

UDC 663.252

DOI: 10.15587/1729-4061.2026.349976

EVALUATION OF THE FACTORS INFLUENCING THE EXTRACTION AND COMPOSITION OF BIOLOGICALLY ACTIVE SUBSTANCES FROM SECONDARY RAW MATERIALS

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The object of this study is the technology for producing bioactive compound-rich extracts and high-quality wines. Although several studies have been conducted in winemaking on resource-saving technologies, the factors affecting the amount and extraction of bioactive compounds obtained from residues have not been evaluated.

The content of phenolic compounds in sweet pomace obtained from red grape varieties was higher compared to other variants (19.8 g/kg); however, in acidic pomace from the same varieties, this value was significantly lower, at 11.2 g/kg. The Merlot and Madrasa grape varieties were found to contain higher amounts of bioactive compounds and antioxidants.

The extraction of compounds from the peel, seeds, and whole pomace was carried out at 60°C with a 1:3 solid-to-solvent ratio. For the peel, 95% ethanol and 0.5% tartaric acid were used as the solvent; for the seeds, water with 2% tartaric acid; and for the whole pomace, a 30% water-alcohol mixture (wine-alcohol or juice-alcohol) was applied.

The extracts obtained from the peel, seeds, and whole pomace contained vitamin C (0.98–6.6 mg/g), dietary fiber (1.81–3.00 mg/sm³), phenolic compounds (1.04–2.70 mg/sm³), and antioxidant activity (1265.3–2550.3 μmol Trolox equivalents/dm³).

Investigating the factors affecting the extraction of bioactive compounds from secondary raw materials, selecting appropriate technological methods and regimes that ensure high extract yield and antioxidant properties, and rationalizing the composition and quality of the extracts depending on the preparation method and the ingredients provide a solution to the problem. The results offer a practical solution through the production of bioactive compound-rich extracts that can improve the quality of juices and wines

Keywords: extract, hydromodule, phenolic compounds, pomace, stem, peel, wood, water-alcohol, CO₂-extract, seedless extract, tartaric acid, wine-alcohol

Received 05.11.2025

Received in revised form 23.12.2025

Accepted date 12.01.2026

Published date 27.02.2026

How to Cite: Fataliyev, H., Huseynova, S., Gadimova, N., İsmayilov, M., Heydarov, E., Rushanov, A. (2026).

Evaluation of the factors influencing the extraction and composition of biologically active substances from secondary raw materials. *Eastern-European Journal of Enterprise Technologies*, 1 (11 (139)), 34–45.

<https://doi.org/10.15587/1729-4061.2026.349976>

1. Introduction

The majority of global grape production is utilized in winemaking, which generates substantial agricultural resi-

dues, including grape pomace, a by-product rich in bioactive compounds. The reuse of these residues is recommended to prevent environmental problems associated with their improper disposal [1]. Grape stems and grape pomace contain

bioactive substances, including phenolic compounds and anthocyanins, as well as other compounds with potential health benefits [2, 3]. Due to its rich composition and antioxidant properties, grape pomace can play a significant role in the sustainable development of the wine industry and the circular economy. It has been found that grape pomace contains nutrients and bioactive compounds such as polyphenols, organic and fatty acids, vitamins, and more. In particular, polyphenols are recognized for their technological and health-promoting effects in various food and biological systems. Thus, the effective utilization of grape pomace contributes to the development of new functional food products and addresses the problem of residue management in the wine industry. Based on these considerations, the use of grape pomace as a food additive or as an ingredient for the development of new products with technological and functional advantages has recently been proposed [4, 5]. In this context, it is important to study effective processing methods for by-products generated during grape processing that enable the recovery and reuse of valuable substances, particularly bioactive compounds.

2. Literature review and problem statement

In the paper [6], where extracts obtained from grape pomace and individual phenolic compounds were studied through *in vitro*, *in vivo*, or *in silico* methods, and it was noted that phenolic extracts derived from grape pomace could potentially be incorporated into biologically active supplements or even pharmaceutical formulations, attempts were made to explain the mechanisms of these effects. However, the biological activity pathways have not yet been fully clarified. Furthermore, there remains a need for *in vivo* studies to obtain more reliable and consistent scientific results.

In the paper [7], which emphasized that the Negru de Dragasani grape variety has great potential for nutritional and functional applications and in which several bioactive compounds were identified by HPLC analyses, only the pomace of this particular variety was examined, while pomace from other grape varieties and their nutritional value were not addressed.

In the paper [8], which demonstrated that the application of pulsed electric fields (PEF) can selectively enhance the extraction of bioactive compounds from grape pomace—one of the most abundant by-products of winemaking—PEF was investigated as a mild electrotechnological method. However, other extraction methods, particularly the use of different solvents, were not considered.

In the paper [9], where five commercial cellulolytic enzyme preparations were tested under different conditions and differences in extract composition were confirmed through PCA models, other enzyme preparations as well as factors influencing the amount and composition of pomace components were not investigated.

In the paper [10], which found that red grape pomace can be effectively used in the production of turnip juice and enriches its polyphenol content, other bioactive compounds in the pomace and its antioxidant properties were not examined.

In the paper [11], which stated that grape pomace, as easily accessible and low-cost natural source rich in polyphenols, has significant potential for the industrial production of bioactive extracts, the mass fractions of pomace components and their physicochemical properties were not studied.

The paper [12], which provides updated information on the chemical composition of grape pomace, presents sustainable methodologies for extracting phenolic compounds and discusses their potential health benefits as well as possible applications in the food, cosmetic, and pharmaceutical industries. However, the factors affecting the extraction of bioactive compounds were not addressed.

In the paper [13], which discussed the production of beer with different aromas and low alcohol content using non-traditional yeasts and the use of various substrates, including grape pomace, in fermentation, the composition of pomace powder and its comparative analysis were not carried out.

In the paper [14], which reported that Petit Manseng pomace (PAP) enriches the flavanol content and that pre-fermentation juice (PJR) enhances pigment and copigment matrices, and that increasing the pomace ratio by about 10% improves wine color and mouthfeel while having limited impact on aroma, the composition of the structural elements of pomace, optimal extraction conditions, and the extraction process itself were not investigated.

In the paper [15], in which Merlot wine was enriched with phenolic extracts obtained from Cabernet Sauvignon pomace, resulting in increased levels of flavonols and procyanidins, the impact on human health remains uncertain due to the lack of clinical studies. Moreover, the parameters under which enrichment yields the best results were not specified.

In the paper [16], where GS and WL extracts (grape stems: GS; grape pomace: GP; wine lees: WL) were found to significantly influence wine color characteristics, increasing color intensity in wines obtained from treated grapes, the mechanism of solvent use and a comparative analysis of extraction methods were not provided.

In the paper [17], in which rational technological parameters for extracting bioactive compounds from grapes and their structural components were determined, and the use of fermented pomace mixed with a 30% wine-alcohol (juice-alcohol or water-alcohol) solvent at a 1:3 ratio was recommended, extracts obtained using other solvents were not sufficiently studied.

In the paper [18], which examined the effects of clarifying agents on the composition of juices and wines and showed that the Brix index, titratable acidity, and phenolic content changed during the process, extraction issues were not addressed.

In the paper [19], where buckwheat extract powder was recommended as a component of beverages, as an independent product, and as a suitable ingredient for individuals following a gluten-free diet, the extraction and investigation of grape pomace extracts were not considered.

In the paper [20], which reviewed the physiological significance of anthocyanins in grape seeds, their biochemical transformations during winemaking and aging, their potential applications in the food industry, and their health benefits—including their prophylactic and therapeutic potential—modern extraction methods for these compounds were not discussed.

In the paper [21], where HPLC analyses identified epicatechin, p-coumaric acid, and quercetin as major phenolic compounds and where it was shown that PEF treatment caused no degradation, no comparative analysis with other physical extraction methods was provided.

In the paper [22], which reported that ultrasound-assisted extraction is superior to water-bath extraction, that temperature has minimal effect, and that the amount of soluble poly-

saccharides does not correlate with antioxidant extraction capacity, the extraction and investigation of grape pomace extracts were not addressed.

Overall, factors affecting the recovery of extracts and bioactive compounds from secondary raw materials remain unresolved. Specifically, the effects of processing methods and grape variety on pomace components, the physicochemical properties and antioxidant characteristics of pomace and pomace-wood powder, and the factors influencing the release of bioactive compounds and physicochemical properties of the extracts have not been sufficiently studied. Therefore, a significant scientific problem in this field remains to be addressed.

3. The aim and objectives of the study

The aim of the study is to evaluate the factors influencing the extraction and composition of bioactive compounds from secondary raw materials.

To achieve this aim, the following objectives were accomplished:

- to determine the effect of processing methods and grape variety on the quantity and physicochemical characteristics of pomace components;
- to evaluation of the composition of bioactive compounds under the influence of the solid-to-solvent ratio, the type of solvent, and the extraction conditions.

4. Materials and methods

The object of this study is the technology for producing bioactive compound-rich extracts and high-quality wines.

The main hypothesis of the study is to added to wines extracts using residues from grape processing will increase the amount of total phenolic compounds, including anthocyanins, in the wine, thereby improving the total phenolic content, antioxidant properties, and resistance to oxidation and diseases of the wine compared to the control. The sensory parameters of the wine will be higher compared to the control.

The assumptions put forward in the study:

- secondary raw material is not contaminated, moldy, oxidized, or seriously damaged, and is homogeneous in terms of chemical composition, and that the extract represents a common sample;
 - extracted phenolic compounds and antioxidant substances remain stable in the extractant without decomposition or oxidation;
 - technological operations performed during the preparation of the raw material, including drying, chopping, grinding, etc., do not have a significant negative effect on the content of phenolic compounds and do not affect the results;
 - measurement and analysis methods are accurate, repeatable, and do not reflect significant differences between repetitions;
 - extracts do not contain toxic and other harmful substances that would negatively affect the quality of the wine, and that the same results will be obtained if the raw material and extraction conditions are the same.
- The simplifications adopted in the study:
- subjective opinions of tasters have a minimal impact on organoleptic evaluation;
 - preparation and storage of extracts do not have a significant impact on oxidation and compositional changes;

- methods for determining phenolic compounds, antioxidant properties, color intensity, etc. give accurate results, although the influence of a number of factors, including substances, can cause minor errors in the analysis;

- operations carried out during the acquisition of secondary raw materials and their preparation for extraction, and the storage of extracts have a minimal impact on the amount of phenolic compounds and antioxidant properties;

- results apply to a limited range of juices and wines, they are considered to be more widely applicable.

Pomace and other residues obtained from the processing of white and red grape varieties were studied separately under the following variants:

1. Sweet pomace obtained from the “white method” processing of a mixture of Rkatsiteli and Bayanshira varieties.
2. Sour pomace obtained from the “red method” processing of a mixture of Rkatsiteli and Bayanshira varieties.
3. Sweet pomace obtained from the “white method” processing of a mixture of Madrasa and Merlot varieties.
4. Sour pomace obtained from the “red method” processing of a mixture of Madrasa and Merlot varieties.

The quantity and qualitative composition of the obtained residues were analyzed across variants. The extraction process and its influencing factors were evaluated, including hydromodule ratio, duration, temperature, type and composition of extractant, and pre-treatment methods.

For efficient extraction of valuable compounds from pomace, various extractants were tested – rectified alcohol, water-alcohol mixtures, wine-alcohol mixtures of different strengths, and juice-alcohol mixtures, among others. Studies on the extraction of stems and pomace with different types and ratios of extractants showed that extraction conditions, particularly temperature, played a decisive role in the composition and quality of the resulting products.

The extraction was carried out in a thermostat under different temperatures and durations in closed vessels to prevent alcohol loss. To determine the rational hydromodule, the ground raw material was mixed with extractant at different ratios (1:3, 1:5, 1:10, 1:15, and 1:20), kept at room temperature under intermittent stirring, and then separated.

Dried pomace samples were ground using a coffee grinder, sieved through a fine mesh (0.3–0.5 mm), and stored in sealed polyethylene bags at –20°C until analysis. After juice separation, the remaining pomace was used for further study. Pomace was divided into seed and skin fractions for analysis. The fermentation of seeds was carried out similarly to that of stems and pomace.

Analyses performed included pH (Sartorius PB-II, Germany), total acidity, dry matter and ash.

The physicochemical and organoleptic characteristics of raw materials, semi-finished, and finished products were determined according to standard enochemical analysis methods [23, 24].

Modern analytical methods, including high-performance liquid chromatography (HPLC) and computer-assisted statistical data processing, were employed. Statistical analyses were performed using the SPSS 18 software package [25, 26].

The mass concentration of phenolic compounds in wine was determined using the Folin-Ciocalteu method. The Folin-Ciocalteu reagent oxidizes phenolic groups and reduces them to form a blue complex, where the color intensity is proportional to the concentration of phenolic compounds.

5. Analysis of the results of the study of factors affecting the extraction of biologically active substances from secondary raw materials

5.1. Determining the effect of processing method and grape variety on pomace composition

Pomace obtained by pressing the crushed grape mash or fermented mass differs in composition. The processing method of grapes has a significant impact on the quantitative composition of pomace components (Fig. 1).

As can be seen, in the first variant, the amount of peel was higher than in the other samples (67–72%), followed by seeds (21–23%), stems (1–3%), and other impurities (2.1–3.1%). The quantities of seeds and stems were noticeably higher in the third variant compared to the others. Overall, it was observed that a greater amount of grape skin was obtained in the sweet pomace.

During storage of the mash, many components of the skin are transferred into the juice, which was more evident in the second variant. At this stage, the skin mainly retains insoluble polysaccharides, including cellulose, hemicellulose, and others. Such skin can be considered more valuable for the production of dietary fibers.

Study has also shown that the processing method significantly affects the chemical composition of grape pomace (Fig. 2).

In the pomace obtained by the white processing method, the sugar content was 7.6%, while in the sample obtained by the red method, no sugar was detected, and in the fermented mash variant, it was 5.5%. No ethyl alcohol was found in the white method sample, whereas its volume fraction was 4.6% in the red method and 6.1% in the fermented mash sample. Overall, the amount of acidic compounds in wine samples varied between 1.4–2.2%, being the highest in the fermented mash variant, the lowest in the white method, and intermediate in the red method [27, 28].

Depending on the processing method, the amount of seeds in the pomace, as well as the oil content of the seeds, was also studied (Fig. 3).

As shown, the seeds occupy a significant proportion in the composition of the pomace, and their amount varies between 24.9–25.1%, depending on the technological processing method. Among the analyzed parameters, the most notable indicator was the oil content of the seeds, which ranged between 14.02–14.20 %. The seeds obtained from the pomace processed by the white method had a higher oil content compared to the other samples.

The composition and yield of grape pomace depend not only on the grape variety and processing method but also on the type of press used and the quality of pressing (Fig. 4).

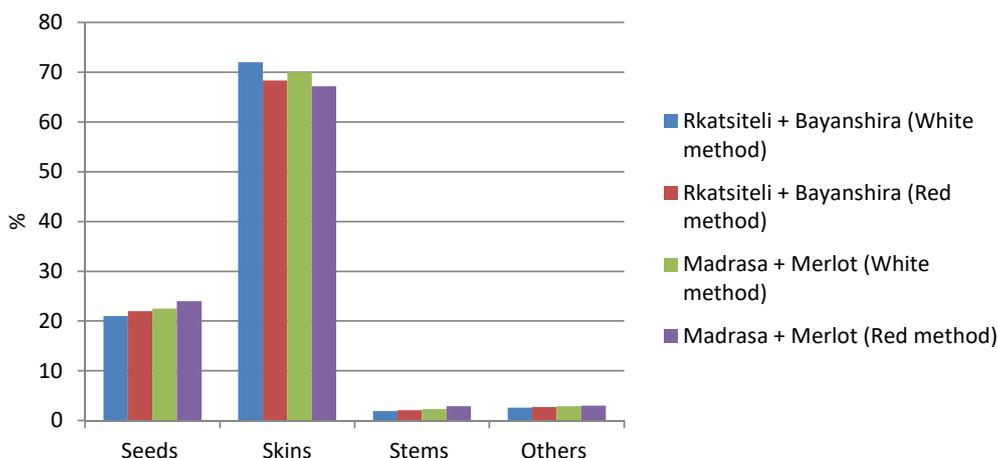


Fig. 1. Effect of processing method on the mass fraction of grape pomace components, $n = 6, p < 0.05$

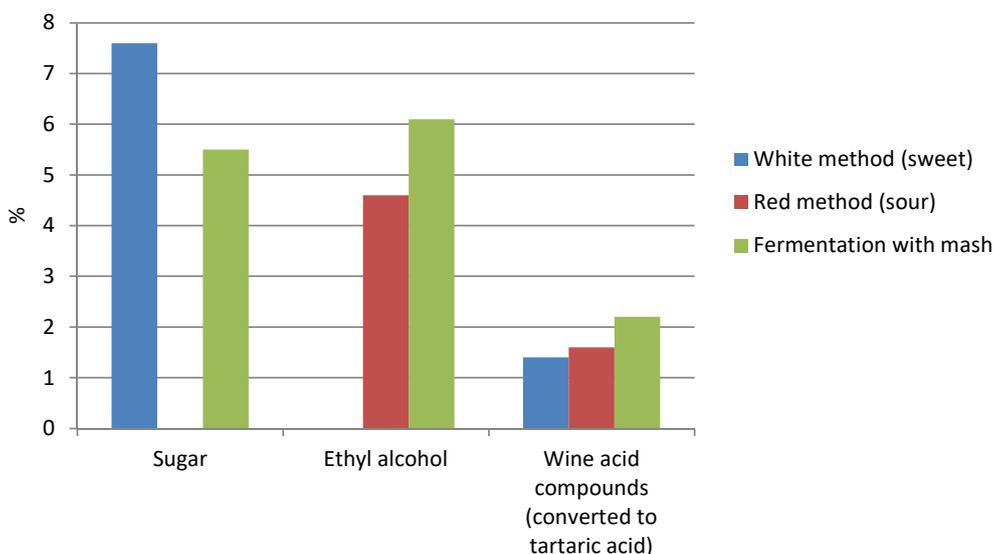


Fig. 2. Effect of processing method on the composition of grape pomace in the "Madrasa" variety, $n = 6, p < 0.05$

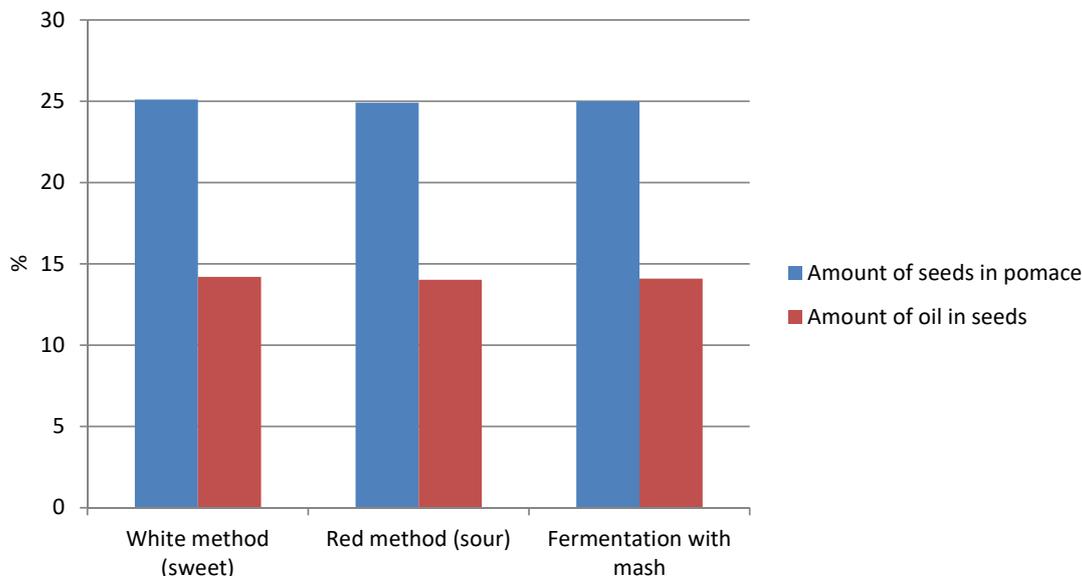


Fig. 3. Effect of processing method on the seed content and oil yield in the “Madrasa” grape variety, $n = 6, p < 0.05$

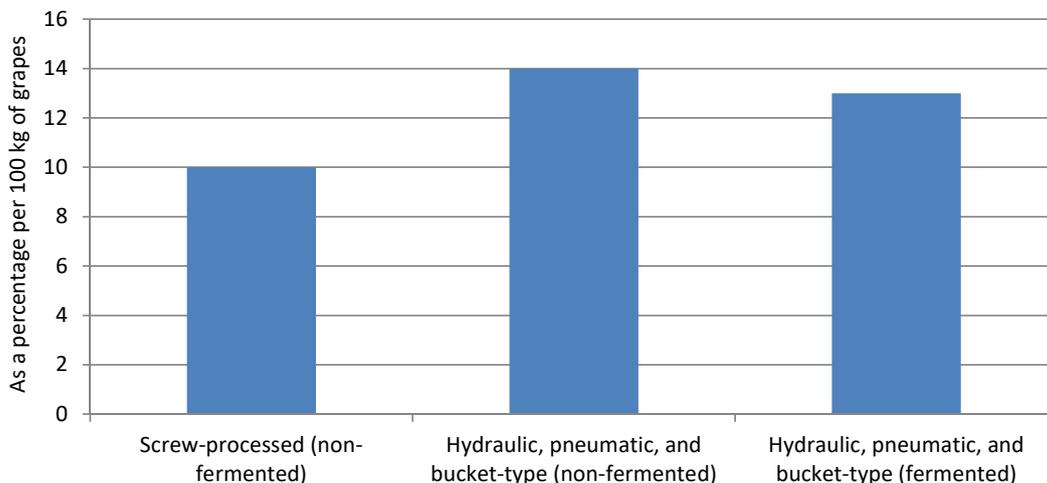


Fig. 4. Effect of pressing method and processing technique on the average amount of pomace in the “Madrasa” grape variety, $n = 6, p < 0.05$

As shown, the amount of sweet pomace varied depending on the type of press and the fermentation process. In the continuously operating screw press, the pomace yield was 10%, while in hydraulic, pneumatic, and basket presses, the amount of unfermented pomace was 14%, and that of fermented pomace was 13%.

The pomace samples obtained from the studied grape varieties were separately fermented, and the mass fraction of structural components, as well as the average moisture and ash content, were determined (Table 1).

Table 1

Composition and ratio of components in fermented pomace samples, $n = 6, p < 0.05$

Grape varieties	Mass fraction, % (of total weight)			Moisture, %	Ash, %
	Skin	Seed	Stem		
Madrasa	85.4	13.1	1.5	7.7	0.42
Isabella	84.9	13.5	1.7	7.9	0.38
Bayanshira	86.7	11.6	1.6	7.8	0.41

As can be seen, the main component of the fermented pomace samples is the grape skin, which accounts for 84.9–86.7% of the total mass. This is followed by the seeds, the amount of which fluctuates between 11.6–13.5%. The proportion of stem fragments in the pomace varied from 1.5–1.7%, depending on the grape variety.

The content of phenolic compounds and polysaccharides in the pomace samples was studied depending on the grape variety and processing method. The white sweet pomace obtained from the Rkatsiteli and Bayanshira blend, the pink sweet pomace from the Isabella variety, and the red sweet and fermented sour pomace obtained from the Madrasa and Merlot blend were analyzed. The results showed the mass concentration of phenolic compounds, polysaccharides, and pectin substances as presented below (Table 2).

As shown, in the sweet pomace obtained from the blend of white grape varieties, 5.5 g/kg polysaccharides, 3 g/kg phenolic compounds, and 1.6 g/kg pectin substances were detected. In the pink sweet pomace derived from the Isabella grape variety, a decrease in polysaccharide content was observed; however, the amount of phenolic compounds

showed an almost threefold increase compared to the previous variant.

Effect of processing method on the mass concentration of phenolic compounds and polysaccharides in pomace, $n = 6, p < 0.05$

Indicators	Mass fraction by variant, g/kg			
	White method (white sweet pomace)	White method (pink sweet pomace)	White method (red sweet pomace)	Red method (red sour pomace)
Polysaccharides	5.5	5.3	5.1	5.2
Phenolic compounds	3.0	8.6	19.8	11.2
Pectin substances	1.6	1.5	1.8	1.8

In the red sweet pomace obtained from the Madrasa and Merlot grape blend, the amount of phenolic compounds reached the highest level (19.8 g/kg) among all variants, while in the fermented sour pomace (the fourth variant), this amount decreased by 8.6 g/kg, reaching 11.2 g/kg. This decrease can be explained by the migration of a significant portion of phenolic compounds from the pomace into the fermenting juice during fermentation. Regarding polysaccharide content, a slight decrease was observed in the third variant and a minor increase in the fourth, compared to the previous samples.

It was also determined that the physicochemical composition of individual pomace components varies considerably. This variation is influenced not only by the grape variety's genetic characteristics, but also by cultivation conditions and agrotechnical practices.

The dry matter content in grape skins varied between 14.0–31.0%, depending on the variety, and consisted of multiple compounds. Among the important components of the pomace samples are pectin substances, which were found to be unevenly distributed in the skin, seeds, and pulp parts of the pomace. The Merlot and Madrasa grape varieties contained significantly higher amounts of pectin substances compared to Bayanshira and Isabella varieties (Table 3).

Variation of physicochemical composition of grape skins depending on the grape variety, $n = 6, p < 0.05$

Composition Indicators	Grape varieties			
	Bayanshira	Isabella	Madrasa	Merlot
Dry matter, %	14.0	17.1	25.1	31.0
Proteins, mg/dm ³	5.4	6.2	6.9	7.5
Total nitrogen, mg/dm ³	6.3	6.9	7.9	8.7
Titrateable acids, %	6.5	6.4	6.7	7.3
Hemicellulose content, %	4.6	5.1	5.8	6.7
Vitamin C, mg/100 g	4.0	5.1	4.8	5.0
Lignin, %	0.11	0.20	0.22	0.25
Pectin, %	0.20	0.30	0.36	0.40
Flavonoids, %	0.35	0.86	1.1	1.40
Anthocyanins	0.30	0.83	1.1	1.40
Phenolic compounds, mg/100 dm ³	0.55	1.4	1.8	2.7

It has been determined that hydrolysis can increase the amount of soluble pectin in grape pomace. The hydrolysis of protopectin in pomace occurs in two stages: first, the bonds between protopectin macromolecules and other structural elements are broken.

Grape seeds are among the by-products of juice and wine production. The seeds constitute 38–52% of the dry pomace, which corresponds to about 3–5% of the total grape mass. The oil content in the seeds, calculated on a dry matter basis, ranges from 7–20% (Table 4).

Table 2

Due to its strong antioxidant properties, grape seed oil is used in cosmetic formulations and as a therapeutic aromatic oil.

Although grape seeds contain a large number of antioxidant polyphenols, primarily monomeric and oligomeric flavonoids, research on grape seeds remains limited.

Hydrolysis of the pomace has also been observed to reduce the content of phenolic compounds. As is well known, phenolic compounds not only contribute to color but also exhibit antioxidant activity. Therefore, considering their role in subsequent processes, careful attention to these compounds is essential.

Table 4

Variation of physicochemical composition of grape seeds depending on the grape variety, $n = 6, p < 0.05$

Composition indicators	Grape varieties			
	Bayanshira	Isabella	Madrasa	Merlot
Dry matter, %	51.2	57.4	59.4	61.6
Total nitrogen, mg/dm ³	22.8	35.6	39.4	41.2
Proteins, mg/dm ³	19.6	29.4	31.3	36.5
Cellulose, %	26.1	28.9	30.2	31.1
Hemicellulose, %	9.2	11.2	10.2	10.6
Vitamin C, mg/100 g	1.96	2.39	2.19	2.16
Tannin substances, %	1.8	2.6	3.7	4.2
Pectin, %	0.30	0.51	0.58	0.66
Lignin, %	16.5	25.1	26.9	28.2
Flavonoids, %	1.2	2.48	3.67	3.96
Anthocyanins, %	0.60	0.75	0.83	0.94

Table 3

Grape pomace is known to be naturally rich in polyphenols. Their concentration varies widely depending on the grape variety and the structural elements of the pomace. Studies indicate that the content of phenolic compounds in white grape varieties is several times lower than in red varieties. Among red varieties, the content is lower in Madrasa compared to Merlot.

Examining the individual components of the pomace, the majority of phenolic compounds are located in the skin and seeds, while a smaller portion is present in the pulp. Most of the lignin is found in the seeds (16.5–28.5%), with a minor amount in the skin (0.11–0.25%). No lignin was detected in the pomace pulp across the studied varieties (Table 5).

Analysis of the structural components of grape pomace revealed that they are rich in bioactive compounds. It was found that the composition of these components varies significantly from one another. As noted, this variability is influenced by the grape processing method, variety characteristics, and the degree of mash pressing. The initial moisture content of the pomace ranges from 50–60%, depending on the quality of pressing.

It is evident that the highest amounts of bioactive compounds (polyphenols, vitamins, organic acids) are found

in the Merlot and Madrasa grape varieties. These bioactive compounds are primarily located in the grape skin and seeds. Based on the analyses, the grape varieties selected for obtaining extracts rich in bioactive compounds include Merlot, Madrasa, and Isabella, while among white varieties, Bayanshira and Rkatsiteli were chosen.

Table 5

Variation of physicochemical composition of grape pulp depending on the grape variety, $n = 6$, $p < 0.05$

Composition Indicators	Grape varieties			
	Bayanshira	Isabella	Madrasa	Merlot
Dry matter, %	13.1	14.9	15.6	18.4
Proteins, mg/dm ³	2.3	3.2	3.9	4.9
Total nitrogen, mg/dm ³	3.1	4.8	5.2	6.3
Titrateable acids, %	5.7	5.6	5.1	5.0
Hemicellulose, %	75.1	81.8	82.5	83.0
Vitamin C, mg/100 g	8.0	11.6	11.3	13.2
Flavonoids, %	0.52	0.63	0.69	0.73
Lignin, %	–	–	–	–
Pectin, %	0.22	0.33	0.36	0.42

5. 2. Evaluation of the composition of bioactive compounds under the influence of the solid-to-solvent ratio, the type of solvent, and the extraction conditions

As noted above, grape pomace is highly enriched with bioactive compounds. Efficient, cost-effective extraction and recycling of these compounds is therefore of special importance.

To this end, factors affecting the extraction process were investigated, including the hydromodule, extraction duration, temperature, type of extractant, extractant composition, and the pre-treatment of the raw material.

To determine the rational hydromodule, the ground raw material was mixed with an extractant (water) in various ratios: 1:3, 1:5, 1:10, 1:15, and 1:20. These mixtures were kept at room temperature under intermittent stirring and then separated (Fig. 5).



Fig. 5. Extracts obtained from pomace and grape seeds

The phenolic bioactive compounds (BFM) in the obtained extracts were quantified. The results are presented below (Table 6).

As seen in Table 6, reducing the amount of extractant in the hydromodule up to a certain point increases the extraction of phenolic compounds. In this regard, the 1:5 hydromodule was effective, and the best results were obtained with the 1:3 ratio.

Table 6

Dependence of the extraction of phenolic BFM on the hydromodule (expressed as free acid, mg/cm³), $n = 6$, $p < 0.05$

Object	Hydromodules				
	1:3	1:5	1:10	1:15	1:20
Peel	1.95	1.27	0.71	0.66	0.60
Seed	2.09	1.46	1.08	0.80	0.49
Whole berry	0.41	0.26	0.18	0.15	0.11

To clarify the effect of extraction duration on the separation of phenolic bioactive compounds (BFM), the material was kept at room temperature under the previously determined rational hydromodule for 20, 40, 60, 120, 180, and 240 minutes. After each interval, the material was pressed, separated, and analyzed individually. The results are presented in Table 7.

Table 7

Effect of extraction duration on the separation of phenolic BFM (expressed as free acid, mg/cm³), $n = 6$, $p < 0.05$

Object	Extraction duration, min					
	20	40	60	120	180	240
Peel	1.69	1.98	1.89	1.78	1.77	1.78
Seed	1.76	1.84	1.98	2.36	2.38	2.37
Whole berry	0.30	0.28	0.40	0.45	0.51	0.52

As seen in Table 7, the rational extraction times are 40 minutes for the grape peel, 120 minutes for the seeds, and 180 minutes for the whole berry.

To determine the optimum extraction temperature, the previously established hydromodule and extraction durations were used, and the materials were maintained at 30°C, 60°C, and 90°C. The efficiency of BFM (bioactive phenolic compounds) separation was assessed by measuring the phenolic compound content. The results are presented in Table 8.

Table 8

Effect of extraction temperature on the separation of phenolic compounds (expressing as free acid, mg/cm³), $n = 6$, $p < 0.05$

Object	Extraction temperature, °C		
	30°C	60°C	90°C
Peel	2.30	2.55	2.05
Seed	2.55	2.80	2.22
Whole berry	0.75	0.76	0.55

The results indicate that (Table 8) the rational extraction temperature for all study materials is 60°C. Further temperature increases lead to the degradation of bioactive compounds, as confirmed by the hot treatment performed at 90°C. At 90°C, the BFM content decreased significantly compared to lower temperatures across all samples.

To identify the most effective extracting agent, the ground raw material was mixed with different solvents while keeping the previously determined rational parameters – hydromodule, extraction duration, and temperature – constant. The solvents tested included water, ethanol, 30%, 50%, and 70% aqueous solutions of wine and juice, and 95% rectified ethanol. The phenolic compound content in the extracts was measured. The results are presented in Table 9.

Table 9

Effect of different extracting agents on the extraction process (expressing as free acid, mg/cm³), *n* = 6, *p* < 0.05

Object	Extractants								
	Water	Water-ethanol, 30%	Water-ethanol, 50%	Water-ethanol, 70 h%	Ethanol, 95%	Wine-ethanol, 30%	Wine-ethanol, 50%	Wine-ethanol, 70%	Ethanol, 95%
Peel	2.45	2.40	2.50	2.59	2.74	1.50	1.50	1.59	1.76
Seed	2.59	2.32	2.19	2.13	2.05	1.11	1.05	1.03	0.97
Whole berry	0.75	1.11	1.08	0.96	0.94	1.10	0.71	0.83	1.09

The results indicate (Table 9) that the most effective extracting agents are as follows: rectified ethanol for the peel, water for the seeds, and 30% water-ethanol or 30% wine-ethanol solutions for the whole grape pomace.

Studies show that the composition of the raw material—particularly the type and quantity of acids—significantly influences the extraction process. Both mineral acids (e.g., hydrochloric acid) and organic acids (e.g., tartaric, citric) are known to have a notable effect. Taking this into account, the influence of acid addition was evaluated in the study.

Using the previously determined rational extraction parameters, tartaric acid was added to the extracting solutions at concentrations of 0.5%, 1.0%, and 2.0%. Rectified ethanol was used for the peel, water for the seeds, and 30% water-ethanol for the whole pomace. The phenolic compound content in the obtained extracts was measured, and the results are presented in Table 10.

Table 10

Effect of adding tartaric acid to the extracting agent on the extraction process (as free acid, mg/cm³), *n* = 6, *p* < 0.05

Object	Initial material (without additive)	Amount of added tartaric acid				
		0.5	1.0	1.5	2.0	2.5
Peel	2.76	2.86	2.08	2.35	2.53	2.51
Seed	2.63	2.71	2.80	2.88	2.90	2.87
Whole berry	1.16	0.62	0.73	0.75	0.80	0.77

As seen from Table 10, the most efficient extraction from the whole grape pomace occurs without the addition of tartaric acid, while for the peel, 0.5% tartaric acid and for the seeds, 2.0% tartaric acid provide the best results.

To evaluate the effect of raw material pretreatment on the extraction process, samples of peel, seed, and whole pomace were taken in triplicate under different conditions: one set remained untreated, the second set was fermented, and the third set was frozen (Table 11).

Table 11

Effect of raw material pretreatment on the extraction process (as free acid, mg/cm³), *n* = 6, *p* < 0.05

Object	Preliminary processing methods		
	Fresh raw material (not pre-processed)	Fermented raw material	Frozen raw material
Peel	2.89	2.96	3.11
Seed	2.96	3.08	2.83
Whole berry	1.17	1.65	0.86

As seen from Table 11, the most effective extraction was achieved by pre-freezing the peel at -18°C, and fermenting the seeds and whole pomace at 60°C. Based on these findings, the rational extraction parameters for peel, seed, and whole pomace were developed as follows: temperature: 60°C,

hydromodule: 1:3 (solid:solvent ratio); extractants: peel: 95% rectified ethanol + 0.5% tartaric acid, seeds: water + 2% tartaric acid, whole pomace: 30% water-ethanol (or wine-ethanol / juice-ethanol) mixture, no tartaric acid.

To maximize the recovery of bioactive and color compounds, the pomace was re-extracted after filtration, and the extracts were combined and dried in an oven at 60°C to 50–55% solids content.

The analysis of the resulting extracts is presented in Table 12, which shows that the extracts exhibit high antioxidant activity, ranging from 1265.5 to 2550.3 depending on the type of raw material.

Table 12

Biochemical characteristics of the extracts, *n* = 6, *p* < 0.05

Indicators	Type of raw material		
	Peel	Seed	Whole grape berry
Dry matter content, %	34.7	6.5	52.1
Titrate acidity, %	7.3	5.7	9.8
Phenolic compounds content (as tartaric acid), mg/ml	2.4	2.7	1.04
Pigment content, g/dm ³	0.44	–	0.27
Vitamin C content, mg/g	5.1	0.98	6.6
Dietary fibers, mg/ml	3.0	Traces	1.81
Light stability, %	76.5	97.5	95.0
Temperature stability, %	95.7	98.8	97.4
Antioxidant activity, μmol Trolox eq./dm ³	2430.2	2550.3	1265.3

The extracts were particularly stable to temperature, with this indicator ranging from 95.7 to 98.8%. Light stability was higher in seeds (97.5%), relatively lower in the peel (76.5%), and at an average level of 95.0% in whole berries.

The biologically active compounds in the extracts were investigated, and the results of the analysis are presented below (Table 13).

As can be seen, a 70% glycerin-water mixture solution provides better extraction of phenolic compounds from whole berries. The extract obtained from the peel using 95% ethanol, on the other hand, favors a higher yield of catechin and epicatechin.

It is well known that one of the promising directions for the efficient use of secondary raw materials is the application of CO₂ extracts. In this case, compressed carbon dioxide is used as the extractant. As a result, the extraction time is shortened, and the amount of bioactive compounds extracted is increased.

CO₂ extracts obtained by various methods were investigated based on several indicators (Table 14).

As shown in Table 14, the acid value of CO₂ extracts varies between 5.66 and 18.21. The lowest acid value was observed in the pomace extract obtained from a mixture of white grape varieties, while the highest was recorded in the pomace extract from red grape varieties.

Table 13

Results of chromatographic analysis of biologically active compounds in the extracts, $n = 6$, $p < 0.05$

Compounds, mg/dm ³	Samples		
	Extract obtained from peel with 95% ethanol	Whole berry extract with 30% hydroalcoholic solution	Whole berry extract obtained with 70% glycerin aqueous solution
Gallic acid	0.1	27.7	31.2
Catechin	186.5	< 0.1	68.4
Epicatechin	173.7	18.1	47.2
Quercetin	< 0.1	< 0.1	< 0.1

Such differences in acid value depend both on the quality of the raw material (decay, mold, etc., sharply increase the acid value) and on the nature of the raw material itself. Regarding the peroxide value, this indicator is higher in the white grape pomace extract, i.e., 26.30 mmol $\frac{1}{2}$ O/kg, and significantly lower in the red grape pomace extract, being 9.74 mmol $\frac{1}{2}$ O/kg in its seedless variant. This suggests that secondary products of white grape varieties contain fewer antioxidants and a higher amount of compounds reactive to oxygen.

Physicochemical characteristics of carbon dioxide extracts, $n = 6$, $p < 0.05$

CO ₂ extracts	Indicators			
	Acid value, mg KOH/g	Peroxide value, mmol $\frac{1}{2}$ O/kg	Anisidine value	Mass fraction of non-washable substances, %
White grape pomace extract	5.66	26.30	12.66	3.05
Red grape pomace extract with seeds	18.21	16.68	23.44	4.09
Red grape pomace extract without seeds	15.31	9.74	12.22	4.26

The anisidine value ranged widely from 12.22 to 23.41. In this case, the minimum anisidine value of 12.22 was observed in the seedless red grape pomace extract, while the maximum of 23.44 was in the red grape pomace extract. It is assumed that more carbonyl compounds are extracted from red grape pomace. Therefore, a high carbonyl value is determined both by the presence of secondary oxidation products and by the nature of the compounds transferred into the extract.

The mass fraction of insoluble substances in the extract was 3.05% for white grape pomace, 4.09% for red grape pomace extract, and 4.26% for the seedless red grape pomace extract. This suggests that the extraction process transfers a significant amount of waxes, high-molecular-weight carbohydrates, sterols, phenolics, terpenes, a certain amount of antioxidants, pigments, and other ether-soluble substances that do not react with alkali during washing into the extract.

The fatty acid composition of the extracts is presented in Table 15.

It is clear from Table 15 that the linoleic acid, which plays a major role in the fatty acid composition of all extracts, ranged from 53.2 to 54.7%. This was followed by oleic acid (14.2–16.7%) and palmitic acid (11.7–20.1%). The first four acids listed in the table belong to saturated fatty acids and are present in relatively small amounts.

Table 15

Fatty acid composition of the extracts, $n = 6$, $p < 0.05$

Fatty acids	Mass fraction of fatty acid		
	White grape pomace extract	Red grape pomace extract	Seedless red grape pomace extract
Caprylic acid	1.1	0.6	Yox
Capric acid	0.6	0.8	0.3
Lauric acid	0.9	1.7	0.8
Myristic acid	2.4	2.8	0.5
Palmitic acid	11.7	16.4	20.1
Palmitoleic acid	1.1	1.0	1.3
Margaric acid	Traces	Traces	No
Stearic acid	2.6	3.4	3.8
Oleic acid	16.7	16.5	14.2
Linoleic acid	53.2	54.7	54.3
Linolenic acid	3.6	2.4	4.3
Arachidic acid	0.1	Traces	0.4

6. Discussion of the results on the study of factors affecting the extraction of biologically active compounds from secondary raw materials

Table 14

Unlike the study [1], which presented phenolic extracts from grape pomace as a component that can be included in biologically active supplements and pharmaceutical preparations, in this work, the amount of residues formed during processing—such as peel, seeds, stems, and other mixtures—was determined depending on the processing method. At the same time, the processing method affects the composition of pomace, its seed content, and fat content (Fig. 1–3).

Unlike the study [2], which identified the amount of bioactive polyphenols in Negrude Dragasani pomace extracts using high-performance liquid chromatography and determined the potential of this variety for food and functional purposes, in this research, it was found that the composition and yield of pomace are influenced not only by the processing method and grape variety but also by the equipment used, particularly the type of press. The amount of sweet pomace varied depending on the press and fermentation: in a continuously operating screw press, it was 10%; in hydraulic, pneumatic, and basket presses, 14%; and for fermented pomace, it was 13% (Fig. 4).

Unlike the study [3], which proposed the use of pulsed electric fields as a soft electro-technological method to increase the efficiency of extracting bioactive compounds from grape pomace, in this work, pomace samples from different grape varieties were fermented, and the amounts of structural components, ash, and moisture in the pomace were determined. It was found that the main part of the pomace consisted of peel (84.9–89.7%), followed by seeds and stem fragments. The mass content of phenolic compounds and polysaccharides in the pomace varied depending on the processing method (Tables 1, 2).

Unlike the study [4], which used five cellulolytic enzyme preparations to extract phenolic compounds from two grape varieties, this work showed that the physicochemical composition of pomace structural components

differed depending on the grape variety. Pectin substances were unevenly distributed in the peel, seeds, and pulp; in Merlot and Madrese varieties, pectin content was higher than in Bayanshira and Isabella varieties. The majority of phenolic compounds were located in the peel and seeds, with a smaller portion in the pulp. The majority of lignin was found in the seeds, with a small amount in the peel. Higher amounts of biologically active compounds (polyphenols, vitamins, organic acids) were observed in Merlot and Madrese varieties, mainly in the peel and seeds (Tables 3–5).

Depending on the grape variety, the content of nutrients in pomace flour varied widely. The raw protein content in Madrasa was 2.5% higher than in Bayanshira. The iodine content in Bayanshira was nearly 2.5 times higher than in Madrese. Considering amino acid composition, the total amount of amino acids in Madrasa pomace flour was 1.15% higher than in Bayanshira (Tables 6, 7).

Unlike the study [6], which determined the use of grape pomace as a low-cost and polyphenol-rich natural source for the production of bioactive extracts in industry, this work also examined the dry perennial parts and one-year shoots after pruning. It was found that the content of easily soluble polysaccharides in the one-year shoot was 41, nearly eight times higher than in the perennial part. Proteins, fats, and other indicators were similar between these parts. The total and amino acid composition of grape cane wood flour was investigated according to grape varieties. Pomace flour was found to be superior to cane flour in terms of richness of composition (Tables 8, 9).

Unlike studies [7, 8], which presented methods for extracting phenolic compounds and investigated fermentation of various materials including grape pomace, in this research, Madrasa and Merlot varieties were superior in flavonoid content, while Merlot was superior in tannin content. Among white varieties, Rkatsiteli showed higher values in both phenolic content and antiradical properties.

The extraction process and its influencing factors – such as hydromodule, extraction duration, temperature, type and composition of extractant, and pre-treatment of raw material – were studied. Extraction from peel, seeds, and whole berries was carried out at 60°C and a 1:3 hydromodule. In this case, the extractant was 95% ethanol with 0.5% tartaric acid for peel, ordinary water with 2% tartaric acid for seeds, and a 30% water–ethanol mixture (wine-ethanol or juice-ethanol) without tartaric acid for whole berries (Fig. 5, Tables 6–11).

Unlike studies [10, 11], which examined the mixing of juice and pomace and enrichment of red wine with phenolic extracts from pomace, in this work, the obtained extracts were analyzed, confirming their high antioxidant activity. The extracts showed stability to temperature and light. The content of biologically active compounds was determined using chromatographic methods. To shorten extraction time and increase the amount of bioactive compounds extracted, CO₂ extraction was applied. The composition of extracts obtained this way was analyzed (Tables 12–14).

Unlike studies [13–15], which investigated phenolic compounds and antioxidant activity in pomace from four grape varieties, the production of juice beverages with added extracts, and the determination of pomace and peel composition, antioxidant potential, fermentative activity,

and sensory evaluation of functional juices, this work examined the fatty acid composition of white and red grape pomace extracts, including seedless red grape extracts. Linoleic acid was present in high amounts, followed by oleic and palmitic acids. Four identified saturated fatty acids were present in relatively low amounts (Table 15).

The results of the study possess practical significance for enterprises engaged in both food production and winemaking. At the same time, these findings may be applied in medicine and pharmacy for the study and extraction of biologically active compounds.

The research outcomes can be utilized in the fields of winemaking and food technology. The obtained results may be implemented in scientific studies in these areas, as well as in family-farm enterprises and industrial winemaking facilities.

The study is considered appropriate for the recycling and processing of technological residues formed during “white method” wine production. However, the use of pomace with high acidity generated during the processing of juices and wines produced by the “red method” is associated with certain technological limitations.

A limitation of the study is the requirement for favorable environmental conditions or specialized equipment and production facilities for the fermentation of secondary raw materials. In particular, due to the seasonal nature of grape processing, the storage of secondary raw materials for later use creates additional challenges.

In addition, investigating changes in the composition of extracts obtained using various extractants during storage – including the variation of antioxidant and antiradical properties is considered a promising research direction.

7. Conclusion

1. Comparative studies were conducted on white sweet pomace (I) obtained from a mixture of Rkatsiteli and Bayanshira varieties, pink sweet pomace (II) from the Isabella variety, red sweet pomace (III) from Madrasa and Merlot varieties, and red fermented pomace (IV). In the fourth variant, the content of polysaccharides was 5.5 g/kg, phenolic compounds 3 g/kg, and pectin substances 1.6 g/kg. In the second variant, although the polysaccharide content decreased, the amount of phenolic compounds increased nearly threefold compared to the first variant. The highest content of phenolic compounds was observed in the third variant (after pressing – 19.8 g/kg), while the lowest was in the fourth variant (11.2 g/kg).

2. The rational parameters for the extraction of biologically active compounds were experimentally determined. Extraction from peel, seeds, and whole berries was carried out at 60°C and a 1:3 hydromodule. The recommended extractants were: 95% ethanol with 0.5% tartaric acid for peel, ordinary water with 2.0% tartaric acid for seeds, and a 30% water-ethanol mixture (wine-ethanol or juice-ethanol) without tartaric acid for whole berries. The radical-scavenging capacity was higher in Merlot and Madrese seeds (58.01 and 30.01 mmol Trolox/100 g DW, respectively) and in Madrasa peel (26.25 mmol Trolox/100 g DW). The antioxidant properties were highest in the pulp of Rkatsiteli (79.2%) and Bayanshira (75.6%), as well as in Madrasa (87.1%) and Rkatsiteli seeds (86.9%).

Conflict of interest

The authors declare that they have no conflict of interest in relation to this study, whether financial, personal, authorship or otherwise, that could affect the study and its results presented in this paper.

Financing

The study was performed without financial support.

Data availability

Data will be made available on reasonable request.

Use of artificial intelligence tools

The authors confirm that they did not use artificial intelligence technologies in creating the submitted work.

Authors' contributions

Hasil Fataliyev: Writing – review & editing, Project administration, Writing – original draft; **Shafiga Huseynova:** Conceptualization, Investigation, Writing – original draft; **Natavan Gadimova:** Validation, Investigation, Writing – review & editing; **Mehman Ismayilov:** Investigation, Methodology, Conceptualization; **Elnur Heydarov:** Investigation, Methodology, Conceptualization; **Asaf Rushanov:** Conceptualization, Methodology, Investigation.

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