

Chokeberry Pomace: Waste, or a Valuable Source of Bioactive Compounds?

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Abstract

Berry pomaces, byproducts of the food industry, are rich in bioactive polyphenolic compounds, which are well recognized for their strong antioxidant and antimicrobial properties. Unfortunately, chokeberry pomace (CBP) often ends up as environmentally harmful waste. In this work, it is shown that CBP is a significant source of polyphenolic compounds, which exhibit high antioxidant activity and can therefore be used as a raw material. The total content of polyphenols, flavonols and flavanones, and anthocyanins in the chokeberry pomace extract was determined spectrophotometrically. Antioxidant activity of chokeberry pomace extract was determined using ABTS and DPPH tests. The contents of total polyphenols, flavonols and flavanones, and anthocyanins, determined in CBP, were 204.61, 14.74, and 14.62 mg g⁻¹ of dry matter, respectively. The antioxidant activity of chokeberry pomace extract was determined using the DPPH and ABTS methods (66.44% and 78.20%), indicating that it can effectively combat free radicals. Rather than being a waste, the rich content of various polyphenols, not only anthocyanins, makes chokeberry pomace an excellent starting material for cosmetic products, with potential applications in the food industry.

Key words: chokeberry pomace, total polyphenols content, DPPH test, ABTS test

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Introduction

Aronia fruit (*Aronia melanocarpa* (Michx.) Elliott, Rosaceae), commonly known as chokeberry, and often categorized as a forest or wild fruit, is a rich source of vitamin C (1, 2). Additionally, aronia berries are high in polyphenols, which contribute to their various health benefits, particularly in combatting chronic diseases (3). Flavonoids and other polyphenolic compounds found in chokeberry itself, its juice, and CBP, contribute to its high antioxidant activity. According of Slimestad *et al.* (4), the main flavonoid subgroups of black chokeberry fruits are anthocyanins, flavonols and flavanones. The total phenolic content in aronia is about 690 mg per 100 g (as gallic acid equivalents), while the anthocyanin content is around 400 mg per 100 g (5). Furthermore, aronia berries demonstrate a higher antioxidant scavenging capacity compared to other traditional fruits, such as blackberries (6). Especially in recent years, chokeberry has been recommended for its beneficial effects on human health and well-being, including cardio protective, antihypertensive, antineoplastic, antidiabetic, etc. (Figure 1).

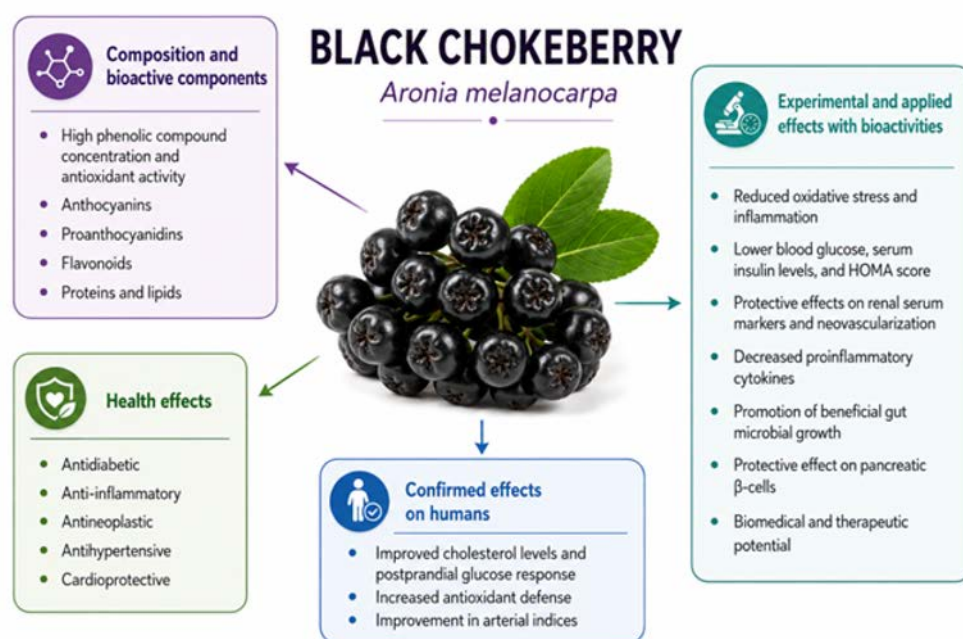


Figure 1. Bioactive components and the bioactivity effects of aronia

Slika 1. Bioaktivne komponente i bioaktivni efekti aronije

Unfortunately, black chokeberry isn't usually eaten fresh because it tastes bitter (7). Instead, it's primarily used in the food industry to produce juices, wines, jams, and dietary supplements (8). When the fruit is processed, there's leftover material called aronia pomace. This pomace includes the fruit's pulp, peels, and seeds and usually accounts for 10–35% of the fruit (9). Aronia pomace is very nutritious and contains many beneficial compounds, sometimes even more than can be found in the final food products. Sadly,

much of this valuable pomace is often discarded, composted, or used as animal feed, and in some cases, it ends up in the environment (10). The negative environmental impact of fruit pomace disposed of includes groundwater pollution, increased soil acidity, reduced soil oxygen levels, and an increased risk of attracting insects and other pests.

The fruit pomace, due to its properties that we have examined and shown in the results, is an important source of bioactive compounds, so in addition to being used in the food industry, it can also be used in the cosmetic industry (fruit seeds found in the pomace are a significant source of oils that are rich in bioactive compounds (11, 12).

The aim of this work is to evaluate the differential polyphenol content of chokeberry pomace (CBP), which reflects its antioxidative potential and may suggest further uses of CBP.

Experimental

Sample Collection and Preparation

Chokeberries (*Aronia melanocarpa*) were obtained directly from local agricultural producers in the region of Kragujevac, Republic of Serbia, in 2024. The berries were cold-pressed below 30 °C to separate the juice from the solid residue (pomace). The wet pomace, consisting of skin, seeds, and pulp residues, was collected and dried at 45 °C to a constant mass. The dried pomace was ground in a laboratory mill, sieved through a 0.5 mm sieve, and stored in the dark at 4 °C until further extraction.

Approximately 5 g of homogenized aronia pomace powder was extracted with 200 mL of 70% V/V ethanol. The extraction was assisted by ultrasound for 30 minutes at 60 °C. The extract was centrifuged for 5 min at 6000 rpm, diluted to a 1:1 extract:water ratio, and used for spectrophotometric determinations of total phenols, flavonols, flavanones, and anthocyanin content, as well as for the DPPH test.

Materials and Methods

Reagents

The following reagents were used: 2,2-diphenyl-1-picryl hydrazyl (DPPH), trolox, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), phosphate buffer (PBS), Folin-Ciocalteu reagent, gallic acid, potassium acetate manufactured by Sigma-Aldrich, USA, aluminum (III) – chloride hexahydrate (Alfa Aesar, USA), quercetin dihydrate (Merck, Germany), methanol, ethanol, potassium chloride, sodium acetate, sodium carbonate, potassium persulfate (Centrohem, Belgrade), and concentrated hydrochloric acid (La Chema, Czech Republic). All reagents used were of *p.a.* purity and were used without purification.

A stock solution of AlCl_3 ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) was prepared by dissolving the appropriate mass of AlCl_3 in double-distilled water, and the solution was standardized gravimetrically. A stock solution of quercetin dihydrate (concentration $1.0 \times 10^{-4} \text{ mol L}^{-1}$)

was prepared by dissolving the appropriate mass of quercetin dihydrate in 80% V/V ethanol.

Instruments

A Beckman DU650 Spectrophotometer was used for spectrophotometric measurements, using 1 cm diameter quartz cuvettes. A Thermo Scientific Orion Dual Star pH meter with a Ross combination electrode (sensitivity ± 0.002 pH units) was used for pH measurements. A Bendelin Sonorex Super Model RK 512K ultrasonic bath was used to accelerate quercetin. A WCX 750 ultrasonic probe, SONICS, Australia, was used for ultrasonic extraction of aronia berries, and a Dynamica Velocity 14 Centrifuge, Livingston, United Kingdom, was used for centrifugation of the obtained extract.

Methods for Determination

Determination of total phenol content according to Folin-Ciocalteu

The total polyphenolic content (TPC) of the extract was determined spectrophotometrically using the Folin-Ciocalteu method (13, 14). This method is based on an oxidation-reduction reaction which phenolic components react with the Folin-Ciocalteu reagent, which is non-selective for phenolic compounds. Under basic conditions, the phenolic proton dissociates to form the phenolic anion, which is reduced by the Folin-Ciocalteu reagent. In a 25 mL volumetric flask, 1 mL of standard solution of gallic acid, 9 mL of distilled water, and 1 mL of Folin-Ciocalteu reagent were added. After five minutes, 10 mL of 7% Na_2CO_3 was added, and the volumetric flask was made up to the mark with distilled water. The mixture was shaken and incubated for 90 minutes in the dark at room temperature. After that, the absorbance of each solution was measured at $\lambda = 765$ nm. The same procedure was used for the extract, but instead of 1 mL of solution of gallic acid, 1 mL of extract was added. The blank sample contained 1 mL of Folin-Ciocalteu reagent, 10 mL of 7% Na_2CO_3 in a 25 mL volumetric flask, which was made up to the mark with distilled water.

For quantification, a linear calibration curve was constructed using a series of gallic acid standard solutions at concentrations of 25–400 mg L^{-1} . The polyphenol concentration in the extract was calculated based on a calibration curve equation of gallic acid (Table I), and expressed as gallic acid equivalents (GAE).

Content of Flavonols and Flavanones

The content of flavonols and flavanones in the chokeberry extract was determined and expressed as quercetin equivalents (QE) (15). Quantification was performed using the calibration curve method based on the absorbance measurements of quercetin standard solutions (12.5, 25.0, 50.0, 80.0, and 100.0 mg mL^{-1} prepared in 80% V/V ethanol) after the addition of the AlCl_3 reagent.

Briefly, 0.5 mL of the chokeberry extract was mixed with 1.5 mL of absolute ethanol, 0.1 mL of 10% AlCl_3 solution, and 0.1 mL of 1 mol L^{-1} CH_3COOK solution. The formation of a yellow-colored complex indicated the presence of flavonoid compounds,

and the absorbance was measured at $\lambda = 415$ nm after 30 min of incubation. The blank sample contained the same reagents, with 0.5 mL of distilled water replacing the extract.

The flavonol and flavanone content in the extract was calculated using the calibration curve equation obtained and expressed as mg QE per unit of sample.

Anthocyanin Content

The anthocyanin content in the sample was determined using the pH differential method with potassium chloride buffer (pH 1.00) and sodium acetate buffer (pH 4.50), prepared with hydrochloric acid (16). The pH values of the buffers were adjusted to 1.00 ± 0.05 and 4.50 ± 0.05 , respectively.

For both buffer systems, the same procedure was applied. Briefly, 0.5 mL of the extract was mixed with 2.0 mL of the corresponding buffer solution in a 10 mL beaker. The buffer solutions without extract were used as blanks. The prepared mixtures were incubated in the dark at room temperature for 20 min. Subsequently, the absorbance of each solution was measured at 520 nm and 700 nm.

The concentration of monomeric anthocyanins was calculated as cyanidin-3-glucoside equivalents (mg L^{-1}) according to equation (1)

$$c (\text{mg L}^{-1}) = \frac{A \cdot MW \cdot DF \cdot 10^3}{a \cdot l} \quad (1)$$

where $A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}=1,0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}=4,5}$, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, 10^3 is the factor for converting g to mg, a is the molar absorption coefficient for cyanidin-3-glucoside, which is $26\,900 \text{ L mol}^{-1} \text{ cm}^{-1}$, l is the optical path length, i.e., the thickness of the cuvette (1 cm).

The parameters and experimental conditions of the spectrophotometric determination of bioactive compounds are summarized in Table I.

Table I Analytical parameters of the spectrophotometric methods

Tabela I Analitički parametri spektrofotometrijskih metoda

Method	λ (nm)	Calibration equation	Range	Correlation coefficient (R^2)	Standard deviation (SD)
TPC (Folin–Ciocalteu) GAE	765	$A = 0.0052c - 0.093$	25–500 mg L^{-1}	0.9988	0.0032
Flavonols and flavanones (AlCl_3) QE	415	$A = 0.062c + 0.065$	12.5–100 mg mL^{-1}	0.9987	0.0062
Anthocyanins (pH diff.)	520/700		—	—	

Antioxidative Activity Tests

DPPH

The antioxidant activity of the extract was evaluated using the DPPH assay (17). This method is based on the transfer of protons or electrons between antioxidant molecules and the stable DPPH free radical (2,2-diphenyl-1-picrylhydrazyl). Due to electron delocalization over the nitrogen atom, DPPH exhibits an intense purple color. Upon reduction by an antioxidant, DPPH is converted to the corresponding hydrazine form, resulting in a decrease in color intensity from purple to yellow.

The extent of discoloration, corresponding to the radical scavenging activity of the extract, was determined spectrophotometrically at $\lambda = 514$ nm. For the analysis, 0.2 mL of the extract and 5.0 mL of 0.004% solution DPPH in 80% V/V ethanol were transferred into a 10 mL volumetric flask and diluted to the mark with 80% V/V ethanol. A solution containing 80% V/V ethanol was used as the blank. The reaction mixture was incubated in the dark at room temperature for 60 min, after which the absorbance was measured.

The degree of DPPH radical scavenging activity was calculated according to equation (2)

$$I = \frac{(A_{control} - A_{sample})}{A_{control}} \cdot 100\% \quad (2)$$

where I represents the degree of DPPH neutralization, $A_{control}$ is the absorbance of the control solution (5 mL of DPPH solution was measured into a 10 mL volumetric flask, the volumetric flask was filled to the mark with 80% V/V ethanol), and A_{sample} is the absorbance of the extract.

ABTS

The antioxidant activity of chokeberry extract was also determined by the ABTS method. As a quick screening method, the chokeberry extract was tested for its capacity to scavenge ABTS free radicals ($ABTS^{\bullet+}$).

The stock solution of $ABTS^{\bullet+}$ was prepared as described by Hsu *et al.* (18). According to our previous work (19), for determination of antioxidant activity, the 0.1 mL of CBP extract was dissolved in 5 mL of $ABTS^{\bullet+}$ solution, mixed thoroughly, and then diluted to 10 mL with PBS buffer, mixed thoroughly. Reaction mixture incubated in the dark at room temperature. After 3 min, the absorbance was recorded at 734 nm with PBS buffer as blank. According to the relation (3), the results as a percentage of ABTS inhibition were given:

$$\% \text{ Inhibition of ABTS} = \frac{A_{ABTS} - A_{sample}}{A_{ABTS}} \quad (3)$$

where A_{ABTS} is the absorbance of the 5 mL of ABTS solution diluted up to 10 mL with PBS buffer and A_{sample} is the absorbance of the reaction mixture.

Results and Discussion

The total polyphenol, flavonols, flavanones, and anthocyanin contents were determined in chokeberry pomace, obtained from locally sourced chokeberries from producers in the region of Kragujevac, Central Serbia. The results are presented in Table II. Additionally, the antioxidant activity of the obtained extracts was determined using the DPPH and ABTS assays, and results are expressed in the percentage of radical scavenging activity (Figure 2).

The accuracy and reproducibility of spectrophotometric methods used to determine the content of total polyphenols and total flavonols and flavanones is good, as can be concluded from the low values of the standard deviation of the determination.

Table II Content of total polyphenols, flavonols and flavanones, and anthocyanins in the ethanolic extract of chokeberry pomace

Tabela II Sadržaj ukupnih polifenola, flavonola i flavanona, i antocijana u etanolnom ekstraktu komine aronije

Content	<i>mg g⁻¹ pomace ± SD</i>	<i>g L⁻¹ extract ± SD</i>
total polyphenols	204.706 ± 0.031	5.1177 ± 0.0042
flavonols and flavanones	14.728 ± 0.036	0.3682 ± 0.0031
anthocyanins	14.608 ± 0.027	0.3654 ± 0.0022

The results show that the polyphenol content of the tested pomace is very high (204.71 mg g⁻¹), representing about 20% of the dry weight. This directly indicates that pomace is an excellent source of bioactive molecules and natural antioxidants, suggesting its high potential for use in the food or pharmaceutical industries. Although a large amount of anthocyanins is lost during berry processing and juice extraction, the results indicate that pomace retains relatively high concentrations of these compounds (14.61 mg g⁻¹) (20). Various flavonoids are natural stabilizers of anthocyanins and contribute to the total antioxidant capacity and their concentration in the tested sample is 14.73 mg g⁻¹.

The large difference in the content of total polyphenols relative to the total content of anthocyanins, flavonols, and flavanones suggests that pomace also contains other specific phenolic compounds, such as proanthocyanidins or tannins (21) and that could be the subject of future research.

The literature states that the content of total polyphenols in chokeberry pomace is 55 mg g⁻¹ of dry matter, the amount of anthocyanins is 18 mg g⁻¹ of dry matter, and the content of flavonols alone is 1.84 mg g⁻¹ of dry matter. After treating the berry pomace in a process similar to extrusion (performed at elevated temperature and pressure), these values decrease to 33 mg g⁻¹ for total polyphenols, while the content of anthocyanins decreases to 6.3 mg g⁻¹ of dry matter (22). The average anthocyanin content in chokeberry

fruit varies from 4.10–14.80 mg g⁻¹ of dry matter, while in chokeberry pulp these values range from 18.00–48.60 mg g⁻¹ dry matter (22–24).

The differences in the content of total polyphenols and anthocyanins can be attributed to the fact that the chokeberry pomace was treated differently compared to the procedure applied in this experiment (different extraction temperature, different composition of the solvents used for extraction), while it should also be borne in mind that the total polyphenol content in natural sources varies depending on the variety, geographical climate, number of sunny days, soil properties, climate and other conditions (25).

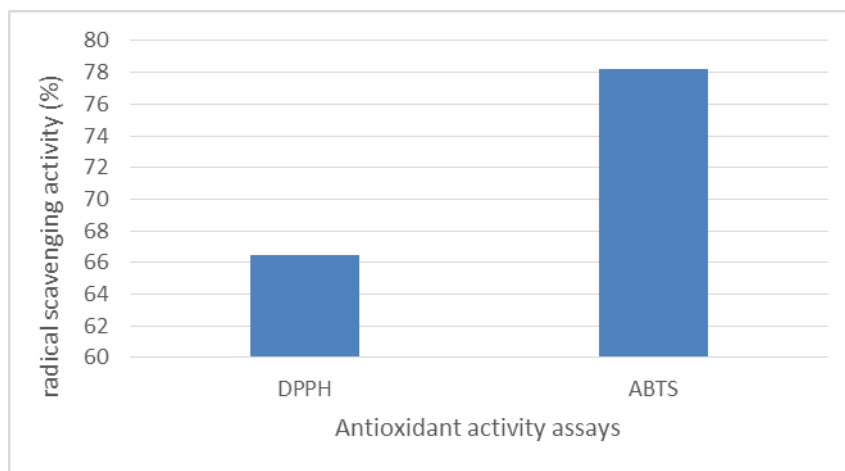


Figure 2. Percentage of radical scavenging activity assays (DPPH and ABTS) in the examined chokeberry pomace sample

Slika 2. Procentualna aktivnost uklanjanja radikala (DPPH i ABTS testovi) u ispitivanom uzorku komine aronije

In accordance with the high total polyphenol concentration, the DPPH (66.44%) and ABTS (77.8%) tests showed significant antioxidant activity in the tested sample. The differences between the DPPH and ABTS assays are likely due to the different reactivity of the extract compounds toward the two radicals. Because 80% V/V ethanol mainly extracts polar phenolic compounds, the antioxidant activity primarily reflects the activity of these compounds. The higher antioxidant activity measured by the ABTS assay may be associated with the presence of anthocyanins and other polar phenolics in chokeberry pomace.

Similarly, Dae-Woon *et al.* (26) concluded that the antioxidant activity of berries is highly correlated with their phenolic and anthocyanin content. Additionally, antioxidant compounds in chokeberry mainly include cyanidin and quercetin derivatives.

In the study conducted by Ugrinović *et al.*, chokeberry extract was used to produce functionalized fabrics of varying compositions (27). Given that anthocyanins predominate in the chokeberry extract, from which the purple color of the extract comes, the content of individual types of anthocyanins was determined using the HPLC method.

The calculated total anthocyanin concentration in the extract is 0.3961 g L^{-1} , which agrees well with the spectrophotometrically determined value of 0.3654 g L^{-1} reported herein. The authors showed that fabrics treated with CBP have the potential to be developed into medical textiles, offering therapeutic, preventive, and protective benefits, but mainly rely on the high anthocyanin content in CBP.

Furthermore, Lipińska *et al.* (20) conducted an interesting study on the impact of adding chokeberry pomace to the diet of Polish Merino and Wrzosówka breed lambs to enhance cellular active defense against oxidative stress, with a perspective on new trends in the feeding of farmed animals. The results indicate that dietary supplementation with CPE improves liver function and supports more effective growth in young animals.

Conclusion

In this study, based on the confirmed high content of total polyphenolic content, separately determined, we suggest the possibility of innovative applications of cosmetic products with CBP or covering films, which could pave the way for new health-supporting solutions through everyday products.

The results of this study demonstrated that chokeberry pomace (CBP) extract is a rich source of bioactive compounds, including flavonoids, with strong antioxidant activity. These results indicate that CBP represents a promising basis for future research on its potential applications in food, cosmetic, and packaging products. Furthermore, using chokeberry pomace may help in the reduction of food waste and pollution, supporting a circular economy.

Important benefits of such application include reducing food waste and pollution from the disposal of berry pomace. Owing to their high levels of bioactive compounds and strong antioxidant capacity, CBP can be given a new, useful value rather than being considered just a food industry waste.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

L.P.: Investigation, Writing – original draft; **S.D.M.:** Data interpretation, Validation; **A.J.L.:** Writing – review and editing; **S.U.M.:** Writing – review and editing, Supervision.

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Komina aronije: otpad ili vredan izvor bioaktivnih jedinjenja?

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Kratak sadržaj

Komine bobičastog voća, nusproizvodi prehrambene industrije, bogate su bioaktivnim polifenolnim jedinjenjima, koja su dobro poznata po svojim jakim antioksidativnim i antimikrobnim svojstvima. Nažalost, komina aronije (CBP) neretko završava kao otpad štetan po životnu sredinu. U ovom radu, ukupan sadržaj polifenola, flavonola i flavanona, antocijanina u ekstraktu komine aronije, određen je spektrofotometrijski. Antioksidativna aktivnost ekstrakta komine aronije određena je ABTS i DPPH testovima. Sadržaj ukupnih polifenola, flavonola i flavanona, i antocijanina, određen u CBP, iznosi 204,61, 14,74, i 14,62 mg g⁻¹ suve materije, respektivno. Antioksidativna aktivnost ekstrakta komine aronije određena je DPPH i ABTS metodama (66,44% i 78,20%), što ukazuje na to da se može efikasno upotrebiti protiv slobodnih radikala. Stoga, umesto da komina aronije završi kao otpad, bogat sadržaj raznih polifenola, ne samo antocijanina, čini kominu aronije odličnim početnim materijalom za kozmetičke proizvode, a takođe postoji i potencijal za njenu primenu u prehrambenoj industriji.

Ključne reči: komina aronije, ukupan sadržaj polifenola, DPPH test, ABTS test
