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Study of Secondary Metabolites of Georgian Grape Wine Processing Waste Using UPLC-PDA-MS Methods and Prospects for Using Products Obtained from It

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Abstract

There is increasing interest in harnessing natural biologically active compounds for preventive and therapeutic purposes. However, the byproducts of wine production from endemic Georgian grape varieties have not been extensively studied using modern analytical methods and are typically left unutilized.This study aimed to analyze the chemical composition of grape wine production residues from cultivated grape varieties in Western Georgia and to evaluate their potential applications. An optimized processing technology was established for grape wine production residues, enabling the extraction of cold-pressed buckwheat oil (rich in unsaturated fatty acids) and hydrophilic preparations enriched with proanthocyanidins, catechins, and phenolic acids. The extraction process utilized green technologies, including high-pressure and ultrasonic-assisted extraction with a water-alcohol solvent system. The obtained extracts were concentrated under vacuum and subsequently freeze-dried to preserve biologically active compounds.This study provides valuable insights into the sustainable utilization of grape processing residues, demonstrating their potential for the production of functional food ingredients and bioactive preparations.

Keywords: Grape processing waste, UPLC-PDA-MS, Green extraction, Antioxidant activity, Anthocyanins.

Introduction

Georgia is home to more than 500 indigenous grape varieties, each displaying distinct morphological traits and unique chemical profiles. This diversity extends not only to the grape berries themselves but also to by-products such as grape skins and seeds, which constitute approximately 25–30% of winemaking waste. The chemical composition of these by-products is influenced by a range of factors, including grape variety, viticultural practices, soil type, climatic conditions, and processing technologies (Vanidze et al., 2020).

In Georgian winemaking, two primary fermentation methods are employed: the European method and the traditional Kakhetian method. The European approach involves fermenting only the grape juice, with solid residues such as skins and seeds removed early in the process. As a result, the chemical composition of the removed materials remains largely unchanged by fermentation. In contrast, the Kakhetian method involves the fermentation of the juice along with skins, seeds, and stems (pomace), leading to extended contact between the solid residues and the fermenting must (Kharadze et al., 2020).

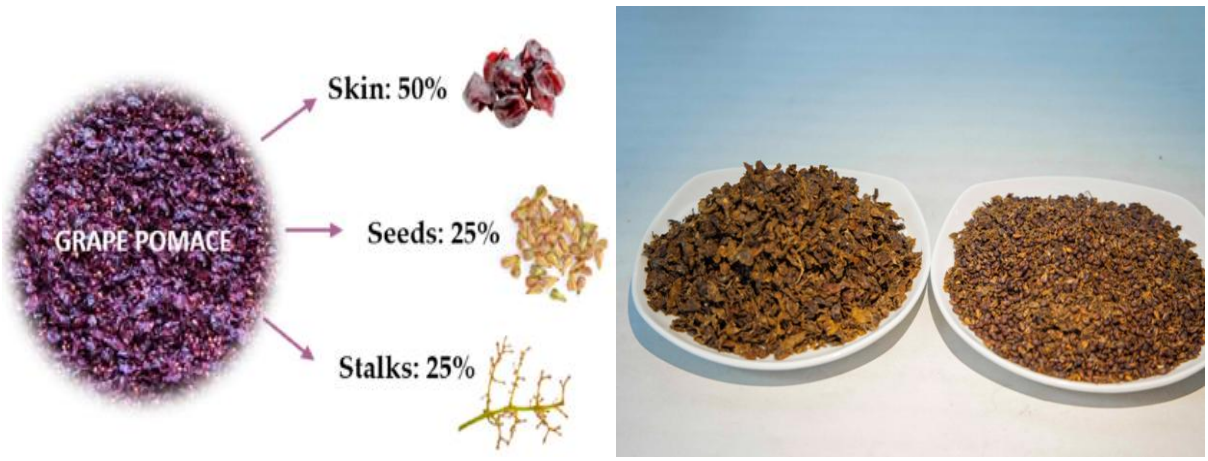
In this latter method, several fermentation-dependent factors—such as initial sugar concentration, alcohol content, and fermentation duration—can significantly alter the chemical profile of the grape skins and seeds. This prolonged exposure to alcohol and enzymatic activity often enhances the extraction and transformation of phenolic compounds, especially in red grape varieties, where the skins are rich in anthocyanins and other bioactive constituents.

Notably, anthocyanins are predominantly located in the grape skin and contribute to the coloration and antioxidant properties of red wines. These compounds are more prevalent in unfermented or minimally processed grape skins. Therefore, understanding the interplay between grape variety, processing method, and chemical composition of winemaking by-products is critical for optimizing their valorization in food, cosmetic, and pharmaceutical applications.

Grape seeds are distinguished by their high content of biologically active compounds. (Kadri, S.et al.,2019), Interest in such studies is very high (Morazzoni et al.,2021), studies focus on obtaining biologically active compounds through the so-called "green extraction" (Beilankouhiet al.,2024), biologically active compounds have been discovered (Montero et.al. 2013; Dabetic et al., 2022; Beilankouhi et al., 2024). Grape seeds also contain lipids (Pérez-Navarro et al., 2019), unfortunately, the chemical composition of the seeds of Georgian grape varieties and their potential uses have been poorly studied.

Plant Material and Sample Preparation

The object of the research was the seeds and skins of grapes (“Ockhanuri saphere”) grown in western Georgia (42.1874° N, 42.9357° E), which underwent various degrees of extraction.



Extraction Methods

Ultrasonic Extraction (USE)

Ultrasonic extraction leverages the dual impact of mechanical vibration and acoustic cavitation generated by ultrasonic waves to enhance the mass transfer during the extraction process. The mechanical aspect involves the transmission of ultrasonic energy through the solvent, which promotes particle agitation, enhancing solvent diffusion and penetration into plant matrices. Cavitation—the formation and implosion of microbubbles—produces localized high temperatures and pressure differentials that rupture cell walls, thereby liberating bioactive constituents into the solvent medium. This approach significantly boosts extraction efficiency and rate while preserving thermally sensitive compounds (Surmanidze et al., 2024).

Pressurized Hot Water Extraction (PHWE)

PHWE operates by employing water under high temperature and pressure to remain in a liquid state above its standard boiling point, thus modifying its solvent characteristics. In this state, water's polarity decreases, enabling it to extract a broader spectrum of compounds, from polar to moderately nonpolar. The technique is considered sustainable due to its minimal environmental impact and effective energy use. Precise temperature regulation is crucial, as excessive heat (above 160 °C) may lead to degradation of heat-sensitive antioxidants. Enhancements in selectivity and yield can be achieved through the inclusion of natural deep eutectic solvents (NADES) or by coupling the process with pulsed electric field (PEF) treatments.

Anthocyanin Analysis

The characterization of anthocyanin compounds was carried out using ultra-performance liquid chromatography coupled with photodiode array and mass spectrometry detection (UPLC-PDA-MS). Separation was performed on a BEN C18 column under positive electrospray ionization (ESI+) conditions. The mobile phase consisted of 2% formic acid (Solvent A) and methanol (Solvent B), applied in a gradient elution mode. Chromatographic parameters included a constant flow rate of 0.3 mL/min and a column temperature set at 30 °C. The mass spectrometer scanned ions in the m/z range of 100–1200 Da, with a probe temperature of 500 °C, a spray voltage of 0.8 kV, and a capillary voltage of 1.5 kV. This analytical setup enabled accurate detection of cyanogenic anthocyanins, compounds with established anticancer potential (Abashidze et al.,2024).

Identification of Non-Anthocyanin Phenolic Compounds

To analyze non-anthocyanin phenolics, ultra-high-performance liquid chromatography (UHPLC) paired with a photodiode array and mass spectrometric detector (PDA-MS) was used. The separation process employed a BEH C18 column (1.7 µm) with a binary solvent gradient: 0.2% formic acid in water (A) and acetonitrile (B), at a consistent flow of 0.3 mL/min and a column temperature of 30 °C. The MS operated in negative ionization mode, with parameters set to enhance sensitivity and resolution (e.g., 500 °C probe temperature, 0.8 kV spray voltage). Data were acquired across a mass range of m/z 100–1200 and matched with standard compounds and the METLIN database. All samples and eluents were filtered through 0.45 µm membranes before injection (Sarker et al., 2013).

Determination of Antioxidant Activity

The antioxidant properties of extracts were evaluated using the DPPH radical scavenging assay. In this assay, a 1 mL sample of the extract was combined with 3 mL of DPPH solution and incubated in the dark for 15 minutes to allow the reaction. The absorbance was then measured at 517 nm using a UV-Vis spectrophotometer. Antioxidant effectiveness was expressed as the IC_{50} value, representing the concentration necessary to neutralize 50% of DPPH radicals, calculated from absorbance differences and sample mass (Abashidze et al., 2024).

Table.1 Influence of extraction methods on the content of phenolic compounds

Sample name (dray)	Total phenol mg/g	Phenol carbonic acids mg/g	Flavonoids, mg/ml	Catechins mg/ml	Proanthocyanins mg/ml
Grape "Ockhanuri saphere" seeds - SWE	68,694	8,5490	48,599	13,613	24,033
Grape "Ockhanuri saphere" seeds - US	74,902	8,7329	54,699	16,039	30,477
Grape "Ockhanuri saphere" - maceration	61,429	7,8685	42,344	12,228	24,051
Grape "Ockhanuri saphere" skine - SWE	59,704	6,8134	49,523	11,855	13,274
Grape "Ockhanuri saphere" skine - US	62,885	6,8670	53,998	13,125	14,781
Grape "Ockhanuri saphere" skine - maceration	47,725	6,5610	38,423	10,618	11,708

The pods taken for the study were prepared in advance. They were divided into pods and skins. The samples were extracted by maceration (classical method), Ultrasonic Extraction (USE) and Pressurized Hot Water Extraction (PHWE). Pod extraction by the US method gives the best results (68.7 mg/g), respectively, such an extract contains more phenolic acids (8.7 mg/g), flavonoids (54.7 mg/g), catechins (16.0 mg/g) and proanthocyanidins (30.5 mg/g). The results are relatively lower under PHWE conditions (total phenols 68.7 mg/g), respectively, other compounds are relatively less. Maceration achieves the worst results (61.4 mg/g). (Table 1)

Similar results are obtained in grape skin extraction. The best results are obtained here by the US method (62.9 mg/g), respectively phenolic acids (6.9 mg/g), flavonoids (49.5 mg/g), catechins (13.1 mg/g) and proanthocyanidins (14.8 mg/g). Relatively lower results are obtained under PHWE conditions (total phenols 59.7 mg/g), respectively other compounds are relatively less. The worst result is achieved by maceration (47.7 mg/g). The content of anthocyanins is not observed in the seed extracts. The extracts are distinguished by high antioxidant activity. This indicator is proportional to the content of phenolic compounds, the higher their content, the less sample is required 50% inhibition of 0.1 mM DPPH (0.414 mg).

Table 2. Effect of extraction methods on anthocyanin content and antioxidant activity

Sample name (dray)	**Total anthocyanins mg/g**	**Monomeric anthocyanins mg/g**	AA, mg Sample **50%** inhibition of 0.1 mM DPPH	**1/ AA,** mg Sample **50%** inhibition of 0.1 mM DPPH
Grape “Ockhanuri saphere” seeds - SWE	-	-	0,720	1,389
Grape “Ockhanuri saphere” seeds - US	-	-	0,414	2,415
Grape “Ockhanuri saphere” seeds - maceration	-	-	0,783	1,277
Grape “Ockhanuri saphere” skine - SWE	1,480	1,079	0,361	2,771
Grape “Ockhanuri saphere” skine - US	2,467	2,127	0,240	4,170
Grape “Ockhanuri saphere” skine - maceration	1,764	1,511	0,419	2,387

US extracted grape skins have a high content of anthocyanins (2.5 mg/g), therefore it is distinguished by a higher antioxidant activity (0.24 mg Sample 50% inhibition of 0.1 mM DPPH) than other extracts (0.36 mg SWE and 0.42 mg-maceration) (Table 2.).

The dominant anthocyanin was identified in the composition of the seeds ESI [M+H] - m/z 493.04 is recorded on the chromatogram with a retention time of 5.437 min, with absorption maxima at 282.7 nm and 518.4 nm. The obtained results, as well as the METLIN (https://metlin.scripps.edu) mass database of compounds, were compared with the standard compound malvidin-3-O-glucoside (C17H13O7).

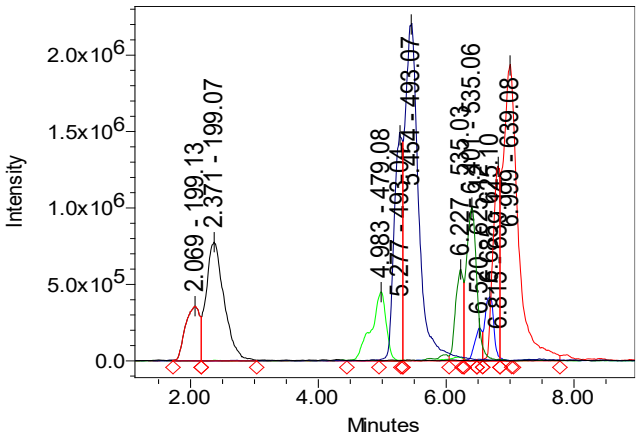


Figure 1. ESI [M+H] –anthocyanidins chromatograms of grape seeds

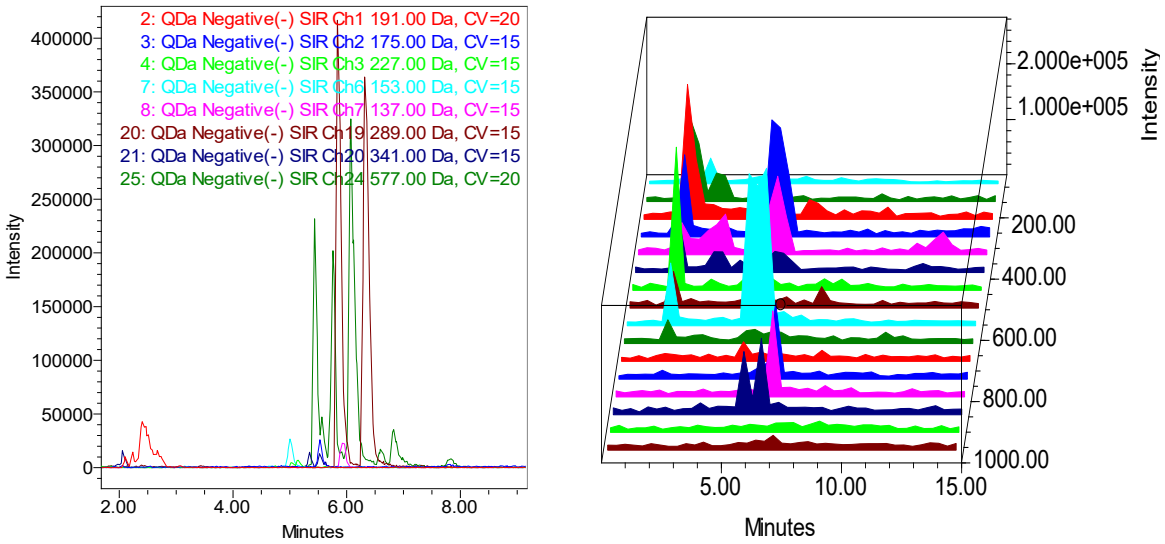


Figure 2. ESI [M-H] – chromatograms of grape seeds

ESI [M-H] - m/z 288.87 and 288.91 are recorded on the chromatogram with retention times of 6.748 min and 7.379 min, with an absorption maximum at 281.7 nm. By comparing the obtained results, as well as according to the mass database of compounds (https://metlin.scripps.edu), these compounds correspond to catechin, (MF C15H14O6, MW: 290.27g/mol Catechin (2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol) and epicatechin MF C15H14O6, MW: 290.27g/mol epi-Catechin (2R,3R)-2-(3,4-dihydroxyphenyl)- 3,4-dihydro-2H-chromene-3,5,7-triol). (Figure 3)

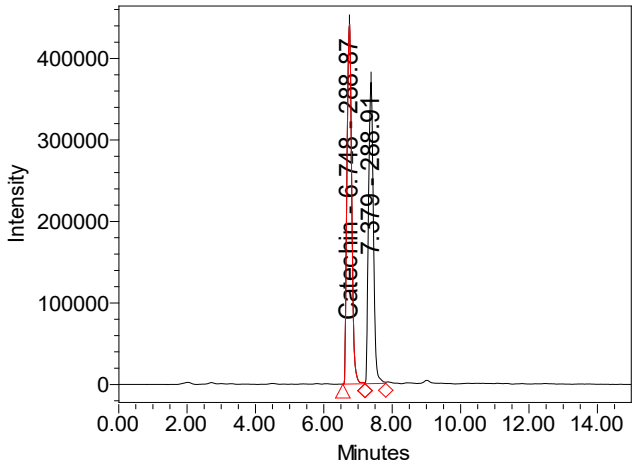


Figure 3. ESI [M-H] – 289 chromatograms of grape seeds

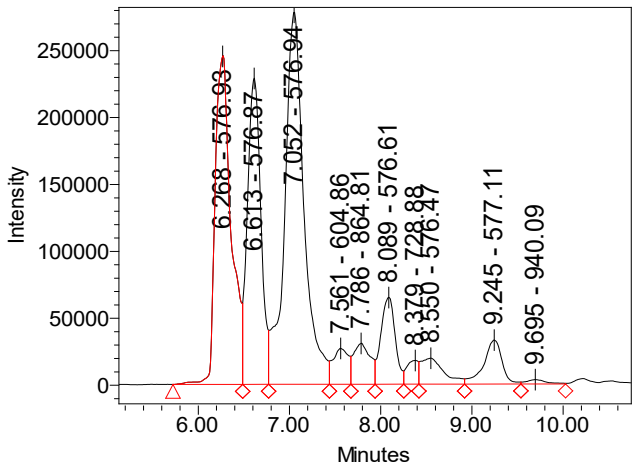


Figure 4. ESI [M-H] – 577 chromatograms of grape seeds

ESI [M-H] - m/z 576.93 is recorded on the chromatogram with a retention time of 6.268 min, with an absorption maximum at 278 nm. By comparing the obtained results, as well as according to the mass database of compounds (https://metlin.scripps.edu), procyanidin B1, (Procyanidin B1) MF: C30H26O12 MW: 578.5 g/mol, IUPAC Name: (2R,3S)- 2-(3,4-dihydroxyphenyl)-8-[(2R,3R,4R)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro2H-chromen-4-yl]-3,4-dihydro-2H-chromene-3,5,7-triol, and ESI [M-H] - m/z 576.87 is recorded on the chromatogram with a retention time of 6.613 min, with an absorption maximum at 278 nm. According to the results obtained, as well as the mass database of compounds (https://metlin.scripps.edu), procyanidin B2 is (Procyanidin B1) MF: C30H26O12 MW: 578.5g/mol, IUPAC Name: (2R,3S)- 2-(3,4-dihydroxyphenyl)-8-[(2R,3R,4R)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro2H-chromen-4-yl]-3,4-dihydro-2H-chromene-3,5,7-triol. (Fig. 4.)

Conclusion

Catechin, epicatechin, procyanidin B1 and procyanidin B2 were identified in the composition of the seeds of the grape variety "Otskhanuri Sapere", which is widespread in Western Georgia, and the dominant anthocyanidin in the composition of the skin is malvidin-3-O-glucoside. Ultrasonic Extraction is used for the extraction of biologically active compounds from seeds and skin.

Recommendations

The grape variety "Otskhanuri Sapere" is a good raw material for the production of preparations rich in biologically active catechins and procyanidins, and in the case of the skin, it is additionally possible to obtain a natural red dye. Biologically active compounds are obtained using Ultrasonic Extraction "green technology".

Conflict of Interest

No conflict of interests is declared.

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