Investigating the antioxidant potential of chokeberry (*Aronia melanocarpa*) products

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Summary

Several studies have reported on the flavonoid and phenolic acid contents of chokeberry. However, limited reports have been focused on the anthocyanins and antioxidant activity of chokeberry products, although chokeberries are generally consumed as processed. In order to determine the health-related constituents of different chokeberry products, total phenolics, flavonoids, anthocyanins and antioxidant activity were examined in fourteen chokeberry products. The highest total phenolics and anthocyanin contents were found in chokeberry pomace with the values of 63.1 $g \cdot kg^{-1}$ expressed as gallic acid equivalents, and 4.5 $g \cdot kg^{-1}$ expressed as cyanidin-3-glucoside equivalents. Total flavonoid content and total antioxidant activity analysed by three different methods (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), diphenyl-(2,4,6-trinitrophenyl) iminoazanium (DPPH), and cupric ion reducing antioxidant capacity (CUPRAC)) were higher in dried chokeberries compared to the rest of the products. The anthocyanin profile was determined by high performance liquid chromatography. Cyanidin-3-galactoside was found to be the major anthocyanin in all samples, but some differences were observed in the contents of individual anthocyanins.

Keywords

chokeberry products; antioxidant activity; phenolics; flavonoids; anthocyanins

Berries are recommended for a healthy diet because of their contribution to provide protection against health problems including degenerative diseases, cardiovascular diseases or cancer [1]. Their role in protection is connected with some biologically-active compounds such as phenolic acids, anthocyanins or flavanols [2]. Among berries, chokeberries have recently drawn attention because of the health claims associated with their consumption [3, 4].

Black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) is a member of the *Rosaceae* family, which originates in North America. Today, chokeberry is also cultivated in Eastern European countries and in Germany [5]. There are two more known chokeberry species – red chokeberry (*A. arbutifolia*) and purple chokeberry (*A. prunifolia*), the latter being a natural hybrid of red and black chokeberries [6].

Chokeberry shows high resistance to frost, mechanized harvesting, damage during transportation and cold storage [5, 7]. Due to these advantages, popularity of chokeberry has raised recently. Although chokeberries are not popular table fruits because of their astringent taste, they are used in the production of many food products such as juices, jams, concentrates, spirits, preserves, puree, tea and wine [3, 5]. They are also used for natural-food colouring purposes due to their strong dark violet colour [8].

Chokeberries contain a wide range of polyphenolic constituents that have been reported to show anticancer, antioxidative, antiinflammatory, antiatherogenic and antidiabetic effects [9]. In

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comparison to other black berries, higher anthocyanin contents [10, 11] and antioxidant capacities were reported in chokeberries [8, 9, 11]. In recent years, several data have been reported on polyphenol constituents in a variety of fruits, including chokeberries [8, 11–13]. Phenolics, flavonoids, anthocyanins and total antioxidant activities were investigated in chokeberry and some chokeberry products, but the products were limited to chokeberry juice and pomace [14]. To the best of our knowledge, there is no previous study which evaluated the antioxidant potential of a wide range of chokeberry products such as jam, compote, syrup, dried fruit or concentrate. The aim of this study was to investigate the total antioxidant capacity, total anthocyanins, total phenolics and total flavonoid contents as well as individual anthocyanins of chokeberry in its products.

MATERIALS AND METHODS

Chokeberry material

Fourteen black chokeberry samples were used for the analyses (Tab. 1). Eight of them were obtained from different regions in Czech Republic. Five of the samples were obtained from Germany, and one sample from Poland. Compote, two jam samples and dried chokeberry products were prepared according to traditional recipes. For drying process, chokeberry fruits were placed on trays and exposed to sunlight. Chokeberries that were used to prepare the dried samples were harvested in Czech Republic. Chokeberry pomace containing skin and seeds of chokeberry fruit was provided from a company located in Germany. Compote was prepared by adding sugar (12%), citric acid (0.5%) and chokeberry to water. Chokeberry jam was produced by cooking chokeberry with sugar and pectin. Chokeberry jam 2 was prepared by mixing chokeberry with sugar (40%) and rum (3%). Raspberry and sour cherry syrups were obtained from a Czech company and contained 5% and 10% chokeberry, respectively. Chokeberry concentrate was obtained from a Czech company. After the preparation or the arrival of samples, they were immediately extracted using the procedure described below and stored at –20 °C.

Preparation of extracts

For the spectrophotometric assays, 2 ± 0.01 g of each sample was extracted in a cooled ultrasonic bath (Tesla VC 006 DMI, Vráble, Slovakia) for 15 min using 5 ml of 75% aqueous-methanol

Tab. 1. Fruit content, country of origin, composition, and expiration date of chokeberry products.

Classification	Sample	Fruit content [%]	Country of origin	Composition	Expiration date
Pure chokeberry products	Chokeberry fruit	100	Czech Republic	Chokeberry fruit	NA
	Dried chokeberry 1	100	Poland	Chokeberry fruit	February, 2014
	Dried chokeberry 2	100	Czech Republic	Chokeberry fruit	NA
	Chokeberry juice 1	100	Germany	Chokeberry fruit	January, 2014
	Chokeberry juice 2	100	Germany	Chokeberry fruit	May, 2013
	Chokeberry juice 3	100	Germany	Chokeberry fruit	July, 2013
	Chokeberry pomace	100	Germany	Skin and seeds of chokeberry	August, 2014
	Chokeberry concentrate	500	Czech Republic	Chokeberry fruit	NA
Products with significant chokeberry addition	Chokeberry syrup	53	Germany	Chokeberry fruit, saccharose	October, 2013
	Chokeberry compote	70	Czech Republic	Chokeberry fruit, water, saccharose, citric acid	NA
	Chokeberry jam 1	50	Czech Republic	Chokeberry fruit, saccharose, pectin	NA
	Chokeberry jam 2	70	Czech Republic	Chokeberry fruit, saccharose, rum	NA
Products with small amount of chokeberry addition	Raspberry-chokeberry syrup	30	Czech Republic	Raspberry (20%,), apple concentrate (5%), chokeberry concentrate (5%), water, glucose-fructose, citric acid	November, 2012
	Sour cherry-chokeberry syrup	30	Czech Republic	Sour cherry (20%,), chokeberry concentrate (10%), water, glucose-fructose, citric acid	November, 2012

NA – not applicable

containing 0.1% (v/v) formic acid (Penta, Strakonice, Czech Republic). Samples were then centrifuged (Eppendorf 5430, Eppendorf, Hamburg, Germany) for 10 min at 83 Hz and the supernatant was collected in a tube with conical bottom. This procedure was repeated four times until the total volume reached 20 ml [15]. Each sample was extracted immediately to obtain two extracts from two independent bottles of samples (2 replications and 2 parallels). Prepared extracts were stored in tubes with conical bottom at –20 °C until analyses.

Determination of total phenolic (TP) compounds

Total phenolic content was determined by the Folin–Ciocalteu method [16]. A volume of 100 μl of the extract was mixed with 750 μl of 10% Folin–Ciocalteu reagent (Sigma-Aldrich Chemie, Steinheim, Germany) (1 : 10, v/v in distilled water). The mixture was allowed to stand for 5 min and 750 μl of 6% sodium carbonate solution was added to the mixture and mixed well. The solution was incubated at room temperature for 90 min. The absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Thermo Spectronic Genesys 20, Thermo, Madison, Wisconsin, USA) and the results were expressed in grams of gallic acid equivalents (GAE) per kilogram of fresh weight (FW).

Determination of total flavonoid (TF) content

In total flavonoid assay, 0.3 ml of 5% $NaNO_2$ was added to 1 ml of extract at zero time. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At the 6th min, 2 ml 1 mol·l^{-1} NaOH was added to the mixture. The mixture was diluted by the addition of 2.4 ml of distilled water and mixed. Absorbance of the mixture was measured at 510 nm versus blank solution. The total flavonoid content was determined by a (+)-catechin (Sigma Aldrich Chemie) standard curve and was expressed as grams of catechin equivalents (CE) per kilogram of fresh weight [17].

Determination of total anthocyanin (TA) content

The total anthocyanin content was determined by the pH differential method [18]. Extracts were diluted according to appropriate dilution ratios by adding both 0.025 mol·l^{-1} KCl (with pH adjusted to 1.0 by adding 35% HCl) and 0.4 mol·l^{-1} $CH_3COONa{\cdot}3H_2O$ (sodium acetate; pH was adjusted to 4.5 by adding 35% HCl) buffer solutions. Diluted samples were mixed well and left in the dark for 15 min. Absorbance (A) of each diluted sample was measured against water at both 520 nm and 700 nm. Absorbance was calculated as follows:

$$A = (A_{520} - A_{700})_{\text{pH 1.0}} - (A_{520} - A_{700})_{\text{pH 4.5}} \quad (1)$$

where A_{520} is the absorbance measured at 520 nm and A_{700} is the absorbance measured at 700 nm.

The total anthocyanin content was expressed as grams of cyanidin-3-glucoside equivalents (cy-3-glu, molar extinction coefficient of 26 900 l·cm^{-1}mol^{-1} and molecular weight of 449.2 g·mol^{-1}) per kilogram of fresh weight.

Determination of total antioxidant capacity

Total antioxidant levels were estimated by three different methods. In ABTS, DPPH and CUPRAC assays, Trolox (Fluka Chemie, Buchs, Switzerland) was used as standard and results were expressed as grams of Trolox equivalent (TE) per kilogram of fresh weight.

In the ABTS method, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma Aldrich Chemie) and potassium persulfate solutions were mixed and stored overnight at room temperature in the dark to complete radicalization. ABTS stock solution was diluted in 50 mmol·l^{-1} potassium phosphate buffer (pH 8.0) to an absorbance of 0.90 ± 0.02 at 734 nm to prepare the ABTS-working solution. The pH of the mixture had to be 7.4 in the end. A volume of 1 ml of the ABTS solution was added to 100 μl of extract and mixed for 10 s. Decolourization by antioxidants was measured at 734 nm against water after 1 min [19].

The DPPH method was performed according to a previous publication [20]. A volume of 2 ml of 0.1 mmol·l^{-1} diphenyl-(2,4,6-trinitrophenyl) iminoazanium (DPPH, Sigma Aldrich Chemie) was added to 100 μl of extract and the absorbance of the mixture was measured at 517 nm against methanol after incubation in the dark for 30 min.

The cupper reducing antioxidant capacity (CUPRAC) assay was used to measure copper ion reducing ability of polyphenols. A volume of 100 μl of the extract, 1 ml per each of 10 mmol·l^{-1} $CuCl_2$, 7.5 mmol·l^{-1} Neocuproine (Nc, Sigma Aldrich Chemie) solution and 1 mol·l^{-1} ammonium acetate (NH_4Ac) was mixed and 1 ml of pure water was added. Absorbance was measured at 450 nm against reagent blank after incubation in the dark for 1 h [21].

Determination of anthocyanin profile

Anthocyanin profile of chokeberry was examined by a reverse phase HPLC method [22]. For quantitative analysis of specific anthocyanins by HPLC, samples were extracted by adding 20 ml of formic acid (1 : 10, v/v in distilled water) to 4 g of sample (1 g for concentrate samples). After

centrifugation at 100 Hz for 10 min, extracts were purified on DSC-18 SPE columns (Discovery 52606-U, Sigma Aldrich Chemie). Detained anthocyanins were leached by washing with 2 ml of methanol in evaporating flasks. Methanol was evaporated in a vacuum evaporator and the residue was dissolved in 1 ml of 0.01% HCl. Prepared samples were injected and analysed by a HPLC system (Dionex 680; Dionex, Sunnyvale, California, USA) with PDA detector (Ultimate 3000, Dionex). A 5 μm Purospher STAR RP-18e 4×250 mm column (Phenomenex, Torrance, California, USA) was used. The mobile phase consisted of solvent A, water : formic acid (9 : 1) v/v and solvent B, water : formic acid : acetonitril (4 : 1 : 5) v/v/v. A linear gradient was used as follows: at 0 min, 88% solvent A and 12% solvent B; at 1 min, 88% solvent A and 12% solvent B; at 26 min, 70% solvent A and 30% solvent B; at 35 min, 100% solvent B; at 38 min, 100% solvent B; at 41 min, 88% solvent A and 12% solvent B; at 43 min, 88% solvent A and 12% solvent B. The flow rate was 1 ml·min^{-1}. Detection was done at 525 nm and identification was based on the retention times and characteristic UV spectra. Quantification was done by external standard curves. Performance parameters for determination of cyanidin-3-glucoside, limit of detection (*LOD*), limit of quantification (*LOQ*) and recovery were determined according to a previous study [23].

Statistical analysis

Data were collected from two independent extractions for each sample and reported as mean ± standard deviation (*SD*). Data were subjected to statistical analysis using SPSS software (version 16.0 for Windows; SPSS, Chicago, Illinois, USA) for the analysis of variance (ANOVA). Duncan's new multiple range test was used to analyse differences between samples ($p < 0.05$).

RESULTS AND DISCUSSION

Chokeberry fruits are not popular as table fruits because of their bitter taste, and they are generally consumed as processed chokeberry products including juice, jam, syrup and compote. Data on the anthocyanin and phenolic acid contents of chokeberry have been reported in several studies, and the present study contributed to the existing knowledge by providing new data on different chokeberry products. In our study, the findings associated with chokeberry and chokeberry products were generally compatible with the existing literature data. However, we analysed different chokeberry products, which were produced from different raw materials. So, the differences between results might be related to variety/cultivar, growing conditions, climatic conditions and ripening stage [24–25]. Comparing antioxidant potential of chokeberry samples indicated that processed chokeberries had different levels of key compounds compared to fresh chokeberry. Supporting this information, it has been reported that pre- and post-harvest stresses had significant effects on the total antioxidant capacity and phenolic levels of several fruits and vegetables such as tomatoes, strawberries, raspberries and blueberries [26–28]. In the view of fruit processing, there are several steps that include heat treatment, such as boiling, drying, pasteurization, etc. Heat treatments are well known for their effects on nutritional value of processed fruits. Thermal processing at determined temperatures deactivates oxidative and hydrolytic enzymes that may cause loss of phenolics [29]. Some thermolabile compounds may be decomposed by heat treatment [30]. Even though it was reported that heat treatment results in the loss of antioxidants, contradictory results were obtained by different researchers during last years. For example, the amount of phenolics was found to increase during processing of grape [31] or tomato [32]. There are different approaches to explain the reasons for the effect of heat treatment on nutritional compounds. During fruit processing, release of bound phenolic compounds may occur due to the breakdown of cellular constituents. The oxidative and hydrolytic enzymes released by the disruption of cell walls can trigger degradation of antioxidants in fruits. Another explanation of this increase may be emerging of novel compounds with an antioxidant activity, such as high-molecular-weight melanoidins formed by Maillard reaction, which might be formed after heat treatment and also during storage. On the other hand, it is also known that the extraction ability, pH, acid content, saccharides and presence of other additives may also affect the analysis of flavonoids and antioxidant activity. Other food constituents, in particular non-antioxidant constituents, may interfere with phenolic compounds in the sample and deflect real antioxidant value [33]. Extraction ability may be affected by solvent type in case of antioxidant assays [34]. Previous publications indicated that extraction performance was not affected only by solvent type, temperature and extraction time, but also by food matrix that contained a complex mixture of compounds [35].

Total phenolic content

Total phenolic content (TP) of chokeberry and

chokeberry products are presented in Tab. 2. The highest total phenolic content (63.1 g·kg^{-1}) was observed in chokeberry pomace that contained skin and seeds of chokeberry. For pure chokeberry products, total phenolic content was found to be higher in dried chokeberries compared to chokeberry fruit, juice and concentrate, as expected as a result of their higher dry matter content. There was no significant difference among three different batches of chokeberry juices. The lowest total phenolic content (0.8 g·kg^{-1}) among all samples was observed in raspberry syrup as expected, since it contained only 5% of chokeberry. Recently, different cultivars of chokeberries were analysed and total phenolic values ranging from 8.6 g·kg^{-1} to 10.8 g·kg^{-1} fresh weight were reported [25]. Lower or higher values were also reported in the literature, which 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. Polymeric procyanidins were identified as the major class of polyphenolic compounds in chokeberries (51.8 g·kg^{-1} dry weight, DW). In addition, chlorogenic (3.0 g·kg^{-1} DW) and neochlorogenic acids (2.9 g·kg^{-1} DW) represented 7.5% of chokeberry polyphenols [14]. Lower levels of (–)-epicatechin compared to chlorogenic and neochlorogenic acids were also determined [36]. On the other hand, gallic acid (0.016 g·kg^{-1} DW), cinnamic acid (0.34 g·kg^{-1} DW), caffeic acid (0.75 g·kg^{-1} FW) and *p*-coumaric acid (0.069 g·kg^{-1} FW) were also reported as contributors to the phenolic content [37].

Changes in phenolic compounds as a result of processing were reported in several studies [32, 38]. It was demonstrated that the total phenolics in hot-air-dried tomatoes increased up to 29% compared to the corresponding levels in fresh tomatoes [39]. In comparison with chokeberry, chokeberry juice had lower phenolic values, which might be related with the differences in their moisture content. The effect of processing and storage on phenolic value of fruits including berries was demonstrated in previous studies [40]. The level of phenolics of fruits and vegetables were reported to be influenced by various factors such as ripeness, post-harvest storage, and climatic conditions. For example, it was reported that total antioxidant activity and phenolic content decreased during ripening of highbush blueberries [41]. On the other hand, ripe sour cherries were reported to have higher phenolic contents. It was also observed that post-harvest storage may decrease or increase the total phenolic content depending on the ripeness stage of sour cherries [42].

Tab. 2. Total phenolic, flavonoid, anthocyanin contents and total antioxidant capacities of chokeberry and chokeberry products.

Sample	Total phenolics [g·kg^{-1}]	Total flavonoids [g·kg^{-1}]	Total anthocyanins [g·kg^{-1}]	ABTS [g·kg^{-1}]	DPPH [g·kg^{-1}]	CUPRAC [g·kg^{-1}]
Chokeberry fruit	13.3 ± 0.03^{e}	5.3 ± 0.2^{d}	4.5 ± 0.20^{b}	11 ± 0.04^{e}	11.3 ± 0.5^{d}	67.7 ± 1.3^{e}
Dried chokeberry 1	39.9 ± 0.3^{c}	19.9 ± 0.9^{b}	3.1 ± 0.1^{e}	74 ± 2^{b}	36.3 ± 1.2^{a}	257.2 ± 1.9^{a}
Dried chokeberry 2	50.1 ± 0.4^{b}	12.5 ± 1.1^{a}	1.4 ± 0.1^{d}	54.4 ± 1^{a}	30.5 ± 1^{b}	233.2 ± 1.3^{b}
Chokeberry juice 1	6.6 ± 0.1^{gh}	2.7 ± 0.1^{e}	0.7 ± 0.01^{f}	9.8 ± 0.3^{ef}	5.7 ± 0.2^{f}	33.8 ± 1^{g}
Chokeberry juice 2	6.5 ± 0.03^{h}	2.9 ± 0.2^{e}	0.4 ± 0.02^{h}	10.8 ± 0.4^{e}	6.2 ± 0.7^{f}	35.1 ± 0.3^{g}
Chokeberry juice 3	6.3 ± 0.04^{i}	2.8 ± 0.1^{e}	0.6 ± 0.02^{g}	10.8 ± 0.2^{e}	5.8 ± 0.2^{f}	30.7 ± 0.4^{h}
Chokeberry concentrate	29.6 ± 0.1^{d}	6.1 ± 0.2^{d}	3.6 ± 0.1^{c}	22 ± 0.1^{d}	10.8 ± 0.3^{d}	74.5 ± 1.5^{d}
Chokeberry pomace	63.1 ± 0.5^{a}	9.3 ± 1.4^{c}	10 ± 0.4^{a}	49.6 ± 1.3^{c}	25.2 ± 1.1^{c}	192.4 ± 2.3^{c}
Chokeberry jam 1	6.9 ± 0.03^{g}	2.9 ± 0.1^{e}	0.4 ± 0.07^{h}	9 ± 0.2^{f}	5 ± 0.1^{f}	33.6 ± 2.7^{g}
Chokeberry jam 2	12 ± 0.02^{f}	6.4 ± 0.2^{d}	0.2 ± 0.03^{i}	9.8 ± 0.1^{ef}	8.7 ± 0.3^{e}	57.4 ± 0.8^{f}
Chokeberry compote	6.7 ± 0.03^{gh}	3.3 ± 0.1^{e}	0.2 ± 0.02^{i}	9.4 ± 0.04^{f}	4.8 ± 0.1^{f}	33.2 ± 0.8^{g}
Chokeberry syrup	2.6 ± 0.03^{j}	1 ± 0.02^{f}	0.1 ± 0.003^{j}	3.7 ± 0.02^{g}	2.2 ± 0.2^{g}	13.4 ± 0.5^{i}
Raspberry-chokeberry syrup	0.78 ± 0.02^{l}	0.04 ± 0.01^{f}	0.01 ± 0.002^{k}	1.2 ± 0.03^{h}	0.7 ± 0.01^{g}	3 ± 0.1^{j}
Sour cherry-chokeberry syrup	1.4 ± 0.03^{k}	0.2 ± 0.01^{f}	0.03 ± 0.001^{k}	2 ± 0.1^{h}	2 ± 0.1^{g}	5.2 ± 0.8^{j}

Data represent average values ± standard deviation of two independent samples. All contents are expressed per kilogram of fresh weight (FW). Different letters in the columns within each sample represent statistically significant differences ($p < 0.05$). Total phenolics are expressed as grams of GAE. Total flavonoids are expressed as grams of CE. Total anthocyanins are expressed as grams of cy-3-glu. Total antioxidant capacities (ABTS, DPPH, CUPRAC) are expressed as grams of TE.

Total flavonoid content

The results of total flavonoid content are presented in Tab. 2. The highest flavonoid content was found in dried fruits (19.9 g·kg^{-1} and 12.5 g·kg^{-1}). There were statistically significant differences between total flavonoid levels of fresh chokeberry and dried chokeberries ($p < 0.05$). Raspberry-chokeberry syrup had the lowest flavonoid content (0.04 g·kg^{-1}) compared to all samples. For other samples, flavonoid content ranged from 0.2 g·kg^{-1} for sour cherry-chokeberry syrup to 9.3 g·kg^{-1} for chokeberry pomace. Chokeberry syrup had significantly higher flavonoid content in comparison to raspberry and sour cherry syrup ($p < 0.05$). To our knowledge, there is only one study reporting the total flavonoid content of chokeberry (0.7 g·kg^{-1} DW), which was significantly lower than our result (5.3 g·kg^{-1} FW) [16]. According to the literature, the main contributor of total flavonoid content is quercetin with an average content of 0.11 g·kg^{-1} DW, while myricetin and kaempferol was not detected in chokeberry [37]. The flavonols that were identified as five different quercetin derivatives were quercetin-3-vicianoside, quercetin-3-robinobioside, quercetin-3-rutinoside, quercetin-3-glucoside and quercetin-3-galactoside [43]. Content of quercetin-3-galactoside was found to be higher compared to quercetin-3-glucoside in chokeberry fruit, but lower compared to quercetin-3-rutinoside in chokeberry juice [44].

Total anthocyanin content

Total anthocyanin content is shown in Tab. 2. The pattern of change in total anthocyanin content was different from that observed in total phenolic content except for the result of raspberry-chokeberry syrup. Raspberry-chokeberry syrup had the lowest anthocyanin content, while the highest anthocyanin content (9.9 g·kg^{-1}) was found in chokeberry pomace compared to chokeberry fruit and other samples. Total anthocyanin content of chokeberry fruit (4.5 g·kg^{-1}) was found to be ten times higher compared to the jam samples, and was significantly higher than that of compote and syrups. 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. Total anthocyanin content of chokeberries reported in several studies was found to be between 4.3 g.kg^{-1} and 18.2 g.kg^{-1} FW expressed as cy-3-glu [10, 11], while anthocyanin contents for blackberry and blueberry were reported to be 1.0–2.0 g.kg^{-1} and 1.0–1.2 g.kg^{-1}, respectively [10, 45]. Result for chokeberry juice was found to be lower than those reported by other authors, who determined the total anthocyanin content by using an HPLC method rather than the pH differential method [44], indicating that the differences in the anthocyanin content may result from differences in cultivars, harvesting time as well as the analytical technique used.

In contrast to the total phenolic content, anthocyanin level in dried chokeberry was lower than in fresh chokeberry. In a previous study, half of the chokeberry anthocyanins were recovered after drying for 72 h [38]. It is well known that anthocyanins are susceptible to many factors including 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 pasteurization [1].

Total antioxidant capacity

The total antioxidant capacity values of samples are presented in Tab. 2. The antioxidant capacity of chokeberry fruit analysed by ABTS, DPPH, and CUPRAC were 10.9 g·kg^{-1}, 11.3 g·kg^{-1} and 67.7 g·kg^{-1}, respectively. By all methods, the highest antioxidant capacity was observed in dried chokeberries. In agreement with TP, TA and TF results, raspberry syrup had the lowest antioxidant capacity (0.7–1.2 g·kg^{-1}). Dried chokeberry showed higher antioxidant capacity values than chokeberry fruit and concentrate. Higher antioxidant capacity was observed in chokeberry syrup compared to raspberry and sour cherry syrup containing certain amounts of chokeberry. There was a significant difference between chokeberry fruit and juice samples according to the results of DPPH method ($p < 0.05$), while they had similar values according to the ABTS method. There are several studies on the antioxidant capacity of chokeberry and chokeberry juice measured by oxygen radical absorbance capacity [46] or inhibition of DPPH radical [8] methods, which showed that antioxidant capacity of chokeberry was higher than of other berries such as red raspberry, blackberry, strawberry, gooseberry, black currant or elderberry.

Antioxidant activity was measured by three different methods, ABTS, DPPH, and CUPRAC, which showed similar patterns within samples, but with varying values. The highest antioxidant values were obtained by the CUPRAC assay. Various methods to measure antioxidant capacity are available, but these methods may provide conflicting results as a result of differences in the principles of these assays that vary depending on the radical utilized, reaction time and the way of end-point detection [47]. Even the methods based

Tab. 3. The correlation coefficients (R^2) for spectrophotometric assays.

	Total phenolics	Total flavonoids	Total anthocyanins	ABTS	DPPH	CUPRAC
Total phenolics	–	0.621	0.614	0.785	0.796	0.831
Total flavonoids	0.621	–	0.211	0.917	0.933	0.907
Total anthocyanins	0.614	0.211	–	0.312	0.326	0.332
ABTS	0.785	0.917	0.312	–	0.974	0.973
DPPH	0.796	0.933	0.326	0.974	–	0.995
CUPRAC	0.831	0.907	0.332	0.973	0.995	–

on the same principle, such as ABTS and DPPH, may produce different results. In addition, in the discolouration-based assays, interferences may occur as a result of the presence of coloured compounds, and may result in inaccurate antioxidant activity values. This problem is more common for the methods in which the measurements are performed at lower wavelengths, since interferences may be expected to be observed more in the visible region. Measurements at higher wavelengths, far from visible region, prevent interference [47]. This is the reason why a single method, in most cases, is not enough to evaluate the antioxidant capacity, and so it is recommended to use several antioxidant assays to obtain a reliable result [48].

Correlation between spectrophotometric assays

The correlation coefficients (R^2) for spectrophotometric assays ranged from 0.211 to 0.995 (Tab. 3). DPPH and CUPRAC methods showed a linear relationship with a high correlation coefficient of $R^2 = 0.995$. The highest correlation was demonstrated between TF and DPPH ($R^2 = 0.933$), followed by TP and CUPRAC ($R^2 = 0.933$) methods. Correlation coefficient was found to be low ($R^2 = 0.312$–0.332) between TA

Tab. 4. Contents of individual anthocyanins, percentage distribution of anthocyanins in chokeberry and chokeberry samples.

Samples	cy-3-gal		cy-3-glu		cy-3-ara		cy-3-xyl	
	[mg·kg^{-1}]	[%]	[mg·kg^{-1}]	[%]	[mg·kg^{-1}]	[%]	[mg·kg^{-1}]	[%]
Chokeberry fruit	2917.2 ± 129.3^{c}	63.8	127 ± 5.4^{c}	2.8	1359.4 ± 0.2^{c}	29.7	165.8 ± 1.3^{c}	3.6
Dried chokeberry 1	928 ± 13^{d}	60.7	60.6 ± 2.1^{d}	4.0	477.7 ± 5.6^{d}	31.2	62.5 ± 0.5^{d}	4.1
Dried chokeberry 2	475.7 ± 0.9^{e}	67.7	19.3 ± 1.1^{e}	2.8	186 ± 0.4^{e}	26.5	21.8 ± 1.2^{e}	3.1
Chokeberry juice 1	441.4 ± 3.1^{e}	67.6	19.9 ± 0.1^{e}	3.0	172.6 ± 1.6^{ef}	26.4	19.3 ± 0.4^{e}	2.9
Chokeberry juice 2	286.6 ± 69.1^{fg}	66.0	15.2 ± 4.2^{ef}	3.5	117.8 ± 27.6^{fg}	27.1	14.7 ± 3.5^{ef}	3.4
Chokeberry juice 3	407.1 ± 5.7^{ef}	67.6	19.4 ± 0.1^{e}	3.2	157.1 ± 4^{ef}	26.1	18.7 ± 0.5^{e}	3.1
Chokeberry concentrate	3349.7 ± 11.1^{b}	64.3	214.7 ± 1.1^{b}	4.1	1447.6 ± 3.2^{b}	27.8	201.1 ± 0.7^{b}	3.9
Chokeberry pomace	4600.5 ± 211^{a}	68.5	237.7 ± 11.4^{a}	3.5	1651.1 ± 87.6^{a}	24.6	223.4 ± 11.8^{a}	3.3
Chokeberry jam 1	237.4 ± 5.3^{hij}	69.6	10 ± 0.2^{fg}	2.9	85.2 ± 1.5^{gh}	25.0	8.7 ± 0.3^{fg}	2.5
Chokeberry jam 2	81.2 ± 0.6^{ij}	74.3	3.3 ± 0.1^{g}	3.0	22 ± 0.3^{hi}	20.1	3 ± 0.1^{g}	2.6
Chokeberry compote	120.4 ± 1.4^{ij}	72.8	4 ± 0.1^{g}	2.4	41 ± 0.03^{hi}	24.8	ND	ND
Chokeberry syrup	81.6 ± 0.1^{gh}	70.8	3.6 ± 0.1^{g}	3.1	27.2 ± 0.2^{hi}	23.6	2.8 ± 0.1^{g}	2.4
Raspberry-chokeberry syrup*	7 ± 0.1^{j}	49.8	1.9 ± 0.01^{g}	13.2	5.2 ± 0.5^{i}	37.0	ND	ND
Sour cherry-chokeberry syrup*	29.3 ± 1.8^{j}	60.1	2.1 ± 0.01^{g}	4.3	16 ± 0.1^{i}	32.7	1.5 ± 0.1^{g}	3.0

Different letters in the columns within each sample represent statistically significant differences ($p < 0.05$).
* – The percentages of anthocyanins in raspberry and sour cherry mixtures were given according to ratio of individual anthocyanin to total value of four anthocyanins since other anthocyanins found in these mixtures were not determined.
cy-3-gal – cyanidin-3-galactoside, cy-3-glu – cyanidin-3-glucoside, cy-3-ara – cyanidin-3-arabinoside, cy-3-xyl – cyanidin-3-xyloside, ND – not detected.

and antioxidant activity assays. These results imply that flavonoids were the major contributors to the antioxidant capacity of the investigated chokeberry products.

Anthocyanin profile

Anthocyanins in chokeberry samples were identified by using reverse phase HPLC (Tab. 4). For quantification of individual anthocyanins, cyanidin-3-glucoside was used as a standard and contents of other anthocyanins were calculated according to the ratio of individual anthocyanins to total anthocyanins, as reported by a previous study [8]. Four major anthocyanins including cyanidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3-glucoside and cyanidin-3-xyloside, were detected in chokeberry fruit (Fig. 1), which was in accordance with previous studies [8, 13, 36, 46]. The highest contents of individual anthocyanins were observed in chokeberry concentrate, having 8-fold higher levels of cyanidin-3-galactoside and cyanidin-3-arabinoside compared to chokeberry juice. On the other hand, chokeberry fruit had a higher level of cyanidin-3-galactoside compared to dried chokeberries (927.9 mg·kg^{-1} and 475.7 mg·kg^{-1} FW, respectively). There were also significant differences between the contents of individual anthocyanins in chokeberry pomace and fruit ($p < 0.05$). Cyanidin-3-xyloside was not detected in compote and raspberry-chokeberry syrup. The predominant anthocyanin in chokeberry was found to be cyanidin-3-galactoside. On the other hand, blackberries that are also known to be a good source of anthocyanins, contain cyanidin-3-glucoside as a dominant anthocyanin [49]. Major anthocyanin of strawberries was determined as pelargonidin-3-glucoside [50], while only cyanidin derivatives were identified in chokeberries. The contribution of different anthocyanins to the total antioxidant capacity is known to be different, which may lead to differences in the antioxidant potential of different fruits and vegetables.

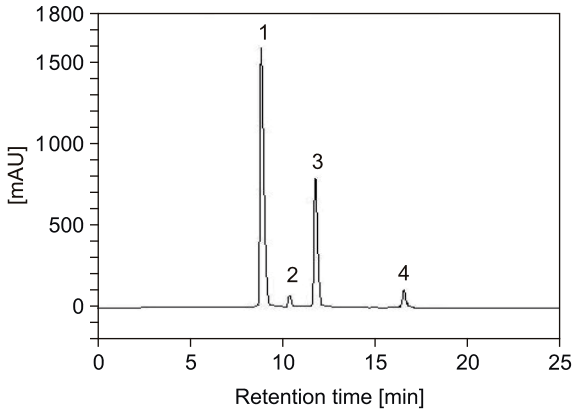

Fig. 1. HPLC chromatogram of chokeberry recorded at 525 nm.

1 – cyanidin-3-galactoside, 2 – cyanidin-3-glucoside, 3 – cyanidin-3-arabinoside, 4 – cyanidin-3-xyloside.

Tab. 5. Performance parameters for cyanidin-3-glucoside determination by HPLC.

Parameters	Cyanidin-3-glucoside
Linearity range [mg·l^{-1}]	0.05–100
Slope [m]	618.4
Determination coefficient r^2	0.999
Limit of detection [mg·l^{-1}]	0.01
Limit of quantification [mg·l^{-1}]	0.02
Recovery [%]	90.5
Relative standard deviation [%]	2.8

Performance parameters for determination of cyanidin-3-glucoside were also calculated (Tab. 5). Linear least-squares regression was used to calculate the slope and correlation coefficient. Correlation was evaluated by the determination coefficient (r^2) and found to be high ($r^2 > 0.999$) for cyanidin-3-glucoside. Limits of detection (*LOD*) and limits of quantification (*LOQ*) were calculated from the amount of cyanidin-3-glucoside required to give a signal/noise ratio of 3 : 1 and 10 : 1, respectively. *LOD* (0.01 mg·l^{-1}) and *LOQ* (0.02 mg·l^{-1}) showed that HPLC method was sensitive enough to quantify individual anthocyanins in chokeberry and chokeberry products. The relative standard deviation (*RSD*) was also calculated to express the repeatability of the assay and the low *RSD* value confirmed that the assay had a good repeatability. The calculated extraction recovery (90.5%) proved that this method was applicable to determine individual anthocyanins in real samples.

CONCLUSION

The results of this research indicated that the highest total phenolic and total anthocyanin contents were in chokeberry pomace, whereas the highest total flavonoid content and antioxidant activity values were in dried fruits. Four major anthocyanins, including cyanidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3-glucoside and cyanidin-3-xyloside, were detected in chokeberry fruit. Different chokeberry products were found to contain different levels of antioxidants, which might be related to the differences in the variety and growing conditions of the fruits, processing methods and parameters, or differences in the

principles of the analytical methods used. In order to fully understand the effect of processing, further research focusing on different processing steps/techniques including cutting, thermal treatments and drying, should be done, starting from the same raw material. In addition, in vivo and in vitro bioavailability studies will be helpful to understand the bioaccesibility and bioavailability of nutritive compounds of chokeberry and its products, and will provide information basis for elucidating the true biological relevance of these data in the context of nutrition and human health.

Acknowledgements

This work was carried out with financial support from the Ministry of Agriculture of the Czech Republic (Research Project MZe QI91B283).

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Received 17 March 2013; 1st revised 1 June 2013; 2nd revised 27 June 2013; accepted 29 June 2013; published online 23 October 2013.