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HARNESSING GRAPE POMACE: NUTRITIONAL ASPECTS, RECOVERY AND EXTRACTION TECHNIQUES FOR HEALTH BENEFITS

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Abstract. Nowadays, grapes represent the third most widely cultivated horticulture crop in the world. For the Republic of Moldova, grapes have been recognized as the most culturally important crop. About 70% of the total production of Moldovan grapes is processed in the wine industry, 30 % of which are by-products that tend to be not fully exploited, being frequently burned or landfilled. Due to its chemical composition, grape pomace is one type of agricultural waste that can be used to achieve sustainability in the food business by converting waste into useful resources. In this sense, the pomace chemical composition, with demonstrated antioxidant potential, is a viable source of biologically active compounds, as a cheap agricultural waste product, for the development of functional products. This paper is an overview of the characteristics and potential uses of wine industry waste, namely grape pomace and explores the implementation of eco-friendly technologies that have the potential to convert this perishable material into a unique ingredient, unveiling fresh opportunities for the grape pomace's utilization and consumption.

Keywords: *extraction, grapes, polyphenols, pomace, sustainability.*

Rezumat. În zilele noastre, strugurii reprezintă a treia cea mai cultivată cultură horticolă din lume. Pentru Republica Moldova, strugurii au fost recunoscuți ca fiind cea mai importantă cultură culturală. Aproximativ 70% din producția totală de struguri moldovenești este procesată în industria vinicolă, dintre care 30% sunt produse secundare care tind să nu fie exploatate pe deplin, fiind frecvent arse sau depozitate. Datorită compoziției sale chimice, tescovina de struguri este un tip de deșeu agricol care poate fi folosit pentru a atinge durabilitatea în industria alimentară prin transformarea deșeurilor în resurse utile. În acest sens, compoziția chimică a tescovinei, cu potențial antioxidant demonstrat, este o sursă viabilă de compuși biologic activi, ca deșeu agricol ieftin, pentru dezvoltarea produselor funcționale. Această lucrare este o prezentare generală a caracteristicilor și potențialelor utilizări ale deșeurilor din industria vinicolă, și anume tescovina de struguri și examinează implementarea tehnologiilor ecologice care au potențialul de a transforma acest material perisabil într-un ingredient unic, dezvăluind oportunități noi pentru utilizarea și consumul tescovinei de struguri.

Cuvinte cheie: *extracție, struguri, fenoli, tescovină, durabilitate.*

1. Introduction

In accordance with the Sustainable Development Goals (SDGs) outlined in the United Nations' 2030 Agenda, which the Republic of Moldova seeks to align with through the discussion of the Environmental Strategy project for the years 2023-2030 [1], one of the major objectives is related to the management of sustainable resources and the reduction of environmental impact. This involves a significant decrease in waste production through prevention, reduction, recycling, and reutilization. In other words, it entails promoting recycling practices of materials such as packaging, agricultural by-products, and other waste generated in agro-industrial processes [2].

At the same time, the European Commission has adopted the sustainability agenda under the framework of the European Green Deal, and the Republic of Moldova, as a country aspiring to integrate into the European Union, has adopted and implemented a series of measures and policies in the field of environmental protection that reflect the principles and objectives of the European Green Deal. As a result, aspects such as Sustainable Agriculture and Circular Economy are incorporated into the legislation of the Republic of Moldova, encouraging sustainability in the agricultural sector and promoting waste reduction and material recycling [3-5].

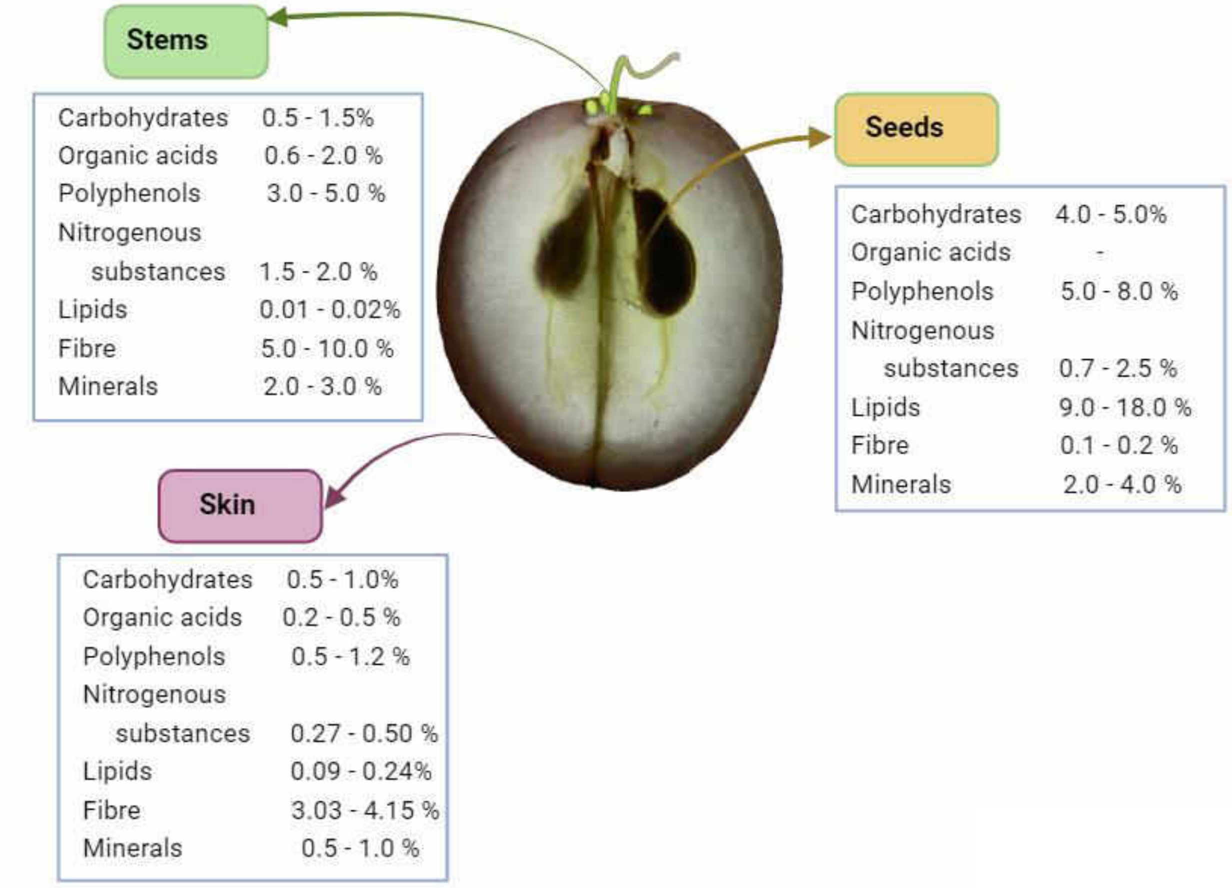
According to several authors and statistics, around one third of the global food production generated by agri-food sector is lost or wasted during processes as handling, processing, transport and final consumption [6,7]. The significant impact of this waste on climate and environment change has been proven by many studies [8,9]. Taking all this into consideration, currently many researches are oriented towards the valorization and reuse of food waste in order to protect the environment and natural resources [10-12].

The Republic of Moldova has a long tradition in wine production, with roots stretching back hundreds of years. The wine sector has always been one of the main pillars of the Moldovan economy[13]. The favorable climatic conditions for the cultivation of vines make the vineyard area cover a significant part of the country's territory[14]. The Republic of Moldova is known for a multitude of native varieties of grapes, which are adapted to the climatic conditions specific to the region. Some of these varieties include: Rara Neagră, Feteasca Neagră, Feteasca Regală, Feteasca Albă, etc. Acording to Bondarciuc et al. (2018), there are 140,000 hectares of grapevine plantings in the Republic of Moldova [15]. It is estimated that about 70% of the grape production in the Republic of Moldova is used in winemaking, thus generating about 30% of their wine waste (20% pomace, up to 7% stalks and 5% wine lees). However, these particular waste parts can serve as the initial raw material for the ingredient production with a high concentration of biologically active compounds [16]. The development of biologically valuable foods and beverages based on secondary grape raw materials containing mineral substances, organic acids, polyunsaturated fatty acids, vitamins, amino acids, pectin substances, etc. is relevant within the context of the modern theory of positive nutrition [17]. The aim of this study is to characterise grape by-product and assess existing sustainable methods for its utilization. This iconic fruit, valued since ancient eras, can be consumed fresh or processed, yet its by-product could be harnessed as a unique food ingredient with untapped potential. The core concept is to make the most of this valuable source of bioactive compounds whenever possible.

2. Grape By-product

Grape by-product could be defined as the solid residue left over after processing grapes to make wine or juices, among other items. It mainly consists of grape skin, seeds, stems, and wine lees. Concerning the proximate composition of grape pomace (Table 1), the main constituents are dietary fibre (grape skin and stems), lipids (seeds), polyphenols and minerals (Figure 1).

Many researches have proven that grape pomace has health-promoting properties (Table 2) being classified as source of biologically active compounds [18-20]. The majority of compounds that exhibit antiradical activity are polyphenols, which are mainly located in skin, stems and seeds, thus most polyphenols are wasted after wine production, according to Moro et al. (2021) this waste can reach up to 70% from the total phenolic content [21]. Phenolic compounds found in grape pomace include phenolic acids, flavonoids, and proanthocyanidins [22]. These compounds exhibit antioxidant, antibacterial and cardioprotective activity [23,24]. Lachman et al. (2015) revealed that linoleic acid was most abundant in grape seed oil, its content ranging between 68.10 and 78.18 g/100 g oil, while the content of linolenic acid was insignificant (0.29 - 0.77 g/100 g oil) [25]. In the same regard, Martin et al. (2020) stated that the share of unsaturated fatty acids from grape seed oil is roughly 90% of the total fatty acid content [26].



Stems	
Carbohydrates	0.5 - 1.5%
Organic acids	0.6 - 2.0 %
Polyphenols	3.0 - 5.0 %
Nitrogenous substances	1.5 - 2.0 %
Lipids	0.01 - 0.02%
Fibre	5.0 - 10.0 %
Minerals	2.0 - 3.0 %

Seeds	
Carbohydrates	4.0 - 5.0%
Organic acids	-
Polyphenols	5.0 - 8.0 %
Nitrogenous substances	0.7 - 2.5 %
Lipids	9.0 - 18.0 %
Fibre	0.1 - 0.2 %
Minerals	2.0 - 4.0 %

Skin	
Carbohydrates	0.5 - 1.0%
Organic acids	0.2 - 0.5 %
Polyphenols	0.5 - 1.2 %
Nitrogenous substances	0.27 - 0.50 %
Lipids	0.09 - 0.24%
Fibre	3.03 - 4.15 %
Minerals	0.5 - 1.0 %

Figure 1. Grape by-product as a source of biologically active compounds [27].
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Regarding fibre, many authors state that fibre have the highest share in the proximate composition grape pomace (60 - 90% of dry matter), the wide range in fibre content being due to variety, soil and clime condition [28–30]. According to Kunzek et al. (2002), there is an ideal fiber ratio, concerning soluble and insoluble fractions (1:3) [31]. In this sense, several studies showed that grape pomace is low in soluble fibre (around 15 % of total fibre amount) [29,32]. However, the higher insolubility of grape pomace fibre opens wide directions in developing functional food products.

Table 1

Proximate composition of grape pomace, % dry matter (DM)

Carbohydrates	Protein	Lipids	Fibre	Mineral	Polyphenols	Reference
29.20±0.02	8.49±0.02	8.16±0.01	46.17±0.80	4.65±0.05	131±0.4 mg/100 g	[33]
1.34 – 55.77	5.4 – 12.3	1.1 – 4.7	17.3 - 53.2	3.3 – 7.6	15.8 – 26.7 mg/g	[29]
19.68	13.8	4.21	51.38	5.55	21.6 – 42.4 mg/g	[34]
2.11 – 50.8	5.3 – 14.0	4.8 – 9.5	26.4 – 59.0	2.9 – 6.3	41.2 ± 1.1 mg/g	[35]

González-Centeno and collaborators [28] determined the configuration of the total dietary fibre of grape pomace indicating pectic substances (40 - 54 % of total dietary fiber) and Klason lignin (20 - 25 %) as principal components. In addition, the study of Deng et al. (2011) demonstrated that white grape pomace was significantly lower in dietary fibre (17.3 - 28.0% DM) than red grape pomace (51.1 - 56.3%) [29].

Although preclinical research on the impact of grape pomace consumption on lipid metabolism, body weight, gastrointestinal health, glucose management and antioxidant activity has found positive effects (Table 2), it has mostly been conducted in animals (mice, rabbits or chickens) [36-40], while few human studies have explored the health benefits of consuming grape pomace.

Table 2

Review of the researches on the grape pomace effect on health

Research characteristics	Results	Reference
Male rats were fed with food comprising 15% grape pomace instead of starchy component.	The presence of grape pomace (15%) in cholesterol diet (0.3%) produced a significant reduction in cholesterol and triacylglycerols in the rat liver and serum.	[41]
The antioxidant activity of pure phenolic compounds from wines and grapes was assessed, through the capacity of inhibition of in vitro oxidation of LDL particles.	Wine and grape phenolic compounds inhibited the oxidation of LDL particles	[40]
Proanthocyanidin from of grape seeds, was tested for its anti-thrombotic effect using *in vitro* and *in vivo* induced thrombosis tests in the mouse carotid artery.	It was shown that grape seed procyanidins, when administered intravenously (20 mg/kg body weight) or orally (2×200 mg/kg body weight), greatly inhibited the formation of laser-induced thrombus in the carotid artery of mice.	[37]
Researchers looked into how grape pomace and seed polyphenol extracts affected the gut microbiota's ability to recover in mice given a high-fat diet following treatment with an antibiotic cocktail.	Compared to the spontaneous recovery group, grape pomace and seed extract improved the relative abundance of gut microbiota. The diversity of the gut microbiota was also significantly changed by grape pomace and seed extract. According to these results, grape polyphenol extracts play a significant role in the gut microbiota's ability to recover following treatment with antibiotics and high-fat diets.	[42]

Continuation Table 2

Mice were given a combination of the usual diet and a mix of grape skin and seeds powder, 14 days prior to the inoculation of Ehrlich ascites carcinoma cells.	The growth of the tumor was tracked, and the effects of extracts from grape skin and seeds on apoptosis and the advancement of the cell cycle were assessed. The results showed that the diet supplementation with mixed seed and skin powders prevented tumor development in the case of 47% of mice inoculated with Ehrlich ascites carcinoma, in the same time a decrease in the tumor volume and weight by 93.9% and 86.3%, respectively was observed.	[38]

Based on the positive properties of grape pomace on human health, industry and scientists have formulated common objectives regarding the creation of new products fortified with grape skin or grape seeds powder in order to increase the biological value of food, Figure 2.

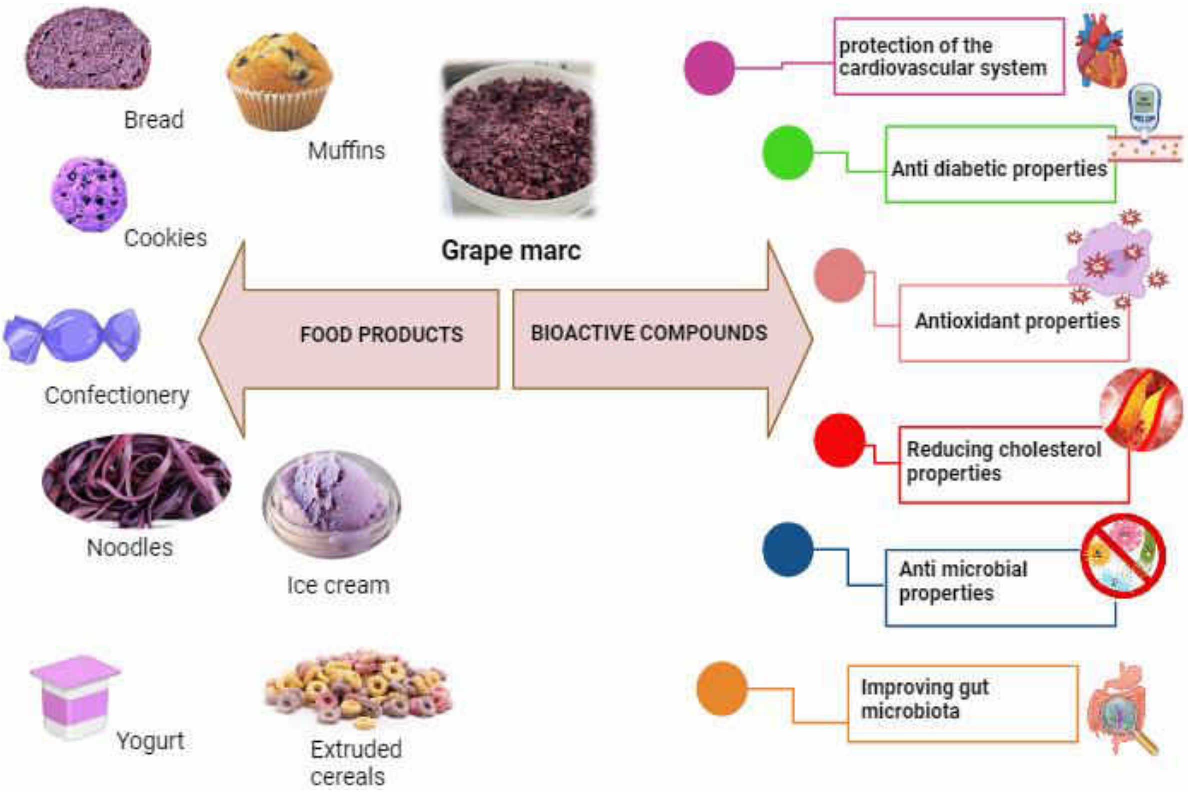

Figure 2. Food products infused with grape skin components and their influence on human health, modified after [43].
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In the food industry, grape pomace and its constituents have traditionally been utilized in powdered form as a nutritional supplement in various foods due to their abundance in phenols, dietary fiber, and anthocyanins [44]. Through a comprehensive review of existing literature, grape pomace and its constituents have been extensively studied for their potential incorporation into a variety of foods including bread [45-48], confectionery [49-51], cookies [52-54], yogurt [55-57], ice cream [58-60], pasta [61-63], noodles [64], fruit candies [65-67], beverages [68-70] and more. Consequently, incorporating grape pomace into foods presents a two-sided outcome, yielding both advantageous and disadvantageous effects on the final products.

2. Innovation for grape pomace recovery and extraction

According to sustainable chemistry, food byproducts are a great source of bioactive substances [71]. The ease of enhancing the functionality of this raw material - that is, making the components that promote health more accessible - suggests that many transformation strategies might be applied.

The nutritional and biological enhancement of food products from the use of by-products is of great relevance due to the benefits of the compounds of these by-products for human health, the economy and the environment [72]. The primary goal of by-product recovery processes is to create new, valuable goods from natural resources while cutting down on waste production and adhering to Green Europe guidelines. Over the past 50 years, new technologies have been created, including pulsed electric fields, enzyme digestion, and ultrasound [39,73,74].

Da Rocha and collaborators [75] demonstrated that utilizing microwave-assisted extraction, employing citric acid solution as solvent system, proved to be a successful method for extracting bioactive compounds from grape pomace. Nevertheless, the levels of phenolic compounds and antioxidant activity were less than those achieved with comprehensive extraction employing methanol solution acidified with acid.

Table 3

Analytical extraction methods of grape pomace compounds

Extraction Methodology	Application	Condition tested	References
Microwave	EXTRACTION OF BIOACTIVE COMPOUNDS	Solvent: 2 % citric acid solution. Microwave power: 600, 800 and 1,000 W. Extraction time: 5, 7 and 10 min. Liquid/solid ratio: 50/1 mL/g.	[75]
	EXTRACTION OF PHENOLICS COMPOUNDS	Solvent types: water or water:ethanol (1:1) solutions. Extraction temperature: 50 ºC. Microwave power: 200 W. Extraction time: 60 min.	[76]
	PECTIN EXTRACTION	Solvent: ultrapure (Milli-Q) water. pH: 1, 2 and 3. Solid−liquid ratio of 1:10 g/mL. Microwave power: 280, 420 and 560 W. Extraction time: 60, 90 and 120 s.	[77]
Ultrasound-assisted extraction (UAE)	Extraction of phenolic compounds	Drying temperatures of 60, 65, 70, 75, 80, and 85 ºC, air velocity of 1.2 m/s. Solvent types - EtOH:H_2O ratios: 50:50, 70:30, MeOH:H_2O ratio: 70:30. Liquid/solid ratio: 8/1 - 24/1 mL/g. Extraction temperature: 20 - 40 ºC. Sonication power: 130W Pulse duration: 5/15 - 2/1.	[73]
	Extraction of pectin	Liquid/solid ratio: 10/1. Extraction temperature: 35, 55, 75 ºC. Extraction time: 20, 40, 60 min. pH of the citric acid solution: 1, 1.5, 2. Sonication power: 140W.	[78]

Continuation Table 3

	Extraction of anthocyanins	Solvent type: 50 % vol. ethanol–water mixture. Liquid/solid ratio: 40:1. Extraction temperature: 20, 45, 65 ºC. Extraction time: (5, 10, 15, 20, 25 and 30 min. Sonication power: 160W. Sonication time: 30 min.	[79]
	Extraction of hemicelluloses	Solvent type: 2M and 4 M KOH solution. Solid:liquid ratio: 1:50 g/mL. Extraction temperature: 20 ºC. Extraction time: 1, 2, 3, 4 and 5 h. Sonication power: 140 W.	[80]
Enzymatic	Extraction of phenolics compounds	Solvent: phosphate buffer saline, pH 7.3. Solid:liquid ratio: 1:9 g/mL. Enzymes: Cellulase and gluco-amylase. Temperature: 55 °C. Time: 24 h.	[81]
	Extraction of phenolics compounds	Enzyme: Pectinex 3XL, Pectinex Ultra SPL, Termamyl, Fungamyl, Pentopan 500BG	[82]
High hydrostatic pressure and enzymatic	Extraction of phenolics compounds	Pressure: 50, 100 and 200 MPa. Extraction time: 0, 5, 10, 15 and 30 min. Enzyme: Carboxymethylcellulase, β-glucosidase, Polygalacturonase. Orbital agitation: 150 rpm. Incubation time: 2, 6, 24 h. Temperature: 24, 30 or 37 °C (depending on the used enzyme).	[83]
Supercritical CO_2	Extraction of anthocyanins	Pressure: 100 bar. Extraction temperature: 95 °C. Extraction time: 30, 60, 90, 120, 150 and 180 min.	[84]
	Extraction o resveratrol	Pressure: 100, 400 bar. Extraction temperature: 35, 55 ºC. Co-solvent: ethanol (5%).	[85]
	EXTRACTION OF OLEANOLIC ACID	Pressure: 25 - 35 MPa. Extraction temperature: 40 - 50 ºC. Co-solvent: ethanol (5%).	[86]

Drosou and collaborators [76] compared the effect of polyphenol extraction methods (using Soxhlet, microwave assisted and ultrasound assisted extraction). The water:ethanol extracts obtained through ultrasound extraction were found to be richest in phenolic compounds (up to 438984 ppm GAE in dry extract) with high Antioxidant activity. Furthermore, Spinei and Oroian [77] applied microwave extraction for pectin recovery from grape pomace and concluded that the ideal parameters for the extraction procedure involved a microwave power of 560 W, a pH level of 1.8, and a duration of 120 minutes. The obtained results suggest that grape pomace holds significant promise as a valuable pectin source, extractable through straightforward and rapid methods, while ensuring comparable quality to traditional pectin sources. Goula, Thymiatis and Kaderides [73] evaluated drying behavior and ultrasound extraction of phenolic compounds from grape pomace and and expressed the combined effect of moisture content and temperature on effective diffusivity by an empirical model. The authors concluded that that employing ultrasound to extract phenolics yielded a maximum of 9.57 mg GAE/g of dry pomace within a 10-minute extraction period. In addition, Minjares-Fuentes and collaborators [78] reported the optimal studied conditions for ultrasound extraction of pectin. Therefore, parameters were established at a temperature of 75 °C for 60 minutes employing a citric acid solution with a pH of 2.0, along with sonication power of 140 W. Specifically, pectic polysaccharides were primarily comprised of galacturonic acid units, accounting for less than 97% of the total sugars.

Bonfigli and colleagues [79] conducted a study on anthocyanin extraction using both conventional and ultrasound-assisted techniques at temperatures of 25, 45, and 65 °C. The results indicated a higher efficiency of ultrasound-assisted extraction, with the maximum concentration of anthocyanins obtained through conventional extraction being 0.475 mg/mL (at 65 °C), while ultrasound-assisted extraction yielded a concentration of 0.479 mg/mL at the same temperatures. Additionally, approximately 80% of anthocyanins were extracted within the first 600 seconds using conventional methods, whereas ultrasound-assisted extraction recovered 90% of anthocyanins within the same timeframe. Another study of Minjares-Fuentes and collaborators [80] imply that ultrasound-assisted extraction may present a viable choice for extracting hemicellulosic polysaccharides from grape pomace on an industrial scale. The optimal conditions for maximizing the extraction yield of hemicelluloses and the levels of xyloglucans, mannans, and xylans were as follows: an extraction time of 2.6 hours, a solid-to-liquid ratio of 1:48 (w/v), and a KOH concentration of 0.4M. These conditions resulted in a maximum extraction yield of approximately 7.9% for hemicelluloses, around 3.6% for xyloglucans, approximately 1.1% for mannans, and roughly 1.2% for xylans.

Enzymatic extraction has been utilised also for phenolic compounds extraction from grape pomace. Kabir et al. [81] found that enzymatic breakdowns, employing cellulase and gluco-amylase, were effective in extracting polyphenols from grape pomace. In addition, the cellulase treatment exhibited notably elevated levels of polyphenolic compounds in the Folin-Ciocalteu's assay, as well as markedly enhanced reductive activities in DPPH radicals, in comparison to the gluco-amylase treated pomace. Ferri et al. [82] conducted a study with the aim of optimizing a two-stage enzymatic and solvent-based process to extract bioactive compounds from white grape pomace. In their research, they utilized six commercial enzymes (Pectinex 3XL, Pectinex Ultra SPL, Termamyl, Fungamyl, Pentopan, Celluclast) for the extraction of both wet and dry pomace, followed by ethanol extraction. The findings indicated that ethanol-based extraction of wet and dry pomace yielded higher amounts of phenols compared to water extraction, with observed variations in their compositions and bioactivities.

Cascaes Teles et al. [83] conducted a study to evaluate the impact of enzyme-assisted extraction and high hydrostatic pressure on the retrieval of phenolic compounds from grape pomace. They applied these methods individually as well as in combination to the pomace. The results revealed that high hydrostatic pressure significantly enhanced the effectiveness of the enzymes used in extraction, increasing their activity by up to 16 times. Techniques incorporating high hydrostatic pressure were found to be more efficient compared to relying solely on enzyme-assisted extraction. Consequently, the findings suggest that employing high hydrostatic pressure could offer an efficient and cost-effective means of recovering phenolic compounds from grape pomace, particularly when compared to more complex and prolonged processes.

Pazir et al. [84] investigated the use of supercritical carbon dioxide extraction for the retrieval of anthocyanins from grape pomace. Conditions were settled at 95 ◦C, whereas pressure was established at 100 bar. The evaluation of the total monomeric anthocyanin content and total antioxidant capacity was performed at the 30th, 60th, 90th, 120th, 150th, and 180th min. Since around 63% of the monomeric anthocyanin content in the red grape pomace samples was extracted by the end of the extraction process (180 min), while 47% was achieved within the initial 30 minutes, the authors conclude, that there is no need to continue the extraction beyond 90 minutes.

Casas and collaborators [85] proposed supercritical carbon dioxide as a method for the extraction of resveratrol from grape components. The impact of varying pressure (100, 400 bar), temperature (35, 55 °C), and the inclusion of a modifier (5% *v/v* ethanol) was assessed to determine the most effective method for extracting resveratrol. The most favorable outcomes, in mg resveratrol/g extractor, (5.97 in grape seeds, 1.12 in stems, 21.35 in grape skin, 10.73 in pomace) were observed when operating at high pressure (400 bar), low temperature (35 °C), and incorporating 5% *v/v* ethanol as a co-solvent. Chronopoulou and collaborators [86] investigated the application of supercritical CO_2 extractions to obtain oleanolic acid from grape pomace and that this method effectively retrieved oleanolic acid from grape pomace samples, with an extraction yield comparable to established extraction techniques like Solid Liquid extraction, which can sometimes have drawbacks.

3. Conclusions

The grape stands as one of the primary crops on a global scale. Annually, the wine industry produces hundreds of tons of grape pomace, typically disposed of as waste. However, this by-product is recognized as a natural reservoir of bioactive compounds with significant potential health benefits. Employing eco-friendly technologies on grape by-products offers a fresh perspective for maximizing the value of grape pomace to preserve and enhance its functionality and nutritional properties. Various methodologies are explored to compare conditions and identify the primary target bioactive compounds. Following processes like enzyme-assisted extraction, supercritical fluids, microwaves, or ultrasound treatments, there appears to be a viable opportunity to convert this perishable material into a valuable source of health-enhancing compounds such as phenols, anthocyanins, and pectins. Consequently, the grape, with its historical significance, can be fully utilized, extending its potential to the often-overlooked grape by-product, thus transforming it into a newly recognized value-added product.

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