygen. These factors may change easily during processing of fruits into juice and other products. It was reported that anthocyanins are affected at several steps of juice processing, namely pressing, clarification and pasteurisation (*47,48*).

Total antioxidant capacity and reducing power

The total antioxidant capacity (TAC) and reducing power (RP) of different chokeberry samples are shown in Table 6. Examined products possess high antioxidant capacity (12.09 to 40.19 mmol of TE per L and 58.49 to 191.31 mmol of TE per 100 g of dm) and reducing power (38.71 to 79.86 mmol of Fe^{2+} per L and 13.50 to 68.60 mmol of Fe^{2+} per 100 g of dm). Highest TAC was reported in dried berries (mean value 187.41 mmol of TE per 100 g of dm), followed by fruit tea (mean value 144.54 mmol of TE per 100 g of dm) and powder (mean value 110.58 mmol of TE per 100 g of dm) samples. The reducing power (FRAP assay) in this study was determined as reduction of Fe^{3+} to Fe^{2+}. The highest RP was observed in P2 sample (68.60 mmol of Fe^{2+} per 100 g of dm), followed by C1 sample (65.82 mmol

Table 6. Total antioxidant capacity (TAC) and reducing power (RP) of chokeberry products

Sample	TAC	RP
Juices		
J1	33.37±0.54	76.14±0.36
J2	12.09±0.93	51.50±0.24
J2	23.03±0.53	48.76±0.48
J4	19.47±0.33	72.43±0.52
J5	18.29±0.68	48.64±0.43
J6	20.66±2.45	79.86±0.14
J7	16.51±0.14	38.98±0.25
J8	34.22±1.61	71.50±0.28
J9	26.25±0.28	38.71±0.41
J10	40.19±2.13	62.92±0.35
J11	28.12±0.88	60.13±0.29
Powders		
P1	95.00±2.94	60.66±2.17
P2	105.68±5.58	68.60±0.99
P3	131.06±0.47	47.38±2.68
Capsules		
C1	58.49±7.30	(65.82±4.20)[a]
C2	80.93±4.56	(60.35±1.70)[a]
Fruit tea		
FT1	149.44±0.89	32.74±1.66
FT2	111.43±2.01	43.12±0.91
FT3	163.33±4.23	13.50±0.22
FT4	153.96±2.99	15.94±1.32
Dried berries		
DB1	183.52±4.20	21.51±2.330
DB2	191.31±0.38	17.4±1.0

The values are presented as mean±standard deviation (S.D.). The same letter in the superscript in the same column indicates no significant differences ($p>0.05$). TAC is expressed as mmol of Trolox equivalent (TE), while RP is expressed as mmol of Fe^{2+} equivalents (FE). For chokeberry juices TAC and RP are expressed as mmol of TE per L and mmol of FE per L, respectively. For powder, capsule, fruit tea and dried berry samples TAC and RP are expressed as mmol of TE per 100 g of dry matter (dm) and mmol of FE per 100 g of dm, respectively
J1–J11=chokeberry juices, P1–P3=chokeberry powders, C=chokeberry capsules, FT=chokeberry fruit tea, DB=chokeberry dried berries

of Fe^{2+} per 100 g of dm), P1 sample (60.66 mmol of Fe^{2+} per 100 g of dm) and C2 sample (60.35 mmol of Fe^{2+} per 100 g of dm). High antioxidant activity of chokeberry fruit and products has been reported in numerous studies (*3,6,14,34,35*). Walkowaik-Tomczak (*48*) showed that antioxidant activity of chokeberry juices is under the influence of pasteurisation and storage. Oxygen availability rate during pasteurisation and storage and storage temperature were found to have the biggest effect on the antioxidant activity of chokeberry juices. Reducing power is generally linked to the presence of reducing substances, which have been shown to exert antioxidant activity by breaking the free radical chain by donating a hydrogen atom (*49*). Antioxidant activity of chokeberry juice concentrate against DPPH radical was stronger than that of black currant, elderberry, red currant, strawberry, red raspberry and cherry concentrate (*3,26*).

The correlation between the antioxidant activity measured by DPPH and FRAP method and total phenolics is presented in Table 7. Different groups of polyphenolic compounds may contribute differently to total antioxidant activity and, therefore, it is necessary to observe the existence of a correlation between the antioxidant activity and individual groups of polyphenolic compounds. The antiradical activity was mostly affected by the content of

Table 7. Correlation coefficients (R) between phenolics and total antioxidant capacity (TAC) or reducing power (RP) in chokeberry products

Phenolics	TAC	RP
Juices		
TPC	0.19*	0.29**
TN	0.47	0.37*
TF	0.09**	0.21**
TA	0.59*	0.47**
Powders		
TPC	–0.45*	0.80
TN	0.74	–0.72*
TF	–0.60**	0.86
TA	–0.94**	0.84
Capsules		
TPC	0.85	–0.67*
TN	0.52	–0.25*
TF	0.84	–0.76*
TA	–0.86*	0.70*
Fruit tea		
TPC	–0.33**	0.70
TN	–0.89**	0.98
TF	0.20**	0.23*
TA	–0.10**	0.48
Dried berries		
TPC	0.76**	–0.78*
TN	0.10*	–0.32
TF	0.71**	–0.68*
TA	0.02*	0.09

*, **significant at $p\leq0.05$ and $p\leq0.001$, respectively
Contents of total phenolics (TPC), total nonflavonoids (TN), total flavonoids (TF) and total anthocyanins (TA) are expressed as mg per 100 g of dry matter (dm) for powder, capsule, fruit tea and dried berry samples. Contents of TPC, TN, TF and TA for juice samples are expressed as mg per L. TPC, TN and TF are expressed as mg of gallic acid equivalent (GAE), while TA is expressed as mg of cyanidin-3-glucoside equivalents (CGE). TAC and RP are expressed as mmol per 100 g of dm for powder, capsule, fruit tea and dried berry samples. TAC and RP for juice samples are expressed as mmol per L. TAC is expressed as mmol of Trolox equivalent (TE), while RP is expressed as mmol of Fe^{2+} equivalents (FE)

phenolic compounds. To see the relationship between the phenolic compounds in chokeberry products and their antiradical activity, TAC and RP values were correlated with the amount of phenolic compounds. This showed that the highest correlation of phenolic compounds and total antioxidant acitivity was between TA and TAC, and between TN and TAC in powder samples, followed by TN and TAC in fruit tea samples. High correlation was also found between TF and RP in powders and between TN and RP in fruit tea. These results imply that flavonoids and nonflavonoids were the major contributors to the antioxidant capacity of the investigated chokeberry products, especially in the case of powders, fruit tea and capsules. Acording to the data presented by others, TPC of various small fruits correlates better with the antioxidant activity than TA does (*3*,*9*). ANOVA showed significant differences between TAC and RP values between groups of chokeberry products and also among individual samples within groups, with the exception of the RP of samples of capsules.

Conclusion

In this investigation, very high contents of phenolic substances and high values of antioxidant properties were observed in different chokeberry products. The presented data show differences in the quality and phenolic composition of chokeberry juices, powders, capsules, fruit tea and dried berries found on the market. Chokeberry capsules and powders have considerably higher amount of total phenolics and total anthocyanins in comparison with other products. Different levels of antioxidants might be related to the differences in the variety and growing conditions of the fruits. To fully understand the effect of processing, research focused on different processing techniques starting from the same material should be done. Chokeberry products can become a valuable source of nutritionally important substances in human nutrition. Due to the high content of natural antioxidants, their consumption could bring health benefits. Besides studies focusing on different processing techniques, future studies should include additional analyses to obtain a complete evaluation of the quality of chokeberry products and also *in vivo* and *in vitro* bioavailability studies. Data from these studies will be helpful to understand the bioaccessibility and bioavailability of nutritive compounds of chokeberry and its products.

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