International Journal of Scientific Research in Science, Engineering and Technology

Print ISSN - 2395-1990
Online ISSN : 2394-4099

OPEN ACCESS

Available Online at :www.ijsrset.com
doi : https://doi.org/10.32628/IJSRSET251261

Antioxidant and Physicochemical Properties of Chokeberry Pomace as Valuable Food Industry By-Product

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ARTICLEINFO

Article History:

Accepted : 01 Sep 2025
Published: 20 Sep 2025

Publication Issue :
Volume 12, Issue 5
September-October-2025

Page Number :
76-83

ABSTRACT

Chokeberry (Aronia melanocarpa) pomace is by-product generated after juice extraction from aronia berries. It represents a solid residue that contains significant amounts of antioxidants, dietary fiber, and other nutritionally valuable ingredients. The transformation of by-products into valuable products and food ingredients should be carried out promptly to ensure a safe and stable product, with drying of the pomace being the first step of the valorization process. In this study, chokeberry pomace was dried at 50°C to a final moisture content of approximately 8%. After drying and cooling, the pomace was ground and subjected to analysis. The greatest value of pomace lies primarily in its high content of bioactive compounds. The results showed that the pomace contained high levels of polyphenols (5923.37±249.31 mg GAE/100 g DM), flavonoids (598.47±30.07 mg CtE/100 g DM), as well as high antioxidant capacity determined by the FRAP assay (88.51±4.40 mmol Fe2+/100 g DM), and DPPH assay (31.93±0.09 mmol TE/100 g DM). The IC50 value was 10.66±3.30 µg/mL, indicating strong radical scavenging ability. In addition to the antioxidant capacity, physicochemical properties (moisture content, water activity, pH value, titratable acidity, ash content, sugar content and color) were also determined. Based on these findings, chokeberry pomace has the potential to be used as a functional ingredient in food products to enhance their nutritional value, antioxidant potential, and sensory properties.

Keywords: Antioxidant Capacity, By-Product Valorization, Chokeberry Pomace, Physicochemical Properties

INTRODUCTION

Fresh, unprocessed chokeberry fruit is rarely consumed due to its characteristic astringent taste, but it is most often used in the food industry for the production of various products, mostly juice. During the production of juice, a large amount of pomace is generated, which is still a good source of, among other things, polyphenols, vitamins and dietary fibers, and at the same time has a low energy value. Therefore, the chokeberry pomace has many useful applications [1].

The first step of the valorization process is the drying of pomace [2]. Drying is the most common way of preserving pomace. The drying process affects the chemical composition of the raw material, its quality and content of bioactive compounds, and its appearance. In addition to drying, the chemical composition of pomace also depends on the quality of the raw material from which it was obtained, which depends on weather conditions, fertilization and agrotechnical treatments [3, 4].

Due to its high content of polyphenols, chokeberry has a high antioxidant capacity [5, 6, 7], high dietary fiber content [8, 9], and high polyphenol bioaccessibility [10].

The high antioxidant capacity of chokeberry pomace contributes to its beneficial effect on the human body, in the prevention of diseases caused by oxidative stress, such as cardiovascular diseases, neurodegenerative diseases, cancer, diabetes and other health problems [11].

Considering these properties, pomace can be an important source of functional ingredients [4, 12] and can be used to enrich food products, especially bakery and confectionery products, in order to enhance the nutritional value, antioxidant capacity, and sensory properties [13, 1, 14, 15, 16, 17, 18]. So, it can be used directly as dried pomace or in further processing to obtain pectin, vitamins, polyphenols, and in the production of fruit teas, fruit distillates, colors, nutritional supplements, aromas and food pigments [3, 19, 2].

The aim of this study was to determine some physicochemical and antioxidant properties of chokeberry pomace as a valuable food industry by-product.

METHODS AND MATERIAL

Chokeberry pomace was sourced in 2022 from a local chokeberry juice producer in Brčko, Bosnia and Herzegovina. The pomace was oven-dried at 50°C for approximately 4 hours, until the moisture content decreased to about 8%. After drying and cooling, it was ground in coffee mill and subsequently analysed.

The content of water was measured using a moisture analyser (KERN DBS 60-3, Germany). Water activity (a_w) was measured using a Novasina LabSwift-aw instrument (Switzerland). Ash content was determined according to ISO 5984 by incinerating the sample in a muffle furnace (Biobase, China) at 550±20°C until all organic matter was burned off, followed by weighing the remaining ash. The pH value was measured using a digital pH meter (XS Instruments PC52+DHS, Italy) in an aqueous solution of the pomace. Titratable acidity was determined by potentiometric titration of an aqueous pomace powder suspension with 0.1 M NaOH, using an end point at pH 8.1±0.2. The titratable acidity was expressed as percentage of citric acid using a conversion factor of 0.070 [20]. Content of directly reducing and total sugars were determined using the Luff-Schoorl method [21]. The color of the chokeberry pomace was measured using a colorimeter (Konica Minolta CR-400, Japan).

For the determination of total polyphenol and flavonoid content, as well as antioxidant capacity, an extract of chokeberry pomace was prepared by accurately weighing 100 mg of the sample (±0.0001 g) into a 300 mL ground-glass Erlenmeyer flask. Then, 100 mL of acidified methanol (containing 1% v/v concentrated HCl) was added. The prepared samples

were extracted using a shaker (Vibromatic, JP Selecta) for 60 minutes at 400 strokes per minute. After extraction, the samples were filtered into a 100 mL volumetric flask, and the volume was adjusted to the mark with methanol.

The antioxidant capacity was determined using the DPPH method, which measures the neutralization of DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, according to the procedure described by Matejić et al. [22], with minor modifications. Briefly, varying volumes of the extract (100-1000 µL) were diluted with methanol to a final volume of 4 mL. To each solution, 1 mL of 0.5 mM DPPH solution was added. The mixture was vigorously shaken and incubated in the dark at room temperature for 30 minutes. Absorbance was then measured at 517 nm (A_1). A control sample (A_0) was prepared by mixing 1 mL of 0.5 mM DPPH solution with 4 mL of methanol. The percentage of DPPH radical inhibition was calculated using the following equation:

$$I\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

Results were expressed as IC_{50} value (µg/mL), defined as the concentration of extract required to inhibit 50% of the DPPH radicals. The IC_{50} value was obtained from the curve dependence of percentage of DPPH radical inhibition (I) and extract concentration.

The antioxidant capacity was additionally evaluated using a second DPPH radical scavenging assay. Briefly, 2.9 mL of methanol, 0.8 mL of 0.5 mM DPPH solution, and 0.3 mL of the sample extract were pipetted into a test tube. The mixture was mixed and incubated in the dark at room temperature for 40 minutes. After incubation, the absorbance was measured at 520 nm. The antioxidant capacity was calculated based on a Trolox calibration curve (6.25-43.75 µM Trolox) and expressed as milimoles Trolox equivalents per 100 grams of dry matter of pomace (mmol TE/100 g DM).

In addition to the DPPH assay, antioxidant capacity was also assessed using the FRAP method according to Benzie and Strain [23]. FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM hydrochloric acid, and 20 mM ferric chloride hexahydrate solution in a 10:1:1 (v/v/v) ratio, respectively. Then, fresh FRAP reagent (3 mL) was added to diluted extract (100 µL), incubated for 30 minutes at 37°C and the absorbance of the samples was measured at 593 nm (Shimadzu UV-1800 UV/Vis). The final results were expressed as milimoles of Fe^{2+} per 100 grams of dry matter (DM) of pomace (mmol Fe^{2+}/100 g DM).

The total polyphenolic content (TPC) was determined using Folin Ciocalteu method as described by Singleton, et al. [24] with slight modifications. In the test tubes 0.2 mL of extract was pipetted and mixed with 2.54 mL of Folin-Ciocalteu reagent (previously diluted 1:10 with deionized water). After mixing, 0.42 mL of 10 % Na_2CO_3 were added, followed by vigorous mixing. After 60 minutes of incubation in the dark at room temperature, 0.91 mL of distilled water was added and mixed. The absorbance of the blue-colored solutions was measured at 765 nm, and the final results were expressed as mg gallic acid equivalents per 100 grams of dry matter (DM) of pomace (mg GAE/100 g DM).

The total flavonoid content (TFC) was determined according to the colorimetric method described by Zhishen et al. [25]. Briefly, 1 mL of methanolic extract was transferred into a 10 mL volumetric flask, followed by the addition of 4 mL distilled water and 0.3 mL of 5% sodium nitrite ($NaNO_2$). After 5 minutes, 0.3 mL of 10% aluminium chloride ($AlCl_3$) was added, and after an additional 6 minutes, 2 mL of 1 M sodium hydroxide (NaOH) was added. The mixture was then diluted with distilled water to a final volume of 10 mL and thoroughly mixed. Absorbance was measured at 510 nm, and final results expressed as mg catechin equivalents per 100 grams of

sample of dry matter of the pomace (mg CtE/100 g DM).

RESULTS AND DISCUSSION

The physicochemical properties of chokeberry pomace are shown in Table 1. Dried pomace has a low moisture content (7.98%) and low water activity (0.439). Water is one of the most important factors controlling the rate of food deterioration, either by microbial or by nonmicrobial effects. Water activity (a_w) is a measure of the availability of water for biological functions and refers to the water present in food in its "free" form. The water activity of chokeberry pomace is below the 0.6 threshold, considered the lower limit for any microbial growth. By reducing a_w in food, the growth of vegetative microbial cells, spore germination, toxin production by molds and bacteria, and enzyme activity are inhibited [26]. Most studies report that the moisture content of dried chokeberry pomace does not exceed 8% [27, 4, 8, 10].

TABLE I PHYSICOCHEMICAL PROPERTIES OF CHOKEBERRY POMACE

Physicochemical parameters	Chokeberry pomace
Water content (%)	7.98±0.11
Water activity	0.439±0.00
pH value	3.65±0.07
Titratable acidity (g citric acid per 100 g)	3.05±0.16
Sugars (%)	
Reducing sugars	13.72±0.23
Sucrose	0
Ash (%)	2.00±0.03
Color	
L*	23.14±0.52
a*	14.64±0.23
b*	8.90±0.16

Values are mean (n=3) ± standard deviation.

Specific conditions are required for the growth and reproduction of microorganisms. Low moisture content and an acidic environment are generally unfavorable for their development. Based on the pH value of chokeberry pomace (3.65±0.07), it can be classified as a highly acidic food (pH<4), which is a result of the presence of organic acids. Most microorganisms grow optimally at near-neutral pH values, typically in the range of 6.0 to 7.5. The low pH value results in high titratable acidity (3.05±0.16% of citric acid). Data for moisture content, water activity, pH value, and titratable acidity imply that there is a low risk of microbiological and enzymatic activity and that the powder can be considered stable. According to Tolić et al. [28], the pH value was determined to be 4.13±0.01, while the total titratable acidity amounted to 2.17±0.07%, expressed as citric acid.

Table 1 also presents the sugar content, specifically the content of reducing sugars (13.72±0.23%), as well as the ash content (2.00±0.03%). A majority of the chokeberry sugars are extracted into juice, whereas its fiber is mainly distributed in the pomace. Fiber is one of the components that give the pomace its high nutritional value. According to Raczkowska et al. [1], out of the total 86.80% carbohydrates in pomace, 69.72% are dietary fiber and 10.81% are total sugars. Lazăr et al. [4] also reported that the total sugar content in chokeberry pomace ranges between 12.72% and 15.89%. Comparable ash content, ranging from 1.6% to 2.73%, has also been reported in other studies [27, 8, 3, 4, 10].

Given the significant role that color plays in determining the overall quality of a product, this study aimed to evaluate the color characteristics of chokeberry pomace. The L* value, which represents lightness, was 23.14±0.52; lower L* values indicate a darker sample. The a* value, which describes the red-green color, was 14.64±0.23 and positive values indicate redness. The b* value, which describes the blue-yellow, was 8.90±0.16 and positive values

indicate yellowness. These color parameters indicate that the pomace powder is dark red, which can be attributed to the high anthocyanin content in chokeberry, one of the richest natural sources of anthocyanins. Lazăr et al. [4] reported that the pomace obtained by lyophilization (freeze-drying) appeared lighter than the one dried by hot air drying. They concluded that the color of chokeberry pomace depends on the drying method, as also observed by Horszwald et al. [29], who dried chokeberry pomace using three different techniques: spray drying, freeze-drying, and vacuum oven drying at 40, 50, and 60°C. Lower L* values were recorded for oven-dried pomace (15.52–17.76) compared to freeze-dried (24.35) and spray-dried (24.03) pomace, indicating that oven-dried samples were darker. Lower a* and b* values were also observed in oven-dried samples. The color parameters obtained through spray drying (L^*=24.03±0.09, a^*=33.61±0.05, b^*=10.19±0.06) were the most similar to the results of this study.

The value of chokeberry pomace lies in its high content of polyphenolic compounds and, consequently, its strong antioxidant capacity. Numerous studies have shown that chokeberry and its pomace contain more bioactive compounds than other investigated species.

Table 2 shows total phenolic content, total flavonoid content and antioxidant capacity of chokeberry pomace. FRAP value shows the ability of the extract to reduce F^{3+} ions to Fe^{2+} ions and for chokeberry pomace it is high (88.51±4.40 mmol Fe^{2+}/100 g DM). According to the study by Tolić et al. [28] the FRAP value for chokeberry pomace was 47.38 mmol Fe^{2+} /100 g DM, which is approximately half the value obtained in the present study. Additionally, compared to that study, the total polyphenol content (4233±234 mg GAE/100 g DM) was slightly lower than in the present research, while the flavonoid content (2327±373 mg GAE/100 g DM) and DPPH value (131.06±0.47 mmol TE/100 g DM) were significantly higher.

According to Mayer-Miebach et al. [30] the total polyphenol content (TPC) ranges from 3100-6300 mg GAE/100 g, while Lazăr et al. [4] reported values between 5026 and 9692 mg GAE/100 g. Similarly, Kapci et al. [31] reported a TPC of 6310 mg GAE/100 g on a wet basis and a TF of 930 mg CtE/100 g wet basis, which is in accordance with present study.

In contrast, some studies have reported lower TPC and TF values. For example, Sărăcilă et al. [10] recorded TPC of 2294 mg GAE/100 g DM and TF of 189 mg QE/100 g DM, while Petrov Ivanković et al. [12] reported a TPC of 1339 mg GAE/100 g DM.

TABLE III TOTAL PHENOLIC CONTENT (TPC), TOTAL FLAVONOID CONTENT (TFC), AND ANTIOXIDANT CAPACITY (DPPH, FRAP) OF CHOKEBERRY POMACE

	Chokeberry pomace
TPC (mg GAE/100 g DM)	5923.37±249.31
TFC (mg CtE/100 g DM)	598.41±30.07
FRAP (mmol Fe^{2+}/100 g DM)	88.51±4.40
DPPH (mmol TE/100 g DM)	31.93±0.09
IC50 (μg/mL)	10.66±3.30

Values are mean (n=3) ± standard deviation.
(TE-Trolox, GAE-gallic acid, CtE-catechin)

The results indicate an exceptionally high content of bioactive compounds and strong antioxidant capacity. Variations in results may be attributed to differences in drying methods, the type of solvent used for extraction, reaction time, pomace particle size, analytical approach, and the quality of the raw material used.

The DPPH free radical scavenging activity of chokeberry pomace was also expressed as the IC_{50} value (μg/mL). Since the IC_{50} represents the concentration of extract required to scavenge 50% of DPPH radicals, a lower IC_{50} value indicates higher antioxidant capacity [32]. The IC_{50} value obtained in this study was 10.66±3.30 μg/mL, demonstrating that

a very low concentration of pomace is sufficient to neutralize 50% of DPPH radicals.

CONCLUSION

Chokeberry pomace, a by-product obtained during juice production, by drying and grinding can be converted into a form suitable for use in food products. Based on its moisture content, water activity, pH value, and titratable acidity, the resulting pomace does not support microbial growth, making it suitable for long storage and as such can be used in the formulation of some food products.

In terms of its polyphenol content and antioxidant capacity, chokeberry pomace can be considered a functional ingredient. It can be used to enrich food products by enhancing their nutritional value and their antioxidant capacity, as well as sensory properties. This is especially important due to the rising demand for functional foods enriched with bioactive compounds in recent years.

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