The object of this study is the extracts from black elderberry fruit pomace obtained using various polyols (glycerol, xylitol, sorbitol), as well as their ability to extract anthocyanins, stability, persistence, and spectral characteristics. It was found that black elderberry fruit pomace is characterized by an increased content of anthocyanins (0.42 ± 0.02%) compared to fresh fruits (0.29 ± 0.02%) and juice (0.23 ± 0.02%), as well as a high concentration of flavonoids: rutin (1.52 ± 0.03%), quercetin (0.26 ± 0.01%), and luteolin (2.77±0.04%). Water-ethyl and water-glycerol extracts demonstrated the highest concentration of coloring substances (57.4–58.3 g/dm^3). Microstructural analysis revealed amorphous formations with a size of 50–300 nm, which contribute to the stable retention of anthocyanins. Spectral analysis confirmed the intense absorption bands of anthocyanins (1625–1725 cm^{-1}) in glycerin extracts. The high extraction capacity of glycerin solvents is due to their polarity, electrostatic interaction with anthocyanins and stabilizing properties of glycerin. Water-sorbitol and water-xylitol extracts were less stable due to their hydrophilicity and weaker ability to retain pigments. The use of glycerin as a solvent ensured maximum stability of the extracts due to the formation of amorphous structures that slow down the oxidation of anthocyanins.

The results could be used in practice in the food industry for manufacturing natural dyes, in particular in beverages, confectionery, dairy and fermented milk products. Practical implementation is possible under the following conditions: extraction temperature 65±1.5°C, pH 3.2–3.5, concentration in a vacuum evaporator and storage at a temperature of 4±2°C to preserve the biological activity of anthocyanins and prevent their degradation. In addition, the results of the study open up prospects for the rational use of berry pomace, which is usually subject to disposal

Keywords: pomace, black elderberry, extraction, polyols, coloring substances, natural dyes, anthocyanins

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INVESTIGATING POLYOLS TO IMPROVE THE EFFICIENCY OF EXTRACTING ANTHOCYANS FROM ELDERBERRY (*SAMBUCUS NIGRA L.*) POMACE

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1. Introduction

Food dyes have been used since ancient times to enhance the aesthetic value of food products and the level of consumer demand. In addition, their widespread use is due to the enhancement of the natural color of food and the preservation of color stability during the shelf life. Food dyes can be divided into three groups: natural, obtained from natural sources; synthetic, identical to natural ones in chemical structure; and artificial or synthetic, which have no natural analogs [1].

Synthetic dyes are used in the food industry due to their low cost, wide range of colors, resistance to technological processing, color stability to oxidation, and different pH values. However, synthetic dyes cause serious disorders in the

human body since the half-life products of artificial dyes are carcinogenic, mutagenic, and teratogenic. In addition, the widespread use of synthetic dyes has a significant negative impact on the environment since their production generates wastewater that enters the environment without proper treatment [2]. Natural dyes are characterized by high cost, less resistance to high temperatures, and a limited palette of colors, but they contain biologically active substances useful for the body and are an alternative to synthetic analogs. Given their properties, it is natural dyes that dominate the market due to the development of the global trend for healthy eating since for most consumers it is very important that products do not contain a high content of sugar, artificial flavors, and dyes [3, 4].

Research on berry pomace as a source of natural dyes and a safe alternative to synthetic analogs is an urgent task. The use of polyols as substitutes for toxic organic solvents helps increase extraction efficiency, extract stability, and expand their application in the production of natural dyes and functional additives for the food industry.

2. Literature review and problem statement

In [5], the results of studies on the extraction of anthocyanins from chokeberry and black elderberry (*Sambucus nigra L.*) fruits using glycerol as an extractant were reported. It was shown that glycerol, due to its lower polarity compared to traditional solvents such as ethanol, increases the extraction efficiency. The highest concentration of anthocyanins was achieved in a system with 50% glycerol at temperatures of 20°C and 50°C, while 65% glycerol was optimal at 80°C. Water-glycerol mixtures were proposed as a safe alternative to ethanol, taking into account its toxicity and flammability, which complicates the technological process [6]. However, issues related to the justification of optimal extraction conditions and the stability of the obtained extracts remained unresolved. The reason for this limitation may be objective difficulties associated with the high concentration of glycerol (over 35%), which makes it difficult to use in standard extraction systems [7]. In addition, there is a fundamental impossibility of avoiding the degradation of anthocyanins during prolonged extraction. For example, at a temperature of 80°C for more than 150 minutes, which leads to the destruction of pigments and the formation of degradation products [8].

An option to overcome these difficulties may be the use of deep eutectic solvents based on polyols, such as xylitol and sorbitol, in combination with choline chloride, which are non-toxic, biodegradable, and environmentally friendly. Polyols, in particular glycerol ($C_3H_5(OH)_3$), xylitol ($C_5H_7(OH)_5$), and sorbitol ($C_6H_8(OH)_6$), are part of eutectic and "green" solvents. They are used to extract dyes from plant materials and do not require removal from the extract due to the formation of hydrogen bonds between the hydroxyl groups of polyols and pigments [9, 10]. This approach was used in [11], which investigated the extraction of anthocyanins using deep eutectic solvents. The study found that xylitol and sorbitol in eutectic solvents are more effective than ethanol and contribute to reducing the degradation of anthocyanins. Moreover, the highest concentration of anthocyanins was achieved in systems with a ratio of choline chloride:xylitol (5:2) and choline chloride:sorbitol (1:1) using ultrasound treatment. However, in [11], the issues of substantiation of extraction conditions, stability of extracts, and the possibility of using xylitol and sorbitol separately, without choline chloride, remain unresolved.

It is worth noting that the natural source of anthocyanins is plant raw materials, in particular black elderberry fruits (*Sambucus nigra* L.), which are characterized by a high concentration of anthocyanins and other polyphenols. In particular, elderberry fruits contain cyanidin glycosides, which have pronounced antioxidant properties and significant potential as natural dyes [12, 13]. Organic solvents (ethanol, methanol, acetone, etc.) and inorganic solvents, in particular water, are traditionally used to extract pigments. However, most organic solvents are dangerous to the human body, and water has a low efficiency of pigment extraction and contributes to the instability of extracts due to oxidation [14]. During the processing of berry raw materials, pomace (skin, seeds, pulp) is formed, which make up 20–40% of the mass of the raw material and is a valuable source of polyphenols, but is currently underused in industry [15, 16].

All this gives grounds to argue that it is advisable to study the development of optimal conditions for the extraction of anthocyanins using polyols as independent extractants. It is also necessary to compare their efficiency with water and ethanol and assess the stability of extracts from black elderberry fruit pomace for further practical application.

3. The aim and objectives of the study

The aim of our study is to determine the choice of the optimal solvent for the extraction of coloring substances from the pomace of black elderberry (*Sambucus nigra* L.), as well as to determine the effect of solvents on the extraction capacity, stability, and persistence of extracts. The results will become the basis for the commercial production of natural dyes from pomace of berry raw materials, including black elderberry.

To achieve the goal, the following tasks were set:

– to investigate the quality indicators of black elderberry fruits and pomace;

– to analyze the infrared spectra of extracts from black elderberry pomace by FTIR IR spectroscopy in the range of 4000–500 cm^{-1};

– to investigate the quality indicators of extracts of black elderberry pomace (*Sambucus nigra* L.);

– to investigate the microstructure of black elderberry (*Sambucus nigra* L.) fruit extracts by transmission electron microscopy.

4. The study materials and methods

4. 1. The object and hypothesis of the study

The object of our study is the extracts from black elderberry fruit pomace obtained using various polyols (glycerol, xylitol, sorbitol), as well as their ability to extract anthocyanins, stability, persistence, and spectral characteristics.

The hypothesis of the study is as follows. Black elderberry pomace (*Sambucus nigra* L.) contains a significant amount of phenolic compounds, in particular anthocyanins and flavonoid glycosides. The use of polyols, as an alternative to replacing conventional solvents, could allow for effective extraction of coloring substances and ensure their stability during storage. The study used a number of assumptions and simplifications, i.e., model conditions of laboratory ex-

traction, fixed temperature and time parameters, as well as a limited amount of raw materials and extracts. The results could be used for further development of more rational approaches to processing plant raw materials and optimization of processes to the principles of circular economy. Therefore, such results could become the basis for the development and implementation of a separate production line for natural dyes based on secondary plant raw materials.

4. 2. Materials

Ripe black elderberry fruits (*Sambucus nigra* L.), collected in the Sumy oblast, Ukraine, during the growing season of 2023, were washed with running water, after which the juice was squeezed, and the resulting pulp was used for further preparation of extracts. To obtain extracts (Fig. 1) from the pulp of black elderberry fruits (*Sambucus nigra* L.), aqueous solutions of ethyl alcohol, glycerin, xylitol, and sorbitol were used.

Fig. 1. Pasteurized extracts from black elderberry pomace (*Sambucus nigra* L.)

Fresh black elderberry (*Sambucus nigra* L.) pomace, weighing 50 g, was mixed with potassium sorbate at a concentration of 2 g per 1000 g of pomace and subjected to extraction in an aqueous solution (control) with a 1:1 hydromodule with the addition of 0.5 g of citric acid. After the completion of the first stage of extraction, the extract was settled and filtered through a sieve with holes with a diameter of 0.35 mm. After that, the pomace was re-filled with water with a 1:1 hydromodule and 0.5 g of citric acid was added.

A second extraction was carried out at a temperature of 65 ± 0.5°C for 1 hour with periodic stirring. After extraction, the mass was settled again and filtered through a sieve with a diameter of 0.35 mm. The resulting extract was poured into a glass container with subsequent pasteurization at a temperature of 75 ± 0.5°C for 20 minutes.

Extraction by a similar methodology was carried out in water-glycerol, water-ethyl, water-xylitol, and water-sorbitol solutions, changing only the extractant component. In the water-glycerol solution, the mass fraction of glycerol was 10% of the total mass of the solution, in the water-ethyl solution – 10% ethyl alcohol, in the water-xylitol solution – 10% xylitol, and in the water-sorbitol – 10% sorbitol. All extraction stages for each solution were carried out under the same conditions: temperature 65 ± 0.5°C, duration 1 hour. After that, periodic mixing, settling, filtering, pouring, and pasteurization at a temperature of 75 ± 0.5°C were carried out.

4. 3. Research on the quality indicators of fruits and pomace of black elderberry (*Sambucus nigra* L.) fruits.

The active acidity of black elderberry (*Sambucus nigra* L.) pomace was determined using a portable pH meter in accordance with the national standard of Ukraine DSTU 6045:2008 (equivalent – ISO 10523:2012). Titrated acidity was determined by the potentiometric method in accordance with DSTU 4957:2008 (corresponding to ISO 750:1999). The content of soluble solids was determined by the refractometric method in accordance with DSTU 8402:2015 (equivalent – AOAC 932.12). The mass fraction of moisture of the raw material was determined by the arbitration method in accordance with DSTU 7804:2015 (equivalent – ISO 1442:1997).

4. 4. Study of infrared spectra of black elderberry fruit extracts (*Sambucus nigra* L.) by Fourier transform spectroscopy (FTIR)

Spectral analysis by Fourier transform infrared spectroscopy (FTIR) was performed using a spectrophotometer (Nicolet IS20, Thermo Fisher Scientific, WI, USA) equipped with an ATR accessory with a diamond crystal. The study was carried out using OMNIC™ v8.2 software in combination with FTIR-ATR technology in the spectral range of wavenumbers 4000–500 cm^{-1}. Each sample was analyzed three times, performing 32 scans under the adsorption mode with a resolution of 4 cm^{-1}, which ensured reproducibility and uniformity of the results.

4. 5. Study of the microstructure of black elderberry fruit extracts (*Sambucus nigra* L.) by transmission electron microscopy

The microstructure and phase composition of black elderberry fruit pomace extracts (*Sambucus nigra* L.) were studied using a TEM-125K electron microscope. Copper microscopic grids with a diameter of 3 mm and cell sizes of 50×50 μm were used as a substrate for the samples. An amorphous carbon film transparent to the electron beam, 50 nm thick, was previously applied to the grids. The extract sample was applied to the grid-substrate by immersing it in a liquid, after which the grid was placed on filter paper to remove excess moisture. The pre-drying process lasted 5 min, after which the holder with the sample was placed in the sluice vacuum chamber of the TEM-125K microscope for final evaporation of the residual liquid under vacuum conditions. Images of the sample structure were obtained under the "bright field" mode using a transmission electron microscope PEM-125K. The electron beam parameters included an acceleration voltage of 100 kV and a beam diameter of 0.5 μm. Image visualization and analysis were performed using a digital processing system integrated with a GT2750 camera for a transmission electron microscope PEM-125K.

4. 6. Research into the quality indicators of black elderberry (*Sambucus nigra* L.) pomace extracts

The active acidity of black elderberry (*Sambucus nigra* L.) pomace extracts was determined using a portable pH meter in accordance with the national standard of Ukraine DSTU 6045:2008 (equivalent – ISO 10523:2012). Titrated acidity was determined by the potentiometric method in accordance with DSTU 4957:2008 (corresponding to ISO 750:1999). The content of soluble solids was determined by the refractometric method in accordance with DSTU 8402:2015 (equivalent – AOAC 932.12). The mass fraction of moisture of the raw material was determined by the arbitration method in accordance with DSTU 7804:2015 (equivalent – ISO 1442:1997).

5. Results of determining the quality indicators of black elderberry pomace and extracts (*Sambucus nigra* L.)

5. 1. Results of determining the quality indicators of black elderberry pomace and fruits (*Sambucus nigra* L.)

At the first stage of the study, the quality of black elderberry fruits, juice, and pomace was analyzed. The results are given in Table 1.

Table 1

Results based on the quality indicators of fresh fruits, juice, and black elderberry extracts (*Sambucus nigra* L.)

Indicator ID	Fresh pomace	Juice	Fresh berries	HIP_{05}
Mass fraction of dry soluble substances,%	$^{c}10.80 \pm 0.2$	$^{a}18.2 \pm 0.2$	$^{b}17.00 \pm 0.2$	0.453
Mass fraction of moisture,%	$^{b}49.3 \pm 0.2$	–	$^{a}70.2 \pm 0.2$	0.453
Active acidity (pH)	$^{a}4.3 \pm 0.1$	$^{a}4.5 \pm 0.1$	$^{a}4.4 \pm 0.1$	0.227
Titrated acidity (%) in terms of malic acid	$^{a}0.73 \pm 0.03$	$^{b}0.47 \pm 0.03$	$^{b}0.53 \pm 0.03$	0.068
Titrated acidity (%) in terms of citric acid	$^{a}0.77 \pm 0.03$	$^{c}0.49 \pm 0.02$	$^{b}0.55 \pm 0.03$	0.057
Titrated acidity (%) in terms of tartaric acid	$^{a}0.82 \pm 0.03$	$^{c}0.52 \pm 0.02$	$^{b}0.59 \pm 0.03$	0.057
Mass fraction of anthocyanins in the raw material in terms of cyanidin-3-O-glycoside,%	$^{a}0.42 \pm 0.02$	$^{c}0.23 \pm 0.02$	$^{b}0.29 \pm 0.02$	0.045
Mass fraction of flavonoid glycosides in the raw material (%) in terms of rutin	$^{a}1.52 \pm 0.03$	$^{c}1.07 \pm 0.02$	$^{b}1.35 \pm 0.02$	0.057
Mass fraction of flavonoid glycosides in the raw material (%) in terms of quercetin	$^{a}0.26 \pm 0.01$	$^{b}0.19 \pm 0.02$	$^{a}0.24 \pm 0.01$	0.034
Mass fraction of flavonoid glycosides in the raw material (%) in terms of luteolin	$^{a}2.77 \pm 0.04$	$^{c}1.97 \pm 0.02$	$^{b}2.49 \pm 0.03$	0.068

The results of analysis showed that black elderberry (Sambucus nigra L.) pomace is a source of a significant amount of biologically active compounds. In particular, they are characterized by an increased content of anthocyanins ($0.42 \pm 0.02\%$), which exceeds their concentration in both fresh fruits ($0.29 \pm 0.02\%$) and juice ($0.23 \pm 0.02\%$). In addition, the maximum concentration of flavonoid glycosides was recorded in pomace (in terms of rutin – $1.52 \pm 0.03\%$, quercetin – $0.26 \pm 0.01\%$, luteolin – $2.77 \pm 0.04\%$). The titrated acidity in pomace turned out to be the highest compared to other samples, which may affect their functional properties (0.73–0.82% depending on the type of acid). Despite the lower mass fraction of dry soluble substances ($10.80 \pm 0.2\%$), compared to fresh berries ($17.0 \pm 0.2\%$) and juice ($18.2 \pm 0.2\%$), black elderberry pomace remains a valuable raw material for obtaining extracts enriched with biologically active components.

5. 2. Results of IR spectra of black elderberry fruit extracts by Fourier transform spectroscopy (FTIR)

Table 2 gives data on the assignment of bands of the studied (IR) spectra for the following samples: sample 1 (control); sample 2 (solvent – ethyl alcohol); sample 3 (solvent – glycerin); sample 4 (solvent – sorbitol); sample 5 (solvent – xylitol).

Table 2 shows the frequency positions of the main bands of the IR spectra.

For all samples (Fig. 2), a broad blurred band is observed in the range of 3500–3200 cm^{-1}, which is due to the increase in the number of bound OH groups and adsorption-bound water, on which the bands of stretching vibrations of the –CH– and $–CH_3–$ bonds in the range of 2960–2850 cm^{-1} are superimposed. In this case, the addition of polyhydric alcohols (glycerol, xylitol, sorbitol) increases the intensity of this band compared to aqueous and ethanol extracts (samples 1 and 2, respectively), reducing the dehydration of the obtained extract due to the strengthening of hydrogen bonds.

Analysis of the spectra in the wavenumber range of 2000...3000 cm^{-1} (Fig. 3) revealed the presence of two characteristic peaks at 2940 cm^{-1} and 2875 cm^{-1} for extracts with polyhydric alcohols (samples 3–5). These peaks reflect the stretching vibrations of C–H in methyl ($–CH_3$) and methylene ($–CH_2–$) groups, which can be associated with aglycones of phenolic compounds, such as anthocyanins, present in the fruits of black elderberry (*Sambucus nigra* L.). The range of stretching vibrations of C–H (2960–2850 cm^{-1}) partially overlaps with this region, while the stretching vibrations of O–H of phenolic hydroxyls are characteristic of the higher range of 3500–3200 cm^{-1}. At the same time, the absence of pronounced peaks at 2940 cm^{-1} and 2875 cm^{-1} in aqueous and ethanol extracts (samples 1 and 2) may indicate less efficient extraction of lipophilic components.

Table 2

Frequency positions (cm^{-1}) of the main bands of the studied IR absorption spectra

Wavenumber, cm^{-1}	Intensity	Band assignment
1	2	3
Sample 1 (black elderberry pomace - water - citric acid)		
3260	weak	O-H jack oscillations in intermolecular hydrogen bonds (3310–3279 cm^{-1}); adsorption-bound water (valence oscillations); 3500–3200 cm^{-1}
2945	weak	-CH-CH_3- (valence oscillations); 2960–2850 cm^{-1}
1610	average	NH_3^+ amino acid band 1; 1300–1400 cm^{-1}
1400	average	-C-O- (symmetrical valence oscillations), carboxylate ion; 1300–1400 cm^{-1}
1250	average	symmetric and asymmetric fluctuations of valence bonds C-O; 1280–1100 cm^{-1}

Continuation of Table 1

1	2	3
1050	average	deformation oscillations of aromatic (C-H) bonds; 900–650 cm^{-1}
975	average	
820	average	
775	average	
Sample 2 (black elderberry pomace - water - citric acid - ethanol)		
3350	weak	O-H jack oscillations in intermolecular hydrogen bonds (3310–3279 cm^{-1}); adsorption-bound water (valence oscillations); 3500–3200 cm^{-1}
2940	weak	-CH-CH_3- (valence oscillations); 2960–2850 cm^{-1}
2370	weak	C≡C (valence oscillations); 2500–2000 cm^{-1}
1725	average	-CH-CH_3- (valence oscillations of carbonyl groups); 2000–1600 cm^{-1}
1625	weak	NH_3^+ amino acid band 1; 1660–1610 cm^{-1}
1400	weak	-C-O- (symmetrical jack oscillations), carboxylate ion; 1300–1400 cm^{-1}
1330	weak	
1225	average	symmetric and asymmetric oscillations of valence bonds C-O; 1280–1100 cm^{-1}
1060	average	deformation oscillations of aromatic (C-H) bonds; 900–650 cm^{-1}
825	average	
775	average	
Sample 3 (black elderberry pomace - water - citric acid - glycerin)		
3300	average	O-H jack oscillations in intermolecular hydrogen bonds (3310–3279 cm^{-1}); adsorption-bound water (valence oscillations); 3500–3200 cm^{-1}
2950	weak	-CH-CH_3- (valence oscillations); 2960–2850 cm^{-1}
2855	weak	
1725	average	C≡C (valence oscillations); 2000–1600 cm^{-1}
1630	weak	NH_3^+ amino acid band; 1660–1610 cm^{-1}
1425	weak	-C-O- (symmetrical oscillations), carboxylate ion; 1300–1400 cm^{-1}
1325	weak	
1210	average	symmetric and asymmetric oscillations of valence bonds C-O; 1280–1100 cm^{-1}
1060	weak	deformation oscillations of aromatic (C-H) bonds; 900–650 cm^{-1}
925	weak	
850	weak	
Sample 4 (black elderberry pomace - water - citric acid - sorbitol)		
3300	average	O-H jack oscillations in intermolecular hydrogen bonds (3310–3279 cm^{-1}); adsorption-bound water (valence oscillations); 3500–3200 cm^{-1}
2940	weak	-CH-CH_3- (valence oscillations); 2960–2850 cm^{-1}
2875	weak	
1725	average	C≡C (valence oscillations); 2000–1600 cm^{-1}
1640	weak	NH_3^+ amino acid band; 1660–1610 cm^{-1}
1435	weak	-C-O- (symmetrical oscillations), carboxylate ion; 1300–1400 cm^{-1}
1325	weak	
1210	weak	symmetric and asymmetric oscillations of valence bonds C-O; 1280–1100 cm^{-1}
1080	average	deformation oscillations of aromatic (C-H) bonds; 900–650 cm^{-1}
1030	average	
930	weak	
875	weak	
Sample 5 (black elderberry pomace - water - citric acid - xylitol)		
3300	average	valence O-H oscillations in intermolecular hydrogen bonds (3310–3279 cm^{-1}); adsorption-bound water (valence oscillations); 3500–3200 cm^{-1}
2940	weak	-CH-CH_3- (valence oscillations); 2960–2850 cm^{-1}
2875	weak	
1725	weak	C≡C (valence oscillations); 2000–1600 cm^{-1}
1640	weak	NH_3^+ amino acid band; 1660–1610 cm^{-1}
1425	weak	-C-O- (symmetrical oscillations), carboxylate ion; 1300–1400 cm^{-1}
1330	weak	
1220	weak	symmetric and asymmetric oscillations of valence bonds C-O; 1280–1100 cm^{-1}
1075	average	deformation oscillations of aromatic (C-H) bonds; 900–650 cm^{-1}
1025	average	
875	weak	
750	weak	

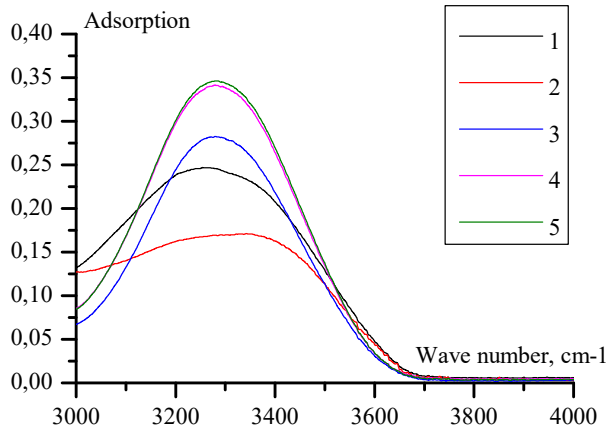


Fig. 2. IR spectra of extracts in the range of 3000–4000 cm^{-1} (samples 1–5)

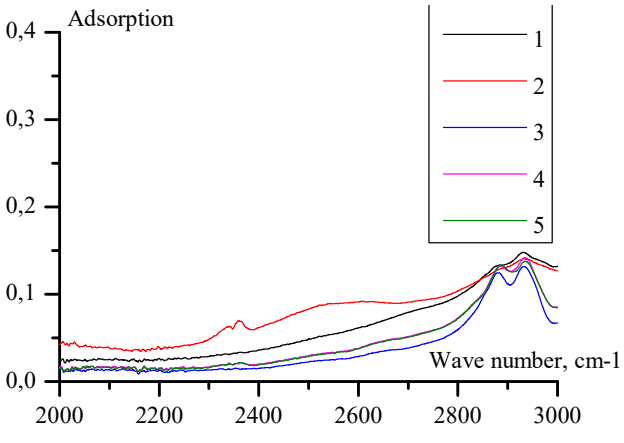


Fig. 3. IR spectra of extracts in the range of 2000–3000 cm^{-1} (samples 1–5)

In all the studied IR spectra (Fig. 4) in the range of wave numbers 1000–2000 cm^{-1}, characteristic bands for extracts from black elderberry pomace (*Sambucus nigra* L.) were detected. These bands are located at frequencies 1050, 1075, 1256, 1220, 1330, 1425, and 1640 cm^{-1} and correspond to vibrations of bonds characteristic of cellulose molecules, fats, proteins, carbohydrates, carboxylic acids, esters, phenols, furans, and phosphate groups, respectively. In the range of wave numbers from 1800 to 1300 cm^{-1}, bands of stretching vibrations of the carbonyl group C = O, alkenes, and aromatic compounds C = C appear in the (IR) spectra. These bands are sharp: C = O is intense near 1725–1730 cm^{-1}, and C = C is weak near 1640 cm^{-1}. This may indicate the presence of characteristic absorption bands of anthocyanins near 1725 cm^{-1} and 1625–1640 cm^{-1} in the spectra of all alcohol extracts, due to double bonds of the benzene nucleus. At the same time, the aqueous extract does not have an absorption band at 1725 cm^{-1}, which confirms the higher extraction capacity of alcohol solvents compared to aqueous ones. In addition, for the sample with glycerin, a shift of the characteristic peak inherent to polyhydric alcohols from 1640 cm^{-1} to a lower wave number – 1630 cm^{-1} is observed.

This indicates a stronger electrostatic interaction of the aromatic ring of anthocyanins with the environment, which leads to its expansion. This is also confirmed by the analysis of the band at 1027–1049 cm^{-1}, which corresponds to the vibrations of the C–H groups of the aromatic ring of anthocyanins. The highest intensity of this peak is observed in the extract obtained with the addition of glycerol.

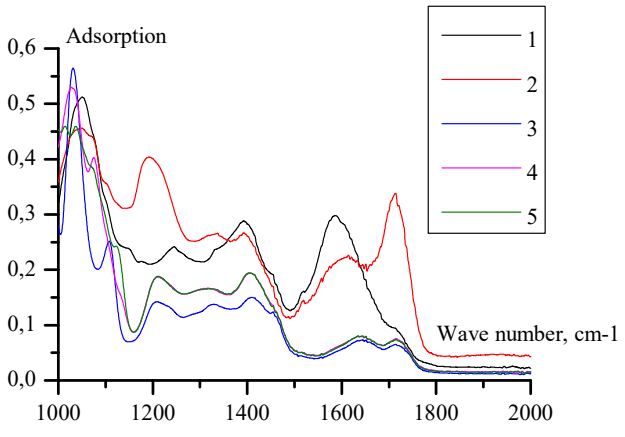


Fig. 4. IR spectra of extracts in the range of 1000–2000 cm^{-1} (samples 1–5)

In all the studied IR spectra (Fig. 5) in the range of wave numbers 500–1000 cm^{-1}, deformation vibrations of aromatic (C–H) bonds in the range 900–650 cm^{-1} were detected, which may be due to the presence of phenolic compounds, in particular anthocyanins, in the extracts. From the analysis of Fig. 5 it is seen that only for the sample with glycerol a characteristic double peak is observed at 925 cm^{-1} and 850 cm^{-1}. These peaks correspond to deformation vibrations of aromatic C–H bonds of the benzene ring of anthocyanins or their interaction with glycerol, and not to stretching vibrations of double bonds.

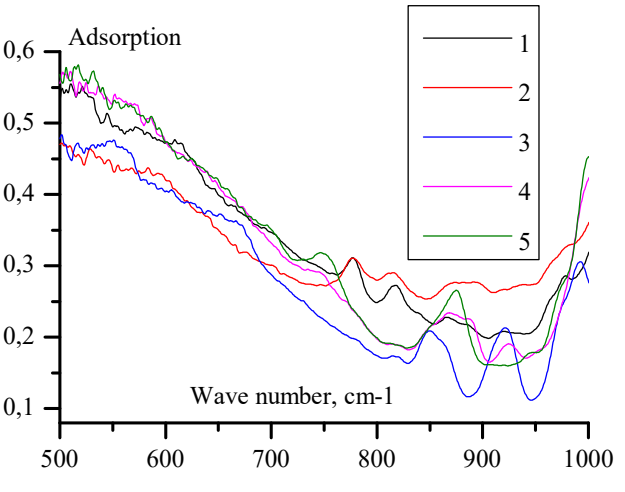


Fig. 5. IR spectra of extracts in the range of 500–1000 cm^{-1} (samples 1–5)

This trend may indicate the stabilizing effect of glycerin, which enhances the interaction with the aromatic structures of anthocyanins, contributing to their stabilization in the extract. Therefore, glycerin provides stabilization of anthocyanins, which increases the efficiency of extraction and contributes to their preservation.

5. 3. Results related to the quality indicators of black elderberry fruit extracts (*Sambucus nigra* L.)

The next stage of the study was to determine the quality indicators of black elderberry fruit pomace extracts (*Sambucus nigra* L.) obtained using aqueous solutions of glycerin, sorbitol, xylitol, and ethanol. The results of the study are given in Table 3.

Table 3

Results related to the quality indicators of black elderberry fruit extracts (*Sambucus nigra* L.)

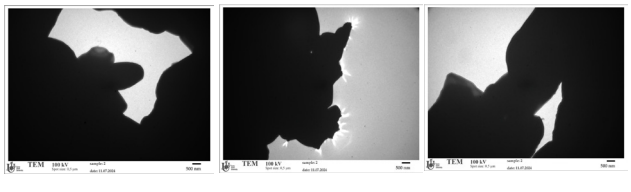
Indicator ID	Extracts of black elderberry fruit (*Sambucus nigra* L.)					HIP_{05}
	Control	Water-xylitol	Water-sorbitol	Water-glycerin	Water-ethyl	
Mass fraction of dry solutes,%	$^{a}6.5 \pm 0.1$	$^{b}12.7 \pm 0.1$	$^{b}12.5 \pm 0.1$	$^{c}12.1 \pm 0.1$	$^{b}12.7 \pm 0.1$	0.227
Organoleptic indicators	The liquid is dark red in color, with a slight odor inherent in the corresponding type of raw material with a sour taste					
Active acidity (pH)	$^{b}3.5 \pm 0.1$	$^{a}3.2 \pm 0.1$	$^{a}3.2 \pm 0.1$	$^{a}3.3 \pm 0.1$	$^{b}3.5 \pm 0.1$	0.227
Titrated acidity (%) in terms of citric acid	$^{a}0.34 \pm 0.05$	$^{a}0.43 \pm 0.05$	$^{a}0.42 \pm 0.05$	$^{a}0.35 \pm 0.05$	$^{a}0.32 \pm 0.05$	0.113
Titrated acidity (%) in terms of malic acid	$^{a}0.32 \pm 0.05$	$^{a}0.42 \pm 0.05$	$^{a}0.40 \pm 0.05$	$^{a}0.34 \pm 0.05$	$^{a}0.30 \pm 0.05$	0.113
Titrated acidity (%) in terms of tartaric acid	$^{a}0.36 \pm 0.05$	$^{a}0.47 \pm 0.05$	$^{a}0.45 \pm 0.05$	$^{a}0.38 \pm 0.05$	$^{a}0.34 \pm 0.05$	0.113
Density at 20°C, kg/m^3	$^{a}1001 \pm 2$	$^{d}1061 \pm 2$	$^{c}1038 \pm 2$	$^{b}1028 \pm 2$	$^{a}1000 \pm 2$	4.533
Concentration of dyes, g/dm^3	$^{a}37.6 \pm 0.3$	$^{b}43.3 \pm 0.3$	$^{c}52.6 \pm 0.3$	$^{d}57.4 \pm 0.3$	$^{e}58.3 \pm 0.3$	0.680
Solubility in water/hydroalcoholic solutions	soluble/soluble					

Our results confirm the effectiveness of water-xylitol, water-sorbitol, and water-glycerol extracts as alternatives to water-ethyl for the extraction of anthocyanins. The mass fraction of dry soluble substances in the control sample is 6.5% – the lowest indicator, while in water-ethyl and water-xylitol extracts it reaches a maximum (12.7%). The active acidity (pH) of the extracts varies within 3.2–3.5, with the lowest values (3.2) in water-xylitol and water-sorbitol extracts. The titrated acidity is the highest in water-xylitol extract (0.43–0.47% in terms of citric, malic, tartaric acids), which indicates a higher content of organic acids.

The density of the extracts depends on the solvent: the highest in water-xylitol (1061 kg/m^3), the lowest in water-ethyl (1000 kg/m^3), which reflects the influence of molar mass and concentration of dissolved substances. The concentration of coloring substances is the lowest in the control sample (37.6 g/dm^3) and the maximum in water-ethyl (58.3 g/dm^3) and water-glycerol (57.4 g/dm^3) extracts. This confirms the high extraction efficiency of the indicated solvents for anthocyanins, which is explained by their polarity and ability to stabilize pigments.

5. 4. Results related to the microstructure of black elderberry (*Sambucus nigra* L.) fruit extracts based on transmission electron microscopy

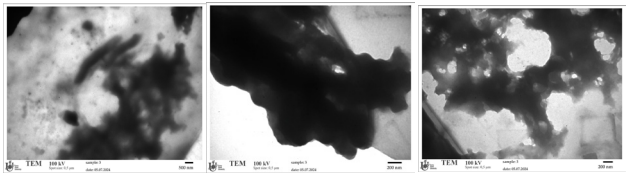
The study of the microstructure of black elderberry (*Sambucus nigra* L.) pomace extracts was carried out by transmission electron microscopy (Fig. 6–10).



a *b* *c*

Fig. 6. Microstructure of black elderberry fruit extract (control sample): *a* – overview of the general structure; *b* – detail of surface particles; *c* – side view of the structure

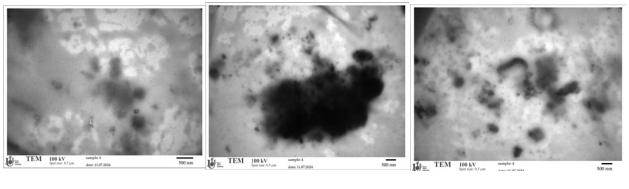
Sample 1 (control) is characterized by the presence of amorphous formations of irregular shape with sizes from 1 to 10 microns. The sample also contains agglomerations of relatively large opaque particles of regular elongated shape, with average sizes of 0.5×5 microns, as well as crystallites and crystallization nuclei with sizes from 50 to 200 nm. The sample demonstrates the stability of its structure.



a *b* *c*

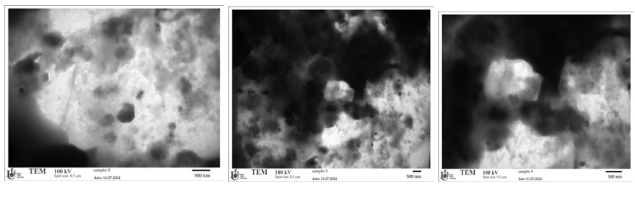
Fig. 7. Microstructure of black elderberry fruit extract (sample 2): *a* – overview of the general structure; *b* – detail of surface particles; *c* – side view of the structure

Sample 2 is characterized by the presence of amorphous formations of irregular shape, in the structure of which agglomerations of crystallites and crystallization nuclei with a size of 50 to 200 nm are observed. The sample demonstrates the stability of its structure.



a *b* *c*

Fig. 8. Microstructure of black elderberry fruit extract (sample 3): *a* – overview of the general structure; *b* – detail of surface particles; *c* – side view of the structure



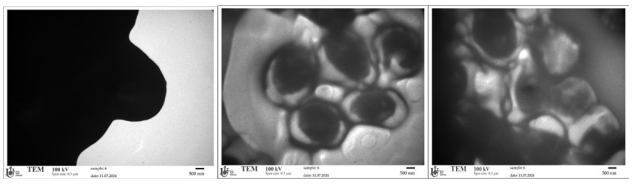
a *b* *c*

Fig. 9. Microstructure of black elderberry fruit extract (sample 4): a – overview of the overall structure; b – detail of surface particles; c – side view of the structure

Sample 3 is characterized by the presence of amorphous formations of irregular shape, which cover almost the entire

surface of the substrate grid. In their structure, clusters of crystallites with a size of 50 to 300 nm were identified. The sample demonstrates the stability of its structure.

Sample 4 is characterized by the presence of amorphous formations of irregular shape, which cover almost the entire surface of the substrate grid. Part of the amorphous substance is opaque to the electron beam and exhibits instability: under the influence of the electron beam (possibly as a result of slight heating in the range of 20–60°C) bubbles are formed in the substance, which demonstrate movement in its volume. The sample also identified clusters of crystallization nuclei with sizes from 50 to 300 nm.



a *b* *c*

Fig. 10. Microstructure of black elderberry fruit extract (sample 5): *a* — overview of the general structure; *b* — detail of surface particles; *c* — side view of the structure

Sample 5 is characterized by the presence of amorphous formations of irregular shape. Part of the amorphous substance is opaque to electron exchange. In places where the substance is exposed to the electron beam, it turns out to be unstable: under its action (probably due to slight heating by 20–60°C) in the substance there are bubbles that move intensively in its volume. In addition, objects located on the surface of the biological matrix of the substance are observed in the sample; these objects are stable and are characterized by low contrast in the electronic image.

Thus, the microstructural analysis of extracts of black elderberry fruit pomace (*Sambucus nigra* L.) revealed that the choice of extractant affects the stability and morphology of coloring substances. Aqueous and water-ethyl extracts are characterized by the presence of amorphous formations, in the structure of which agglomerations of crystals with sizes of 50 to 200 nm were identified. The water-glycerol extract forms dense amorphous shells with crystalline clusters ranging in size from 50 to 300 nm, which helps retain anthocyanins, slowing their release and oxidation. The water-sorbitol and water-xylitol samples exhibit instability, which is manifested in the formation of bubbles and structures similar to cells of biological origin. The high viscosity and hydrophilicity of glycerol provides a stable environment for the preservation of pigments.

6. Discussion of results based on investigating the feasibility of using polyols as extractants for the extraction of coloring substances

Pomace, as a secondary raw material, is characterized by an increased content of phenolic compounds, which are concentrated in the peel and pulp after squeezing juice or puree [15, 16]. Pomace of berries constitutes 20–40% of the total mass of raw materials, which is a valuable source of polyphenols. In contrast to the study [17], in which whole fruits are used to obtain anthocyanins, it is advisable to use processing waste (pomace) to obtain natural dyes with a higher concentration of coloring substances. A limitation of our study is the dependence of the content of biologically active substances of plant raw materials on external factors (growing conditions, processing), which must be taken into account in practice, as well as in further studies. According to the results (Table 1), the maximum concentration of flavonoid glycosides was recorded in black elderberry extracts (in terms of rutin – 1.52 ± 0.03%, quercetin – 0.26 ± 0.01%, luteolin – 2.77 ± 0.04%), which confirms their potential for use.

Glycerin is a polyol that is part of triglycerides, fatty acid esters. It is able to dissolve many organic compounds due to its properties [14]. However, its use is limited by high viscosity and low solubility of hydrophobic compounds. Glycerin is used as a solvent, cosolvent, component of deep eutectic mixtures or simultaneously as a solvent and reagent [14]. The data in Table 2 show that the IR spectra of the samples are classical with high resolution of absorption bands. They have a flat baseline, which indicates the chemical stability of the samples. This also indicates the absence of colloidal systems in the extracts. The spectra are total, with partial overlap of the absorption bands of functional groups [18]. The broadening of the peaks indicates the presence of residual moisture in the samples. According to the literature [19, 20], water has absorption bands: asymmetric (3600 cm^{-1}), symmetric (3450 cm^{-1}), overtones (3290 cm^{-1}). Also, characteristic are deformation (1645 cm^{-1}), torsional (780 cm^{-1}), and total vibrations (2150 cm^{-1}). These bands overlap with the bands of raw materials and solvents, complicating the analysis of the spectrum. The data confirm the higher stability of extracts with polyhydric alcohols [21–23]. Thus, glycerol as a solvent and humectant contributes to the stabilization of anthocyanins in the extract. This increases the resistance of the extract to degradation [23–26].

According to Table 3, the content of coloring substances in the extracts varies. The lowest indicator in the control extract is 37.6 g/dm^3. The highest is in glycerin (57.4 g/dm^3) and ethanol (58.3 g/dm^3). This indicates the effectiveness of water-glycerol mixtures. Water and water-ethyl extracts have amorphous formations with a size of 50–200 nm. Water-glycerol extract forms dense amorphous shells with a size of 50–300 nm. Such structures retain anthocyanins, slowing down their oxidation (Fig. 8–10). An increase in the proportion of glycerin over 35%, xylitol and sorbitol – 10% reduces the extraction capacity. This is due to an increase in viscosity, which worsens diffusion in the "raw material-solvent" system.

According to the literature [14], the use of glycerin as an extractant for dyes is effective. In study [5], the optimal conditions for extraction were determined to be 50% glycerin at temperatures of 20–50°C and 65% at 80°C. In our work, it was found that the extraction temperature of 65 ± 1.5°C ensures the stability of anthocyanins even at lower concentrations of glycerin (up to 35%), which partially solves the problem associated with the high viscosity of glycerin [7]. Also, in study [5], it is indicated that extraction at 80°C ensures the maximum extraction of dyes. However, we found [8] that prolonged extraction at a temperature of 80°C (more than 150 minutes) leads to the degradation of anthocyanins. These results also indicate the need for additional studies, in particular on the mechanisms of thermal stability of anthocyanins.

In [11], deep eutectic solvents with xylitol, sorbitol, and choline chloride outperform ethanol. They contribute to reducing anthocyanin degradation [11]. In our study, water-xy-

litol and water-sorbitol extracts are less stable due to high hydrophilicity. However, we investigated their use without choline chloride, which was not studied in [11]. The concentration of coloring substances in the water-xylitol extract is 43.3 g/dm^3, in the water-sorbitol extract is 52.6 g/dm^3. This is lower than in the glycerin extract, but higher than in the control (37.6 g/dm^3). Such results indicate the feasibility of their use as separate extractants. To increase stability, additional optimization of extraction conditions is required.

The practical significance of our study relates to the use of polyols, in particular glycerol ($C_3H_5(OH)_3$), xylitol ($C_5H_7(OH)_5$), and sorbitol ($C_6H_8(OH)_6$), as extractants for obtaining coloring substances from black elderberry fruit pomace. The advantage of this method is the use of safe solvents – glycerol, sorbitol, and xylitol.

The limitations of the study are due to the physicochemical properties of polyols. In particular, at a concentration of glycerol above 35%, as well as sorbitol above 10%, a significant increase in viscosity is observed, which contributes to the slowdown of diffusion processes and a decrease in the efficiency of mass transfer. Further research could aim at improving the technology for obtaining natural dyes and finding ways to increase the stability of anthocyanins during heat treatment and storage.

7. Conclusions

1. We have found that black elderberry extracts are characterized by an increased content of anthocyanins (0.42 ± 0.02%), which exceeds their concentration in fresh fruits (0.29 ± 0.02%), as well as in juice (0.23 ± 0.02%). In addition, the maximum concentration of flavonoid glycosides was recorded in the extracts (in terms of rutin – 1.52 ± 0.03%, quercetin – 0.26 ± 0.01%, luteolin – 2.77 ± 0.04%).

2. Spectral analysis confirmed intense absorption bands of anthocyanins (1625–1725 cm^{-1}) in glycerin extracts. The high extraction capacity of water-ethyl and glycerin solvents is due to their polarity, electrostatic interaction with anthocyanins, and stabilizing properties of glycerin.

3. It was found that water-xylitol, water-sorbitol, and water-glycerol extracts are an effective alternative to water-ethyl in the extraction of anthocyanins. The highest dry matter content (12.7%) was observed in water-ethyl and water-xylitol extracts, the lowest in the control (6.5%). The water-xylitol extract had the highest titrated acidity (up to 0.47%) and density (1061 kg/m^3). The maximum concentration of anthocyanins was found in water-ethyl (58.3 g/dm^3) and water-glycerol (57.4 g/dm^3) extracts. Our results confirm the feasibility of using polyols as safe, non-toxic, and effective solvents for extracting dyes from plant raw materials, in particular black elderberry, which is promising for the food industry.

4. It has been established that the choice of extractant affects the stability and morphology of colorants. The water-glycerol extract forms dense amorphous shells with crystalline clusters ranging in size from 50 to 300 nm, which helps retain anthocyanins, slowing their release and oxidation. The water-sorbitol and water-xylitol samples exhibit instability, which is manifested in the formation of bubbles and structures similar to cells of biological origin. The high viscosity and hydrophilicity of glycerol provides a stable environment for the preservation of pigments.

Conflicts of interest

The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study, as well as the results reported in this paper.

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Data availability

All data are available, either in numerical or graphical form, in the main text of the manuscript.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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