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ANTIOXIDANT ACTIVITY, POLYPHENOL, AND ANTHOCYANIN CONTENT OF BLACK CHOKEBERRY (*ARONIA MELANOCARPA* L.)

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ABSTRACT

Black chokeberry (*Aronia melanocarpa* L.) attracts scientific interest due to its high content of bioactive compounds with beneficial effects on human health. Presented work aimed to determine the total polyphenol content (TPC), total anthocyanin content (TAC), and antioxidant activity by methods ABTS, DPPH, FRAP of black chokeberry (*Aronia melanocarpa* L.) variety Nero, from 4 localities (Holovousy, Bašnice, Choteč, Veľký Lapáš), fresh, and conserved (with addition of sucrose and subsequent freezing for 3 months). TPC determined by spectrophotometry using the Folin-Ciocalteau reagent were in the range 8646-13213 mg GAE.kg^{-1} (fresh chokeberry) and 5068-7047 mg GAE.kg^{-1} (preserved chokeberry). The TAC values, determined spectrophotometrically by lowering the pH of the extract, were in the range 489-875 mg.kg^{-1} (fresh chokeberry) and 620-1026 mg.kg^{-1} (preserved chokeberry). The AA values (ABTS, DPPH, FRAP) were 2.35-2.97; 1.79-2.18; 2.01-2.67 mmol TE.kg^{-1} (fresh chokeberry) and 2.79-3.45; 2.15-2.57; 2.42-2.60 mmol TE.kg^{-1} (preserved chokeberry). The results obtained show that the location of cultivation has a significant influence on the chemical parameters monitored. The addition of sucrose and freezing resulted in lower TPC and higher TAC in all samples, while it had no clear effect on the AA levels determined by all three methods.

Keywords: black chokeberry, polyphenols, anthocyanins, antioxidant activity

INTRODUCTION

Black chokeberry (*Aronia melanocarpa* L., *Rosaceae*) is mainly known for its beneficial effects on the human body. It is grown as an ornamental shrub, and its fruits are used to make juices, wine, jams, and natural food coloring. It first appeared in Europe in the 20th century but is now widely cultivated for its phenolic composition and vitamin C content. Black chokeberry is rich in various biologically active compounds, especially polyphenols, with strong antioxidant effects. **Dobros et al. (2024)** report that 70% of the polyphenols in chokeberry are flavonoid compounds. Compared to other berries, the black chokeberry is characterised by the richest anthocyanin content, therefore, can be considered as the best plant source of anthocyanins **(Kim et al., 2013)**. Black chokeberry anthocyanins contain high levels of cyanidin glycosides (**Meng et al., 2019)**. Black chokeberry anthocyanins have antioxidant activity, protecting cells from oxidative damage and apoptosis **(Jurendić and Ščetar, 2021).** In addition, black chokeberry contains a wide range of flavonoids and phenolic acids **(Tomas et al., 2024)**. The presence of o-dihydroxy groups of the aromatic B-ring in polyphenol molecules (only in anthocyanins) indicates that these compounds have the ability to protect against the toxicity of Cd^{2+} ions, as polyphenolic compounds can form complexes with metal ions **(Brzóska et al., 2015**). This finding is very important, as Cd is one of the most serious environmental pollutants, and plant foods are the most important non-occupational source of Cd exposure for human health **(Musilová et al., 2015)**.

Current literature suggests that long-term consumption of polyphenol-rich foods protects against certain cancers, cardiovascular diseases, type 2 diabetes, osteoporosis, and neurodegenerative diseases **(Bystricka et al., 2016; Cory et al., 2018; Strati et al., 2018)**. The biological effects of berry fruit consumption can be attributed to the properties of polyphenolic compounds, which can scavenge free radicals, inhibit lipid peroxidation, and influence the stimulation/inhibition of enzyme activity **(Denev et al., 2019)**. Due to their molecular structures, phenolic compounds have inhibitory effects on the formation of glycation end-products (non-enzymatic reactions between the -NH_2 groups of macromolecules and the carbonyl group on the reducing sugar) by forming phenolic compound-glyoxal and phenolic compound-methylglyoxal adducts **(Velichkova et al., 2021)**, and by reducing the conversion of compounds such as glyoxal and methylglyoxal into cross-linked melanoidins **(Jia et al., 2022; Tan et al., 2024)**. The accumulation of glycation products, which can be both endogenous and exogenous (dietary), is a factor in the development of age-related chronic diseases such as Alzheimer's disease, diabetes, and cardiovascular disease. Long-term intake of glycation products can affect kidney function and increase the risk of cancer **(Wang et al., 2021)**. Black chokeberry extract shows geroprotective activity, such as prolonging life, improving glucose and lipid metabolism, antiviral and antibacterial activity, and a protective effect on the gastrointestinal system **(Platonova et al., 2021)**.

This work aimed to evaluate the biochemical composition (total polyphenol content, total anthocyanin content) and determine the antioxidant activity values of black chokeberry (*Aronia melanocarpa* L.) fruits grown in different selected locations, and the effect of conserving fruits on these parameters.

MATERIAL AND METHODS

The analysed fruits of the black chokeberry (*Aronia melanocarpa* L.) variety "Nero" came from 4 locations in the Czech and Slovak Republic (Table 1). The chokeberry fruits were harvested at full ripeness (500 g for each sample) in the first week of September. For each sample, 50 g of fruit and 50 g of sugar (sucrose) as a preservative were weighed into the second container. Aronia fruits with sugar (sucrose) were frozen for 3 months at -18°C. Extracts were prepared by shaking 50 g of homogenized sample in 80% methanol for 16 hours.

At the Holovousy site (1), chokeberry was grown as a gene pool plantation. It is situated at an altitude of 340 m above sea level. It is formed by the sandstone bedrock at the junction of the geological formations of the Česká tabula - Podkrkonoší. The eel is bred here. The major part of the territory lies in the climatic region T3 - warm to moderately humid. Average temperature 8-9 °C, annual precipitation 550-650 mm.

At the Bašnice site (2), chokeberry was grown in an alley orchard along a third-class road. It is situated at an altitude of 300 m above sea level. It is formed by marl on the geological formation of Česká tabuľa. The vegetation is largely unmaintained. The average precipitation is 500-600 mm. The average annual temperature is 9-10 °C.

In the Choteč site (3), chokeberry was grown in a domestic mixed orchard at an altitude of 320 metres above sea level. It is characterised by clay subsoil of the Podkrkonoše geological formation. Planting is carried out in organic mode. The average annual temperature is 9-10 °C and the rainfall varies between 500-600 mm.
In the Veľký Lapáš site (4), chokeberry was grown in a private orchard at an altitude of 160 metres above sea level. The bush were planted in a sunny place. The average annual temperature is 10-11 °C and the average precipitation is about 750 mm.

Table 1 Origin of investigated black chokeberry (*Aronia melanocarpa* L.), variety „Nero“ fruits

Sample No.	Country	Locality	Latitude / Longitude	Altitude
1	CR	Holovousy	50.22°N / 15.34°E	340
2	CR	Bašnice	50.33°N / 15.61°E	300
3	CR	Choteč	50.43°N / 15.51°E	320
4	SR	Veľký Lapáš	48.29°N / 18.18°E	160

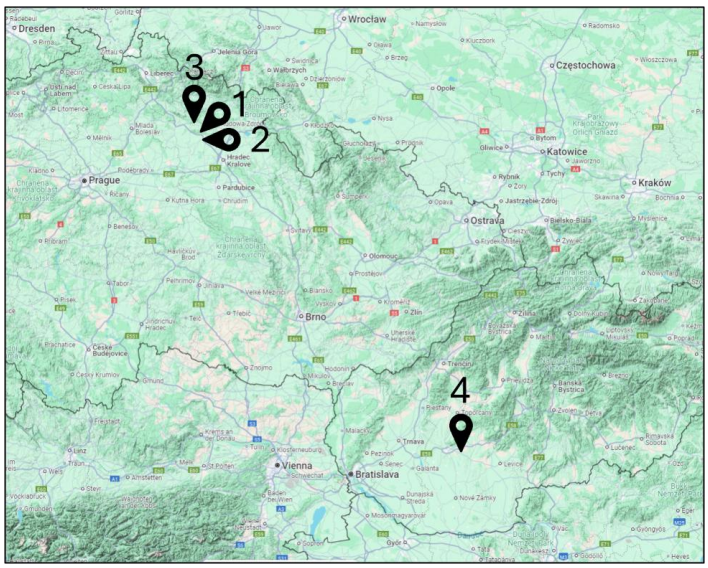

Figure 1 Map of the monitored localities

Determination of total polyphenol content

The volume of 0,03 ml of extract was pipetted into a 50 ml volumetric flask and diluted with distilled water. To the diluted sample, 2.5 ml of the Folin-Ciocalteu reagent was added, and after 3 minutes, 7.5 ml of an aqueous solution of 20% Na_2CO_3 was added and mixed. Then, the flask was filled with distilled water to a volume of 50 ml and the contents were mixed. At the same time as the sample, a calibration curve with standard solutions of gallic acid (5 $\mu g.cm^{-3}$) was prepared. After two hours, the absorbance of the blue-coloured solutions was measured spectrophotometrically against a blank at a wavelength of 765 nm, using a Shimadzu UV- 1800 UV/ Visible Scanning Spectrophotometer (Shimadzu, Japan). TPC in samples was determined from the equation of the calibration curve and expressed as mg of gallic acid equivalent per kg of fresh material **(Lachman, 2003)**.

Determination of total anthocyanin content

The volume of 1 ml of the extract was pipetted into two test tubes and 1 ml of 0.01% HCl in 80% ethanol was added. Then, 10 ml of a 2% aqueous solution of HCl and 10 ml of a buffer solution with pH = 3.5 (c = 0.2 $mol.dm^{-3}$ Na_2HPO_4 and c = 0.1 $mol.dm^{-3}$ citric acid) was added. The absorbance of both samples was measured spectrophotometrically against a blank at a wavelength of 520 nm, using a Shimadzu UV- 1800 UV/ Visible Scanning Spectrophotometer (Shimadzu, Japan). The total anthocyanin content was calculated from the differences in absorbance values and expressed as $mg.kg^{-1}$ **(Lapornik et al., 2005).**

Determination of antioxidant activity using DPPH assay

Antioxidant activity (AA) was determined by the DPPH method according to **Brand-Wiliams et al. (1995)**, using 2,2-diphenyl-1-picryhydrazyl and methanol. DPPH solution (3.9 ml) was pipetted into cuvettes, and the absorbance was measured at a wavelength of 515.6 nm, using a Shimadzu UV- 1800 UV/ Visible Scanning Spectrophotometer (Shimadzu, Japan). After measuring the initial absorbance, 0.1 ml of the extract was added using an automatic pipette and stirred. After ten minutes, absorbance was measured again against a blank sample. The results are expressed as mmol of Trolox equivalent per kg of fresh matter ($TE.kg^{-1}$).

Determination of antioxidant activity using ABTS assay

Antioxidant activity was determined using ABTS assay **(Re et al., 1999).** $ABTS^{\bullet+}$ radical cation - (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma Aldrich, USA), potassium persulfate ($K_2S_2O_8$) (Sigma Aldrich, USA), and acetate buffer (pH= 4.3) were used to produce working ABTS solution. 0.05 mL of extract was pipetted into 3 mL of ABTS solution, stirred, and left in the dark. After 20 minutes, absorbance was measured against a blank solution at 734 nm, using a Shimadzu UV- 1800 UV/ Visible Scanning Spectrophotometer (Shimadzu, Japan). The results are expressed as mmol of Trolox equivalent per kg of fresh matter ($TE.kg^{-1}$).

Determination of antioxidant activity using FRAP assay

Antioxidant activity was determined using the FRAP assay (Pedersen et al., 2000). TPTZ - (2,4,6-tris(2-pyridyl)-S-triazine) (Sigma Aldrich, USA), ferric chloride ($FeCl_3$) (Sigma Aldrich, USA), and acetate buffer (pH= 3.5) were used to produce working FRAP solution. 0.05 mL of extract was pipetted into 3 mL of FRAP solution, stirred, and left in the dark. After 20 minutes, absorbance was measured against a blank solution at 593 nm, using a Shimadzu UV- 1800 UV/ Visible Scanning Spectrophotometer (Shimadzu, Japan). The results are expressed as mmol of Trolox equivalent per kg of fresh matter ($TE.kg^{-1}$).

Statistical Analysis

Statistical analysis was performed using XLSTAT software **(Lumivero, 2024).** The non-parametric ANOVA test (Kruskal-Wallis) was used for the comparison between the tested variables. The Spearman correlation was used to determine the relationships between individual parameters. All analyses were performed in quadruplicate.

RESULTS AND DISCUSSION

Total polyphenol content, total anthocyanin content, and values of antioxidant activity in monitored samples are shown in Table 2. (fresh samples), and Table 3. (preserved samples).

Table 2 Content of bioactive compounds in fresh samples

Sample	TPC (mg GAE.kg^{-1})	TAC (mg.kg^{-1})	ABTS (mmol TE.kg^{-1})	DPPH (mmol TE.kg^{-1})	FRAP (mmol TE.kg^{-1})
1	13213 ± 8.55^{b}	767.1 ± 22.3^{ab}	2.49 ± 0.01^{ab}	1.80 ± 0.01^{a}	2.61 ± 0.01^{ab}
2	10865 ± 12.9^{ab}	874.8 ± 7.03^{b}	2.97 ± 0.02^{b}	2.18 ± 0.01^{b}	2.67 ± 0.02^{b}
3	11651 ± 15.5^{ab}	540.4 ± 16.1^{ab}	2.66 ± 0.02^{ab}	2.14 ± 0.02^{ab}	2.54 ± 0.02^{ab}
4	8646 ± 14.3^{a}	488.6 ± 17.5^{a}	2.35 ± 0.01^{a}	1.79 ± 0.01^{a}	2.01 ± 0.01^{a}

Values shown in the table are expressed as mean ± SD (n = 4); values in the column marked with a different letter mean significant statistical differences ($p < 0.05$) between the studied samples

Total polyphenol content

Table 2 shows the content of phenolic compounds in chokeberry of the Nero variety from the investigated locations. We measured the TPC values from the monitored locations in the interval from 8646±14.3 to 13213±8.55 (mg GAE.kg^{-1}). According to the increasing total content of polyphenols, we can rank the sites in the following order: Holovousy > Bašnice > Choteč > Veľký Lapáš. Between the Holovousy and Veľký Lapáš localities, a statistically significant difference in TPC content in chokeberry fruits was recorded. At the Holovousy site, chokeberry

probably had more favourable conditions for growth (sufficient moisture and sunlight).

Rop et al. (2010) report the total content of polyphenols in chokeberry fruits from 7780 to 12850 mg. kg^{-1} FW, our values correspond to their values. **Wangensteen et al. (2014)** state that chokeberry contains a large amount of TPC, the value of which can be up to 2,994 mg.100 g^{-1} FW. **Zhang et al. (2021)** indicate that in these fruits, phenolic compounds usually range from 2,000 mg.100 g^{-1} to 8,000 mg.100 g^{-1} DW. **Dobros et al. (2024)** reported a TPC content of 25-150 $mg.g^{-1}$ DW in their analyzed samples of black chokeberry fruits. **Meng et al. (2019)** reported TPC of 1900 mg.100 g^{-1} FW, which is slightly higher than our measured values. The concentration of phenolic compounds in berries depends on plant variety, growing conditions, and harvest time **(Zhang et al., 2021). Lidiková et al. (2024)** indicate that genetics is a key factor influencing the formation and concentration of bioactive compounds.

Total anthocyanin content

Anthocyanins are the predominant type of polyphenols in chokeberry. Anthocyanins are a group of flavonoids with exceptionally good scavenging activities. Black chokeberry anthocyanins have attracted the attention of researchers because of their high antioxidant activity, due to their diverse structure. They contain hydrogen donors (-OH groups) that can scavenge free radicals. They are highly unstable compounds whose levels are affected by light, pH, and freezing temperature, but also by the presence of other compounds such as enzymes and metal ions **(Hellström et al., 2013).** A statistically significant highest value of TAC was recorded in Choteč locality (874.8±7.03 $mg.kg^{-1}$ FW) and a statistically significant lowest value was recorded in chokeberry fruits from Veľký Lapáš locality (488.6±17.5 $mg.kg^{-1}$ FW). The lower content of anthocyanins can probably be attributed to the higher content of calcium in the soil, as Aronia does not like limestone soils too much. **Esparza et al. (2004)** state that the total content of anthocyanins is positively influenced by iron and copper. Higher iron and copper contents were recorded in the soil from the Choteč location. TAC values of **Platonova et al. (2021)** ranged from 0.31 to 1.79 $mg.g^{-1}$ FW. **Bushmeleva et al. (2022)** report a TAC value of 93.6 $mg.g^{-1}$ DW, and **Lin et al. (2022)** report total anthocyanins in the amount of 2.996 $mg.g^{-1}$ DW. Most studies on the content of anthocyanins in chokeberry indicate the total amount of anthocyanins in fresh fruits varies between 83 and 370 $mg.100g^{-1}$ (FW **(Meng et al., 2019).** The total anthocyanin content is influenced by the extraction method, genetic information, and changes due to geographical location and environmental factors **(Lin et al., 2022)**. Similarly, **Tolić et al. (2017)** report that individual meteorological parameters such as temperature, precipitation, relative humidity, and sunshine hours affect the level of bioactive compounds in chokeberry.

Antioxidant activity

Black chokeberry shows strong antioxidant effects **(Bontsidis et al., 2021)** and has one of the highest *in vitro* antioxidant activities among fruits. The antioxidant activity of chokeberry goes far beyond radical scavenging. It includes suppression of reactive oxygen species, inhibition of pro-oxidant enzymes, restoration of antioxidant enzymes, and possibly regulation of antioxidant enzyme levels **(Denev et al., 2012).** AA depends on the chemical structure of the phenolic compounds present, and not all compounds may exhibit antioxidant activity. **Grygorieva et al. (2018)** report positive correlations between the content of phenols present and antioxidant activity. Aronia from Choteč locality showed the highest AA value (2.97±0.02, 2.18±0.01, 2.67±0.02 mmol $TE.kg^{-1}$), and the lowest values were recorded in chokeberry from Veľký Lapáš (2.35±0.01, 1.79±0.01, 2.01±0.01 mmol $TE.kg^{-1}$). The different values of AA in chokeberry from individual locations can be attributed to the different agrochemical characteristics of the soils from these locations, such as humus content and soil pH.

Effect of conservation on monitored parameters

In recent years, research has also focused on the effect of sugar addition on bioactive content, as it is thought that externally added carbohydrates can affect the stability of polyphenols. Sucrose has a stabilizing effect on monomeric anthocyanins by reducing their rate of degradation. In this work, we monitored the influence of sucrose as a preservative on the content of TPC, TAC, and AA in chokeberry. Based on our results (Table 3), we can conclude that the addition of sucrose at the level of 50 g of sugar per 50 g of fruit statistically significantly reduced the TPC in all samples. On the other hand, there was a statistically significant increase in the TAC content in all the samples. There was also a statistically significant increase in the AA values determined by the DPPH method after the addition of sucrose. The influence of sucrose addition on the AA values determined by the ABTS and FRAP methods did not show a clear trend.

The effect of the addition of carbohydrates on the content of bioactive compounds was also monitored by **Benedek et al. (2020),** who report that AA was not significantly affected by the influence of sugar in jams and noted a slight increase in TPC content. The authors are not unanimous regarding the influence of added sugars on the anthocyanin content. Works are describing an increased loss of anthocyanins **(Hubbermann et al., 2006)**. **Loncaric et al. (2014)** reported a lower loss of TPC after sugar addition in apple processing. The effect of sugar concentration on TPC and AA content was observed by **Zayapor et al. (2021)**, who reported an equivocal effect of sugar addition on these parameters. In their studies, Zeng et al. (2017) reported only a slight loss of TPC content after adding sugar. **Salar et al. (2022)** found an increase in anthocyanin loss with sucrose addition. According to **Katz et al. (2020),** both carbohydrates and fructose have a protective effect against the degradation of bioactive compounds.

Table 3 Content of bioactive compounds in preserved samples

Sample	TPC (mg GAE.kg^{-1})	TAC (mg.kg^{-1})	ABTS (mmol TE.kg^{-1})	DPPH (mmol TE.kg^{-1})	FRAP (mmol TE.kg^{-1})
1	$6518±13.2^{ab}$	$1026±7.69^{b}$	$2.91±0.02^{ab}$	$2.15±0.02^{a}$	$2.42±0.02^{a}$
2	$5453±12.4^{ab}$	$964.4±23.5^{ab}$	$2.79±0.01^{a}$	$2.51±0.01^{ab}$	$2.74±0.01^{ab}$
3	$7047±7.10^{b}$	$898.6±25.2^{ab}$	$3.45±0.02^{b}$	$2.57±0.02^{b}$	$3.17±0.02^{b}$
4	$5068±19.7^{a}$	$619.8±9.15^{a}$	$3.03±0.02^{ab}$	$2.56±0.02^{b}$	$2.60±0.02^{ab}$

Values shown in the table are expressed as mean ± SD (n = 4); values in the column marked with a different letter mean significant statistical differences ($p < 0.05$) between the studied samples

Relationships between monitored parameters

To determine relationships between monitored parameters, a correlation analysis was performed. Results are shown in Table 4.

Table 4 Relationship between monitored parameters of analyzed samples

Variables	TPC	TAC	AA ABTS	AA DPPH	AA FRAP
TPC	**1**				
TAC	-0.381	**1**			
AA ABTS	-0.524	0.476	**1**		
AA DPPH	-0.643	0.500	**0.929**	**1**	
AA FRAP	-0.095	0.476	0.571	0.714	**1**

Values in bold are different from 0 with a significance level of alpha=0.05

A statistically significant ($p < 0.05$) positive correlation was found between the value of antioxidant activity determined by ABTS and DPPH method. Positive correlations were also observed between total anthocyanin content and antioxidant activity; however, these relationships were not statistically significant. This means that while higher anthocyanin content was generally associated with higher antioxidant activity, the relationship is not strong enough to be conclusive. This could be due to several factors, such as presence of other antioxidants, and variability in the structure of anthocyanins. For a better understanding of the relationship between antioxidant activity and the content of bioactive components in chokeberry, further studies are necessary.

CONCLUSION

The present study suggests that the consumption of black chokeberry *(Aronia melanocarpa* L.), both fresh and preserved, has several health functions and benefits. It is characterised by a high content of bioactive compounds (TPC, TAC) and strong antioxidant properties, although these may be influenced by various internal and external factors, such as location and method of preservation. A

significant difference ($p < 0.05$) in the content of total polyphenols, total anthocyanins, and antioxidant activity values was found between the locations. After adding sucrose as a preservative and freezing of chokeberry fruit for three months, a decrease in total polyphenols and an increase in total anthocyanins was observed. The value of antioxidant activity determined by the DPPH method increased statistically significantly in all analysed samples of black chokeberry, while this preservation method did not show a clear trend for AA values determined by the ABTS and FRAP methods. Black chokeberry (*Aronia melanocarpa* L.) is a source of valuable secondary metabolites with antioxidant activity, which can be incorporated as a functional ingredient in foods and dietary supplements and can be used in both the food and pharmaceutical industries.

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