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BIOCHEMICAL COMPOSITION AND ANTIOXIDANT CAPACITY OF A FUNCTIONAL INGREDIENT OBTAINED FROM ELDERBERRY (*SAMBUCUS NIGRA* L.) POMACE

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Abstract

The elderberry (Sambucus nigra L.) is cultivated worldwide for the production of fruits and flowers. The elderberry fruits are not consumed fresh but are mainly processed into longer shelf-life food products, such as juice, concentrates, syrups, jams, jellies, colorants, and wines. In this paper are presented the results of the research performed to achieve a functional ingredient (powder) from elderberry (Sambucus nigra L.) pomace resulting in the fruit juice industry. For this purpose, Aronia pomace (from the conventional and organic culture of our country) was subjected to a convective drying process at 50 °C or lyophilisation at -55 °C to protect the bioactive compounds to moisture content to allow their milling and turning them into powder and their stability in terms of quality. Powder achieved from elderberry pomace is characterized by total dietary fiber content (46.28-50.50%), minerals (3.13- 3.50%), total sugar (9.73-12.37%), pectic substances (6.88-8.32%), vitamin C (5.21-16.14 mg/100 g), vitamin E (1.50- 3.05 mg/100 g), and total polyphenols (97.84-146.76 mg GAE/g). At the same time, powder achieved from elderberry pomace has antioxidant capacity. Due to its complex biochemical composition and antioxidant potential, the functional ingredient achieved from elderberry pomace can be used to fortify bakery and pastry products.

Key words: *dietary fiber elderberry, pomace, powder, total polyphenols.*

INTRODUCTION

European black elderberry is grown in Europe and in many parts of the world, for fruits and flowers production. Elderberry fruits are rich in nutrients such as carbohydrates, proteins, lipids/fatty acids, organic acids, minerals, vitamins, but also essential oils.
Polyphenols are bioactive compounds known for their antiradical activity, which are found in high concentrations in elderberry fruits. The biochemical composition of elderberry fruits depends on the variety, cultivation area, climatic conditions and their maturation degree (Młynarczyk et al., 2018).
The carbohydrates in elderberry, according with recent research results, contains 7.86-11.50% sugar of total sugar and 2.8-8.55% of reducing sugar. According to Veberic et al. (2009), the total sugar content of elderberry fruits varied between 68.53 and 104.16 g/kg fresh substance (f.s.), depending on the variety and cultivation area.
The main sugars identified were glucose (33.33-50.23 g/kg f.s.) and fructose (33.99-52.25 g/kg f.s.), sucrose being present only in low concentrations in fruits (0.47-1.68 g/kg f.s.).
Carbohydrates found in elderberry fruits (*Sambucus nigra* L.) also include dietary fiber, especially pectin, pectic acid, protopectin, calcium pectate and cellulose. Elderberry is an important source of proteins: 2.7-2.9% in fruits, 2.5% in flowers and 3.3% in leaves. Proteins include sixteen amino acids, nine of which are essential amino acids.
The total content of essential amino acids in elderberry is 9% in flowers and 11.5% in

leaves. Glutamic acid, aspartic acid and alanine have been reported as the main amino acids.

Fats are mostly accumulated in elderberry fruit seeds (fat content: 22.4%) and in elderberry seed flour (fat content: 15.9%). The organic acid content of elderberry fruits is 1.0-1.3%. Veberic et al. (2009) detected four organic acids in elderberry fruits: citric acid (3.08-4.81 g/kg f.s.), malic acid (0.97-31.31 g/kg f.s.), shikimic acid (0.14-0.93 g/kg f.s.) and fumaric acid (0.10-0.29 g/kg f.s.).

The mineral elements are located in both fruits and flowers. The mineral content of elderberry fruits represents 0.90-1.55% of their mass. According to Vulić et al. (2008) the mineral content of the elderberry is: Potassium 391.33 mg/100 g; Phosphorus 54.00 mg/100 g; Ca 28.06 mg/100 g; Magnesium 25.99 mg/100 g; Iron 1.86 mg/100 g; Zinc 0.36 mg/100 g; Manganese 0.27 mg/100 g; Copper 0.14 mg/ 100 g.

Several studies have shown the beneficial effects of elderberry, used in antiviral drugs (Zakay-Rones et al., 1995; Roschek et al., 2009; Barak et al., 2019) efficient supplements in the treatment and prevention of diabetes, cardiovascular diseases, and cancer (Ciocoiu et al., 2009; Jing, et al., 2008). Thus, various nutraceutical *Sambucus nigra* berries products (syrups, drops, tablets, emulsions, suspensions, extracts) are commercially available (Młynarczyk et al., 2018; Barak et al., 2019).

Sambucus nigra fruits are not consumed fresh but are mainly processed into longer shelf-life food products, such as juice, concentrates, syrups, jams, jellies, colorants, and wines (Kaack et al., 2008; Schmitzer et al., 2012). In the case of solid juice processing by-products amounts 20-40% of total elderberry biomass (Seabra et al., 2010). Data on global production of elderberry pomace is not available. Syrups and beverages are indicated as important elderberry products (e.g., the share of beverages was 17.5% in 2017). So, the processing of elderberry fruits generates large amounts of pomace (Technavio, 2019).

The aim of this work was to obtain a functional ingredient (powder) from elderberry (*Sambucus nigra* L.) pomace, a by-product of fruits juice processing.

MATERIALS AND METHODS

Samples

The elderberry (*Sambucus nigra* L.) pomace resulted by processing the elderberry fruits with a juicer extractor (Philips) in the laboratory at IBA Bucharest.

Elderberry fruits used in the study come conventional (II – *Ina* variety) and bio (I- *Nora* variety and III- *Bradet* variety) culture. Till processing, elderberry pomace was stored under refrigeration conditions (at 3°C), for a maximum of 36 hours. Fresh pomace was subjected to hot-air dehydration (at 50°C) or freeze-drying (at -55°C) and then grinding and turned into powder.

The elderberry pomace samples I and III were hot-air dehydrated and freeze-drying. Sample II was not lyophilized, it was only hot-air dehydration.

The milling of the dry semi-finished products was performed using the Retsch mill, at a temperature of 20-22°C, to ensure the quality parameters of the final product.

Figure 1 shows elderberry pomace powders obtained by hot-air dehydration (at 50°C), and Figure 2 those obtained by freeze-drying (at -55°C)

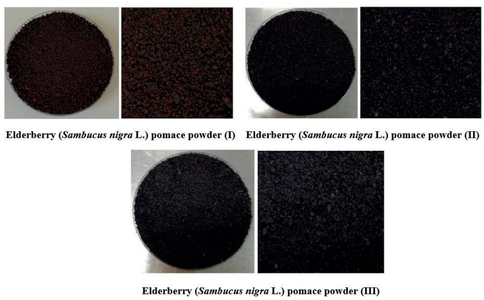

Figure 1. Elderberry pomace powder (hot-air dehydration at 50°C)

Figure 2. Elderberry pomace powder (freeze-drying at -55°C)

Methods

Statistical Analysis

Elderberry pomace powder samples were analyzed in triplicate. Mean and standard deviation are reported for each analytical parameter studied.

Sensory analysis

Sensory analysis (appearance, taste and smell) was performed by descriptive method.

Physico-chemical analysis

Measurement of the colour parameters (L^*, a^* and b^*) of samples was performed using a CM-5 colorimeter (Konica Minolta, Japan) and SpectraMagic NX software.

The water content was determined by infrared moisture balance (Ohaus Moisture Analyzer MB45), a temperature of 105°C.

Chemical composition was determined by AOAC Methods: 979.09 (protein content), 963.15 (fat content), 923.03 (ash content).

The samples were mineralized by calcination. The minerals (potassium, calcium, magnesium and iron were determined by atomic absorption spectrophotometry. Phosphorus was determined by spectrophotometric method (McKie & Mccleary, 2016).

Schoorl method was used to establish the total sugar content.

Enzymatic method using the assay kits: K-TDFR ''Total dietary fibre'' (AOAC Method 991.43) was used for determining the total dietary fibre (TDF). Gravimetric method was used for determining pectic substances (expressed as calcium pectate).

Determination of vitamin C content was performed by by HPLC-HRMS (Catană et al., 2017).

Determination of vitamin E (α-tocopherol) content was performed by liquid chromatography with diode array detection (Popović et al., 2015).

Total polyphenol content

Total polyphenol content was performed by using Folin-Ciocalteau method according to Horszwald and Andlauer (2011) by means of UV-VIS Jasco V 550 spectrophotometer, at a wavelenght λ = 755 nm. The calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The polyphenol extraction solvent was a mixture of methanol and water (methanol: water = 1: 1). Total polyphenol content was expressed as g of Gallic Acid Equivalents (GAE) per 100 g elderberry pomace powder.

Antioxidant capacity

Antioxidant capacity was performed by DPPH (1,1diphenyl-2-picryl hydrazyl) radical scavenging assay, according to Horszwald and Andlauer (2011), using UV-VIS Jasco V 550 spectrophotometer, at a wavelenght λ = 517 nm. The calibration curve of Trolox achieved in the concentration range 0-0.4375 mmol/L. Antioxidant capacity was expressed as mg Trolox Equivalents per g elderberry pomace powder.

Microbiological analysis

Aquaspector AQS-2-TC, Nagy, equipment was used to establish the water activity (*A*w). The measurements were performed at 25ºC. SR ISO 21527-1:2009 method was used to check for the yeasts and moulds. SR EN ISO 21528-1:2017 method was used to check for the presence of *Enterobacteriaceae.* SR ISO 16649-2:2007 method was used to check for the presence of *Escherichia coli*. The method SR EN ISO 6579-1:2017 was used to determine the *Salmonella* presence.

RESULTS AND DISCUSSIONS

Sensory analysis

As a result of the sensory analysis, it was found that the powders achieved from elderberry pomace, have colours from dark cherry to dark brown and, respectively, dark and have pleasant taste and smell, characteristic of elderberry fruits.

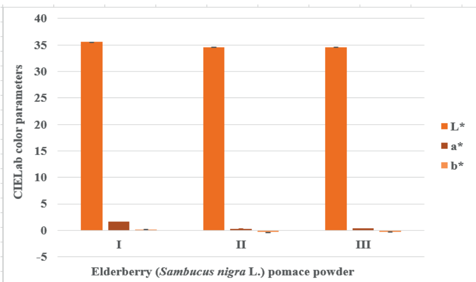

Figure 3. Colour parameters of the powders achieved from elderberry pomace (hot-air dehydration at 50°C)

As a result of the instrumental color analysis, it was found that the powders obtained from elderberry pomace by freeze-drying at -50°C, are lighter in color compared to those obtained by dehydration at 50°C, registering higher luminance values, respectively, L * = 39.75, in the case of sample I (Figures 3, 4).

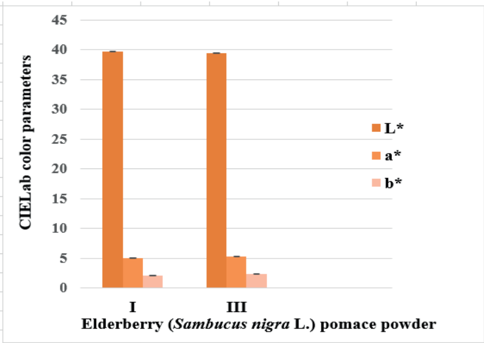

Figure 4. Colour parameters of the powders achieved from elderberry pomace (freeze-drying at -55°C)

Also, the maximum values of the parameters a* (red colour coordinate) and b* (yellow colour coordinate) were recorded for powders obtained from elderberry pomace, by freeze-drying at

-55°C (a* = 5.35 and b* = 2.35, values recorded in the case of sample III).

Physico-chemical analysis

The physico-chemical indicators of the powders obtained from elderberry pomace are presented in Table 1.

Table 1. The physico-chemical indicators of powders achieved from elderberry pomace

Functional ingredient	Water (%)	Ash (%)	Protein (%)	Fat (%)	Total sugar (%)	Total fibre (%)
Dehydration with hot air at 50°C						
Sample powder I	5.03±0.13	3.23±0.04	10.23±0.09	19.18±0.21	10.48±0.052	49.75±0.92
Sample powder II	5.44±0.14	3.13±0.04	11.01±0.10	18.47±0.20	12.37±0.062	47.50±0.88
Sample powder III	4.96±0.14	3.42±0.05	10.31±0.09	19.44±0.21	9.73±0.049	46.28±0.86
Lyophilization at - 55°C						
Sample powder I	3.42±0.09	3.30±0.04	10.47±0.09	19.55±0.21	10.71±0.053	50.50±0.93
Sample powder III	3.85±0.10	3.50±0.04	10.51±0.09	19.74±0.22	9.88±0.049	46.82±0.87

Sample II was not lyophilized, it was only hot-air dehydration. The results show that these powders are distinguished by their content in protein (10.23-11.01%), fat (18.47-19.74%), total ash (3.13-3.50%), total sugar (9.73-12.37%) and total fiber (46.28-50.5%). Water content of the powders obtained from elderberry pomace in this study, is lower compared to that of the elderberry pomace (residual moisture content 6.2%) achieved by Kitrytė et al. (2020).

Powders achieved from elderberry pomace (sample III) has the highest total ash content (3.42% in the case of powder obtained by dehydration at 50°C and 3.50% in the case of that obtained by freeze-drying at -55°C), fat (19.74% in the case of powder achieved by freeze-drying, at -55°C). The powder from elderberry pomace (sample II), by dehydration at 50°C, had the highest content of total sugar (12.37%), protein (11.01%). Also, the powders from elderberry pomace (sample I) has the highest total fiber content (49.75% for the powder obtained by hot-air dehydration at 50°C and 50.50% in the case of that obtained by freeze-drying at -55°C).

The elderberry pomace powders had a high content of pectic substances in the following range: 6.88-8.32 % pectate of calcium (Table 2).

The content in pectic substances of the powders achieved from elderberry pomace, within this study is like that reported by Sidor and Michałowska (7.52%), in case of *Aronia melanocarpa* powder.

Table 2. Content in pectic substances of powders achieved from elderberry pomace

Functional ingredient	Pectic substances (% calcium pectate)
Dehydration with hot air at 50°C	
Sample powder I	7.85±0.20
Sample powder II	6.88±0.17
Sample powder III	7.97±0.20
Lyophilization at - 55°C	
Sample powder I	8.15±0.20
Sample powder III	8.32±0.21

Several studies showed that pectic substances are physiologically active substances with immunomodulating properties and anti-inflammatory activity, with the capacity to lower cholesterol and triglyceride in the blood serum, normalize glucose metabolism, bind and remove toxins and radionuclides from the body. Also, pectin polysaccharides can provide protection for the gastrointestinal tract and have anticarcinogenic and antimetastatic effects.

Powders achieved from elderberry pomace, are important sources of minerals (K, Ca, Mg, Fe, Zn). The mineral content of the elderberry pomace powders obtained by hot-air dehydration at 50°C is shown in Figures 5 and 6.

Elderberry pomace powder from sample III, had the highest potassium content (548.85 mg/100 g).
The calcium content of the elderberry pomace powders was lower than that of potassium and varied in the range of 180.25 - 220.42 mg/100 g (the maximum value being recorded in the case of sample III and the minimum value, in the case of sample II).

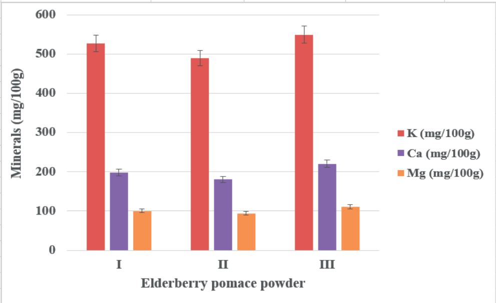

Figure 5. Mineral content (Na, K, Ca, and Mg) of the powders achieved from elderberry pomace (hot-air dehydration at 50°C)

The magnesium content of the powders was lower than that of calcium, representing about 50.21-52.19% of their calcium content.

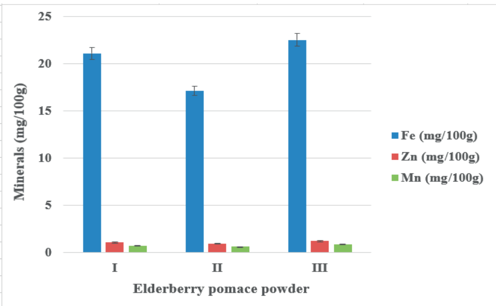

Figure 6. Mineral content (Fe, Zn, and Cu) of the powders achieved from elderberry pomace (hot-air dehydration at 50°C)

The elderberry pomace powders differed by their iron content, which varied in the range 17.17-22.56 mg/100 g (the maximum value being recorded in the case of sample III and the minimum value, in the case of sample II).
The zinc and manganese content of the elderberry pomace powders recorded low values, in the range 0.64-1.25 mg/100 g.
Content in calcium and iron of elderberry pomace powders achieved by dehydration at 50°C, is higher compared to that of the elderberry pomace achieved by Różyło et al. (2019) (calcium: 177 mg/100 g; iron: 4.78 mg/100 g), and the potassium and magnesium content is lower.
The mineral content of powders (samples I and III) obtained by lyophilization at -55°C is similar to that of powders obtained by dehydration at 50°C (Table 3). Sample II was not lyophilized, it was only hot-air dehydration. Very small differences can be explained by the lower water content of the powders obtained by lyophilization.

Table 3. Mineral content of the powders achieved from elderberry pomace (lyophilisation at -55°C)

Minerals	Powders achieved from elderberry pomace	
	Sample powder I	Sample powder III
K (mg/100g)	535.10 ±18.73	555.26±19.43
Ca (mg/100g)	201.48±7.05	222.99±7.80
Mg (mg/100g)	101.95±3.57	111.96±3.92
Fe (mg/100g)	21.51±0.65	22.82±0.68
Zn (mg/100g)	1.11±0.06	1.29±0.07
Mn (mg/100g)	0.74±0.02	0.91±0.03

Bioactive compounds content
The powders obtained in this study were noted for their content in bioactive compounds: total polyphenols, vitamin C and vitamin E (α-tocoferol) (Table 4). Sample II was not lyophilized, it was only hot-air dehydration.

Table 4. Bioactive compounds content of the powders achieved from elderberry pomace

Functional ingredient	Total polyphenols (g GAE/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)
Dehydration with hot air at 50°C			
Sample powder I	10.36±0.26	7.84±0.26	1.62±0.09
Sample powder II	10.09±0.25	5.21±0.17	1.50±0.08
Sample powder III	9.78±0.24	9.22±0.30	1.85±0.10
Lyophilization at - 55°C			
Sample powder I	13.99±0.35	12.39±0.41	2.62±0.14
Sample powder III	14.68±0.23	16.14±0.53	3.05±0.17

The total polyphenol content of the elderberry pomace powders was very high and varied in the range 9.78-14.68 g GAE /100 g.
Also, the elderberry pomace powders are distinguished by their vitamin C (5.21-16.14 mg/100 g) and vitamin E (α-tocopherol: 1.50-3.05 mg/100 g).
Elderberry pomace powders obtained by lyophilization at -55°C recorded a higher retention of bioactive compounds, compared to those obtained by dehydration at 50°C. Thus, their content in vitamin C was 1.58-1.75 times higher and that of total polyphenols 1.35-1.50 times higher, respectively).

Total polyphenol content of the elderberry pomace powders obtained by lyophilisation at -55°C is higher compared to that of the elderberry pomace achieved by Tańska et al. (2016) (13.86 g GAE/100 g D.M.)

Antioxidant capacity

The high antioxidant content (total polyphenols, vitamin C, vitamin E etc.), of the powders obtained in this study, gives them antioxidant capacity (Figure 7).

The highest values of antioxidant capacity were recorded in the case of powders obtained by lyophilization at -55°C: 11.45 mg Trolox Equivalents/g (sample I) and, respectively, 11.95 mg Trolox Equivalents/g (sample III).

The powders obtained by dehydration at 50°C recorded lower values of antioxidant capacity in the range 5.90-6.57 mg Trolox Equivalents/g (the minimum value being recorded in the case of sample III, and the maximum in the case of sample I).

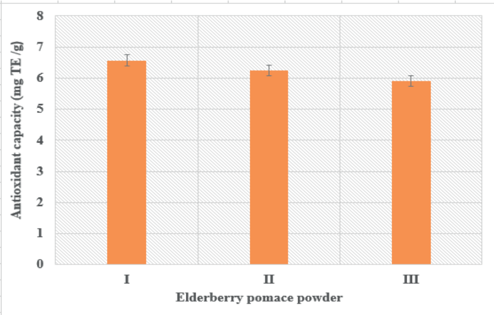

Figure 7. Antioxidant capacity of the powders achieved from elderberry pomace (hot-air dehydration at 50°C)

Microbiological analysis

The microbiological indicators of the elderberry pomace powders are presented in Table 5. Sample II was not lyophilized, it was only hot-air dehydration.

Microbiological analysis shown that the powders are within the legislation into force.

Also, these powders recorded low values of water activity, in the range of 0.285-0.305, which gives them microbiological stability.

Table 5. The microbiological indicators of powders achieved from elderberry pomace

Functional ingredient	Yeast and mold (CFU/g)	*Enterobacteriaceae* (CFU/g)	*Escherichia coli* (CFU/g)	*Salmonella* (in 25 g)	Water activity
Dehydration with hot air at 50°C					
Sample powder I	< 10	< 10	< 10	absent	0.300
Sample powder II	< 10	< 10	< 10	absent	0.305
Sample powder III	< 10	< 10	< 10	absent	0.294
Lyophilization at - 55°C					
Sample powder I	< 10	< 10	< 10	absent	0.267
Sample powder III	< 10	< 10	< 10	absent	0.285

CONCLUSIONS

This study reports valorization of elderberry (*Sambucus nigra* L.) pomace, a by-product of fruits juice processing.

Powders achieved from elderberry pomace are distinguished by their content in minerals (K, Fe, Mg, Ca and Zn), total dietary fibres, pectic substances and bioactive compounds (total polyphenols, vitamin C, vitamin E).

The process of lyophilization of elderberry pomace at -55°C C ensures a higher retention of bioactive compounds compared to hot-air dehydration at 50°C.

Due to its high content of bioactive compounds, these powders have antioxidant capacity (5.90-11.95 mg Trolox Equivalents/g) and their consumption is beneficial in a healthy diet to prevent diseases caused by oxidative stress.

Due to its high nutritional value, bioactive compounds content and antioxidant potential, the powders achieved from elderberry pomace are functional ingredients.

An important practical application of these functional ingredients is the fortification of food products (especially for bakery and pastry products), in order to increase the nutritional value, the content of bioactive compounds, dietary fiber and antioxidant potential.

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