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Phenolic Compounds Recovery from Grape Fruit and By-Products: An Overview of Extraction Methods

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Abstract

Phenolic compounds are considered as bioactive compounds having beneficial effects on human health. Because of their biological properties, they have wide applications on pharmaceutical and food industries, and for this reason, it is important to identify most appropriate procedures, which permits the standardization for recovery of these compounds from several plant materials including grapes. Grape fruit and by-products are excellent sources of bioactive compounds such as pigments, organic acids, and phenolic compounds. Several convectional and emerging technologies have been evaluated in order to recover phenolic compounds from grape fruits and wastes such as chemical, physical, and biotechnological techniques, which offer different advantages related to economic, environmental, time-saving, and yield aspects. Nowadays, there is no updated information, which provides an overview about the techniques applied of these bioactive compound recovery in order to obtain high-quality and high-activity extracts rich in phenolic compounds from grape fruit and by-products. This chapter offers relevant aspects related to the techniques employed during the last five years by researches for phenolic compound recovery from grapes.

Keywords: phenolic compounds, grape fruit and by-products, extraction methods, emerging technologies

1. Introduction

Bioactive compounds are extra-nutritional components that naturally occur in small quantities in plant and food products. Most common bioactive compounds include secondary metabolites such as antibiotics, mycotoxins, alkaloids, food grade pigments, plant growth factors, and phenolic compounds [1]. Phenolic compounds are considered as bioactive compounds having beneficial effects on human health by decreasing the incidence of some degenerative diseases, such as cancer, diabetes, and reducing the risk factors of cardiovascular diseases. In addition, phenolic compounds have other biological properties such as inhibitors of cellular proliferation [2]. Because of their biological properties, phenolic compounds have wide applications on pharmaceutical, chemical, and food industries, and for this reason, it is important to identify the most appropriate procedures, which permits the standardization and/or optimization for recovery from these compounds, which are considered as the most abundant antioxidants in berries including grapes. Grape is the most widely cultivated fruit crop in the world with a global production of around 69 million tons, being Europe the biggest producer [3]. Grape fruit and by-products are an excellent source of bioactive compounds [4] such as pigments, organic acids, and phenolic compounds. Several emerging or conventional technologies have been evaluated in order to recover phenolic compounds from grape fruits and wastes such as chemical, physical, and biotechnological techniques, which offer different advantages related to economic, environmental, time-saving, and yield aspects. These techniques including ultrasound, microwave, micro- and ultra filtration, supercritical fluids, and electric fields assisted extraction. In addition, Soxhlet method, pressurized hot water, and the use of different organic solvents had been reported for this proposal. Moreover, enzyme technology and solid-state fermentation have been successfully applied for phenolic extraction from grape samples with important environmental advantages. Nowadays, there is no updated information, which provides an overview about the techniques applied of this bioactive compound recovery in order to obtain high-quality and high-activity phenolic compounds from grape fruit and by-products. This chapter offers relevant aspects related to the techniques employed during the last five years by researches around the world for phenolic compound recovery.

2. Soxhlet method

Soxhlet is equipment for extracting bioactive compounds, generally from lipid nature. It was invented by Franz von Soxhlet in 1879. Nowadays, the Soxhlet extraction represents the classical methodology for lipophilic compounds extraction [5]. For more of one century, this methodology has been used for different purposes and is described as the universal chemical extraction process [6]. Nevertheless, by itself, it is an optimized extraction process, but the literature offers a high amount of practical examples from bioactive compound extraction using different Soxhlet extraction conditions. However, this methodology requires large extraction times and quantities of solvents. The solvents more used are methanol [7, 8], etano [9, 10], n-hexane [7, 11], petroleum ether [12], toluene, chloroform [13], benzene, diethyl ether,

dichloromethane, acetone, isooctane, cyclohexane [14], isopropanol [15], and water (for comparison only).

Grape variety	Solvent	Conditions	Yield	Antioxidant activity	Reference
Ruby Cabernet	Methanol	1:6 w/v 16 h	8.2% g/100 g d.b.	11.62 µg/mL (IC ₅₀ for AAPH)	[8]
Agiorgitiko	Ethanol	3:100 w/v 2–3 h	15% d.b. (approximately)	1.35 ± 0.02 (IC ₅₀ mg/mL)	[9]
	Water	3:100 w/v 5–6 h	Up to 24.35 ± 0.34% d.b.	2.02 ± 0.02 (IC ₅₀ mg/mL)	
NR (from Serralunga d' Alba, Italy)	Ethanol	6:85 w/v 18 h	7.7 ± 0.2 mg _{GAE} /g from seed fraction 11.9 ± 0.3 mg _{GAE} /g from skin fraction	NR	[10]
NR (from Friuli Venezia-Giulia, Italy)	n-hexane	1:12 w/v	15.6 ± 1.2% d.b.	678 ± 15.5	[7]
	Methanol	6 h at 70°C		mg _{α-tocopherol} 100 g ⁻¹	
Raboso Piave	n-hexane	1:12 w/v 6 h at 70°C	14.64 ± 0.29% d.b.	97.24 ± 0.35 Eq a Toc/g flour	[11]
Tempranillo (GSEJ)	Water	5 h 80–90°C	6.04 ± 0.69 g _{GAE} /L ⁻¹	57.48 ± 3.61% Inhibition (DPPH) 36.57 ± 2.26 mg TROLOX L ⁻¹ (FRAP)	[65]
Tempranillo (GSEW)			2.41 ± 0.34 g _{GAE} /L ⁻¹	40.35 ± 4.64% Inhibition (DPPH) 23.89 ± 5.55 mg TROLOX L ⁻¹ (FRAP)	
Gamay			2.09%	151.8 µg/g for IC ₅₀ DPPH	[66]
Kalecik	Ethanol:	1:1 w/v	2.49%	189.6 µg/g for	
Karasi	water (95:5)	60°C for 8 h		IC ₅₀ DPPH	
Okuzgozu			2.63%	109.8 µg/g for IC ₅₀ DPPH	

NR, non-reported; GAE, gallic acid equivalents; d.b., dry basis; TPC, total phenolic compounds; GSEJ, grape seed extracts from juice; GSEW, grape seeds extracts from wine.

Table 1. Comparison of different organic solvents for phenolic compounds extraction from different varieties of grape residues.

Soxhlet method is based on the separation of a specific fraction from several food or plant materials with the use of a polar solvent depending on the solubility characteristic of the target compounds and the physicochemical nature of source, which can determine the surface contact and diffusivity of the solvent into the samples. Grape pomace is a waste product of grape juice and wine industry. These by-products contain high phenolic compounds because of poor extraction during the winemaking processes; hence, it makes their utilization worthwhile. In the last years, a large number of investigations have been conducted in order to find the best conditions for extraction of bioactive compounds from agroindustrial waste including grape waste as shown in **Table 1**. However, only this extraction method is used as comparison to replace the use of organic solvents.

3. Pressurized hot water extraction (PHWE)

As an alternative to use solvents in the several extraction methods, PHWE promotes the reduction or elimination of organic solvents into extraction processes. It improves the extraction process due to the water is non-flammable, non-toxic, available, and eco-friendly solvent [16]. High-pressure processing is a technology that has shown good prospects to extract bioactive compounds from several agroindustrial wastes [17]. PHWE is a non-conventional extraction method based on the extraction of molecules using hot liquid water as solvent. This technique is based on the use of temperatures above 100°C and 0.1 MPa, but lower than its critical point (374°C and 22.1 MPa) [18]. In addition, it is a highly promising energy-efficient and eco-friendly technique for recovering phenolic compounds from several sources [19]. However, in the beginning, it was not well received as analytical extraction solvent because the water is too polar to efficiently dissolve most target compounds. But, with PHWE, the water properties (polarity, viscosity, and surface tension) can be manipulated to optimize the phenolic extraction [20]. This manipulation improves the mass transfer rate and disrupts the water surface equilibrium, thereby lowering the activation energy required for desorption process [16]. When water is used as solvent, PHWE technology could also be designated as subcritical water extraction (SWE), superheated liquid extraction (SHLE), and pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE) [21]. In recent years, several methodologies have been applied to the extraction of bioactive compounds of grape waste. Among them, the PHWE is a viable alternative, economic, with low-energy consumption, and eco-friendly. Most recently, possible scale up has been proposed by [9] with the use of a 10 l reactor to extract bioactive compounds from *Withania somnifera*. More recently, a new technique called high hydrostatic pressure has been successfully applied for phenolics recovery and microbial control, which could considerate to improve wine quality [22]. **Table 2** summarizes the last 5 years of investigation in the recovery of bioactive compounds from grape pomace. These investigations show that it has been possible to replace organic solvents with high yields and high antioxidant capacity. Therefore, it is an alternative extraction technique for application in pharmaceutical, food, and biotechnological industries.

Grape variety	Solvent	Conditions	Yield	Antioxidant activity	Reference
Cabernet Sauvignon	Hot water	1:10 w/v 5 min at 100°C	65.58 mg/g d.e	10.2 mg AAE/g d.e.	[19]
		1:10 w/v 5 min at 200°C	20.38 mg/g d.e	15 mg AAE/g d.e	
NR	Ethanol/water (70:30 vol%)	100 bars 140°C 5 cycles × 0 min each	7.28 g GAE/100 g d.b.	Up to 49.12% (DPPH) compared to resveratrol 50 µg/mL	[67]
Cabernet Sauvignon	Hot water	1:10 w/v 50–200°C 5–30 min	Up to 4.1 mg GAE/g dp	Up to 4.4 mg AAE/g dp (FRAP) Up to 184 mg TE/g dp (DPPH)	[68]
White Zinfandel	Hot water	Lower than 60 psi 140°C 9 mL/min water flow rate	130 mg/100 g d.b. (anthocyanins) 2077 mg/100 g d.b. (procyanidins)	NR	[69]
Sunbelt	80% aqueous ethanol	10.3 MPa 124°C 1 min	9.65 mg/100 g d.b. (Flavonols)	NR	[70]

NR, non-reported; GAE, gallic acid equivalents; d.b., dry basis; TPC, total phenolic compounds; d.e., dry extract.

Table 2. Summary of conditions and solvents for phenolic compounds extraction from different varieties of grape residues.

4. Ultrasound-assisted extraction (UAE)

Ultrasounds are sound waves of very high frequency (20 kHz to 100 MHz), which are propagated via compression and rarefaction, and require a medium (i.e., tissue) in which to travel. Ultrasound-assisted extraction makes use of high-intensity ultrasonic energy created by the implosion of cavitation bubbles. The bubbles collapse can produce physical, chemical, and mechanical effects. When this energy reaches the surface of the raw material through the extraction solvent, it is transformed into mechanical energy that is equivalent to several thousand atmospheres of pressure [23]. The high-pressure breaks the material particles, destroys cell membranes, improves penetration of solvent, and increases the contact surface area between the solid and liquid faces resulting in the release of phenolics to the extraction solvent in a relative short time [23, 24]. UAE is a simple, environmentally friendly, and efficient alternative to conventional extraction techniques [25]. The method's main advantages are simplicity of use and low instrumental requirements [23, 24, 26]. Ultrasonic devices include an ultrasonic bath, mainly used for small-scale extractions, or an ultrasonic probe system for large-scale industrial extractions (**Figure 1**) [26–29]. There are some previous applications of

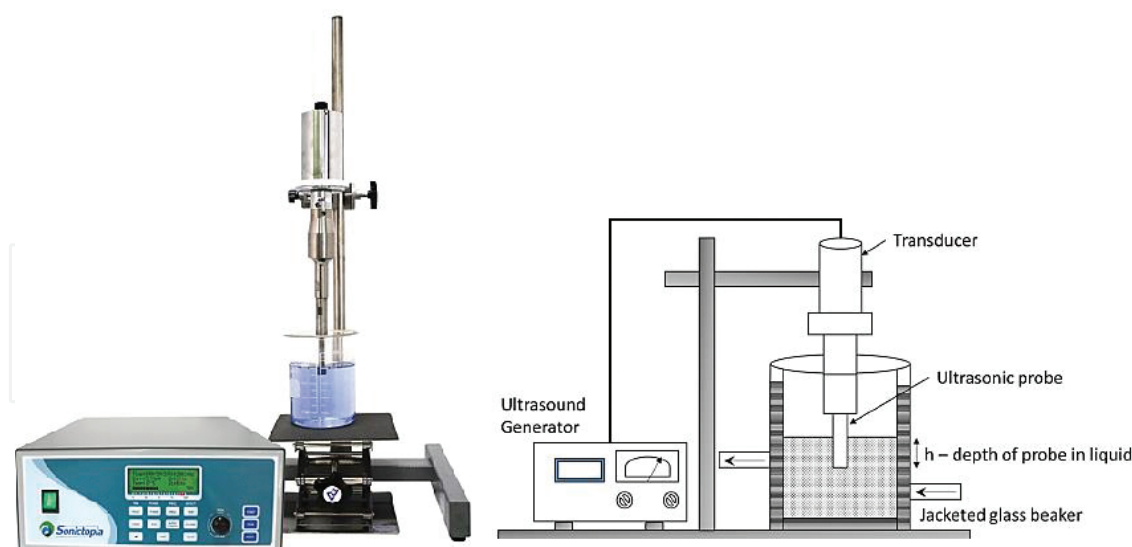


Figure 1. Schematic representation of an ultrasound-assisted extraction equipment.

UAE in the determination of phenolic compounds on specific parts of grapes. Ghassempour et al. [30] worked with red grape skin and the recuperation of anthocyanins. They compared UAE and microwave-assisted extraction (MAE), and the results showed that UAE has a slight lower recovery than MAE. Moreover, in their study, González-Centeno et al. [31] evaluated UAE as an extraction method for the quantification of total phenolic content and antioxidant capacity from grape pomace using an ultrasonic water bath. According to the results, the UAE resulted to aqueous extracts with phenolic and antioxidant characteristics similar to those obtained with conventional extraction, working under lower temperature conditions, and during less operating time (eight times less time than the conventional method).

Grape seeds have also been an interesting sample for UAE-based methods. Tao et al. [32] evaluated the effects of acoustic energy density (6.8–47.4 W/L) and temperature (20–50°C) on the extraction yields of total phenolics and tartaric esters during UAE from grape marc and demonstrated that ultrasound is an effective and promising technology to extract these kind of bioactive substances from this source. Ultrasound energy for extraction also facilitates more effective mixing, faster energy transfer, reduced thermal gradients and extraction temperature, selective extraction, reduced equipment size, faster response to process extraction control, quick start-up, increased production, and eliminates process steps among others [33]. Thus, the advantages of UAE include reduction in extraction time, energy, and use of solvent, which is reflected on economic and environmental aspects in the recovery of bioactive compounds.

5. Microwave-assisted extraction (MAE)

The fundamentals of the microwave extraction (MAE) process are different from those of conventional methods (solid–liquid or simply extraction) because the extraction occurs as the result of changes in the cell structure caused by electromagnetic waves. Microwave energy is

a non-ionizing radiation that covers a third order of magnitude scale from 300 MHz to 300 GHz [34]. The principle of heating using microwaves is based on its direct effects on molecules of the material. Electromagnetic energy is converted to heat following ionic conduction and dipole rotation mechanisms (**Figure 2**) [35]. MAE process is assumed to involve three sequential steps [36]: (i) separation of the solutes from the active sites of the sample matrix under increased temperature and pressure, (ii) diffusion of solvent across the sample matrix, and (iii) release of the solutes from the sample matrix to the solvent. The operating conditions could be related to the success on the efficiency of this process; in this sense, parameters such as solvent extraction, temperature and time of the extraction, microwave power, and the physicochemical characteristics of the material should have special attention as they could potentially influence the recovery of target compounds [37, 38].

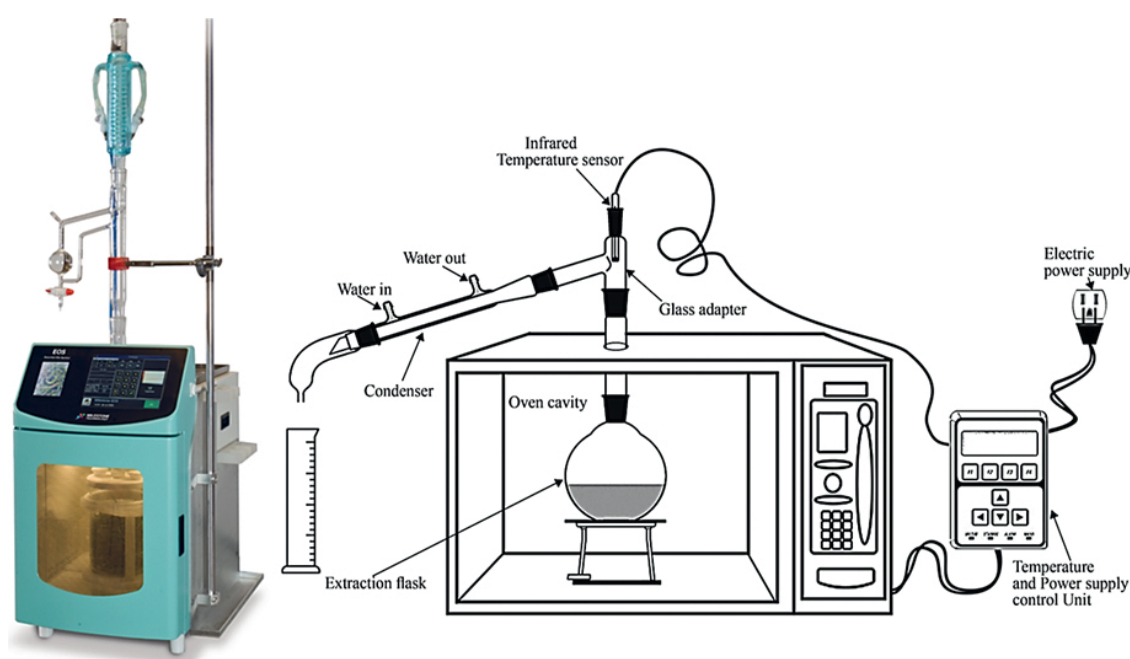


Figure 2. Schematic representation of a microwave-assisted extraction equipment used at laboratory scale.

The potential of MAE to recover high-added value compounds from winery wastes and by-products was investigated by several research groups. For instance, in their study, Liazid et al. [39] shown a remarkable reduction in the time applied from 5 h to 5 min that could be achieved with MAE compared to conventional extraction method when this technology was apply on grape skins for anthocyanins recuperation. Moreover, with this method, three additional acyl derivatives were extracted and quantified, while with the conventional method, it was impossible to measure. Bittar et al. [40] produced grape juice rich in polyphenols by MAE. Microwave-assisted process was evidenced to possess the highest values of TPC (21.41 ± 0.04 mg GAE/g DW) and TAC (4.49 ± 0.01 l g MVGE/g DW). In addition, Li et al. [41] developed a microwave-assisted extraction (MAE) method for the extraction of phenolic compounds from grape seeds of *Vitis vinifera*. To optimize the extraction, it was considered the

ethanol concentration in the extraction solvent, liquid: solid ratio, time, power, and temperature. The results obtained revealed that the optimal extraction conditions were ethanol concentration (47.2%), liquid: solid ratio (45.3:1), and time (4.6 min). The total phenolic content also was determined. Sequential application of the optimal conditions to one sample revealed that approximately 92% of the total phenolics were extracted in the first instance. Concluding that, MAE provides comparable or better extraction and it was very much quicker than other extraction methods.

6. Membrane separation

Membranes can be defined as semipermeable barriers that separate two phases and restrict the transport of defined components in a selective manner. The transport of components through the membrane is achieved by applying a driving force (concentration gradient, pressure, temperature, or electric potential). Thus, membrane separation processes use semipermeable membrane of definite nature to separate the components of a solution based on molecular size differences. In every membrane separation process, there is a membrane that is placed between two phases. One phase is called feed and the other is called permeate. When the feed consists of equal to or more than two components, and some of those components flow faster than others through the membrane, separation of the feed mixture takes place (**Figure 3**) [42, 43].

Microfiltration (MF) and ultrafiltration (UF) have already been widely used in the recovery, concentration, and fractionation of value-added products from agroindustrial wastes [44, 45]. Nevertheless, the use of membrane separation processes for the recovery of value-added products from wine lees is still a matter of research. In general, MF membranes rate according to nominal pore sizes, which are in the range of approximately 0.1–10 μm and operate at very low pressure, typically 10 psi or less, while UF membranes have molecular weight cut-off values between 1000 and 300,000 Da and pore diameters in the range of $\leq 10\text{ nm}$ –0.1 μm and typically operate at pressures ranging from 15 to 100 psi [28, 46].

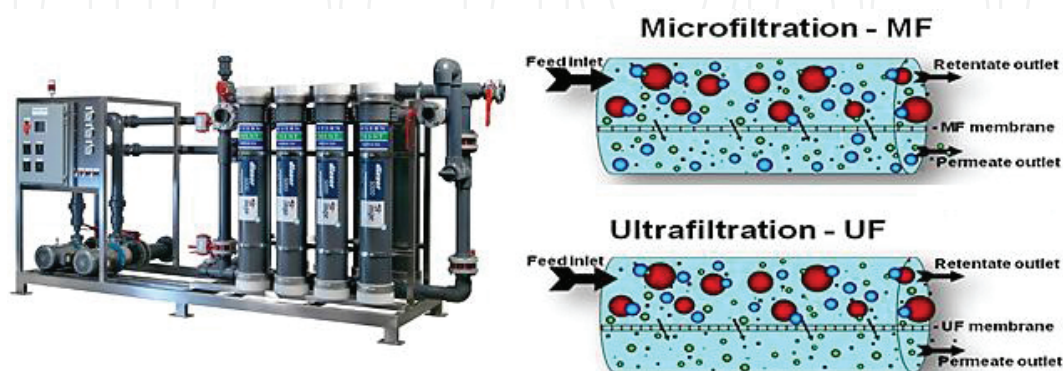


Figure 3. Schematic representation of membrane-based separation process.

In their study, Galanakis et al. [47] evaluated ultrafiltration processes in application of recovery, concentration, and fraction of polyphenolic compounds extracted from winery sludge. Ultrafiltration removes high molecular-weight substances, colloidal materials, and organic and inorganic polymeric molecules (i.e., soluble dietary fibers or polysaccharides) from low molecular-weight organics and ions (i.e., phenols, simple sugars) in a non-destructive way. In the mentioned work were tested three membrane types (100- and 20-kDa poly sulfone, 1-kDa fluoropolymer), and the results indicated that solute retention was affected mainly by severe fouling phenomena due to polar solutes adsorption on membrane surface instead of size exclusion. Finally, it was separated successfully hydroxycinnamic acid derivatives from anthocyanins and flavonols. Giacobbo et al. [48] investigated the aqueous extraction associated with microfiltration for the recovery of phenolic compounds present in the effluent of wine lees. They proposed that effluents are rich in polyphenols and can be potential sources. Therefore, authors worked first in reducing the charge of the suspended solids and then used this permeate in an ultrafiltration process (V0.2 and MFP5 membranes) with dilutions combined with vacuum filtration. At the optimal conditions, a solution diluted 10 (v/v) followed by microfiltration led to the achievement of a limpid permeate, rich in phenolics obtaining a recovery rate of 21% of the total content of phenolic compounds. The results demonstrated that this technology is up to 6 times more efficient than others. On the other hand, Fernández et al. [49] studied the maximization of the permeate flux in the purification by ultrafiltration of a grape seed extract, by evaluating the effect of operating variables: transmembrane pressure and tangential velocity on permeate flux and on the extracts chemical characteristics. The authors concluded that the UF process (10-kDa membrane to 5 bar and 1.3 m/s) reduced the mean degree of polymerization of the extracts from 7.15 up to 1–3 units of flavan-3-ols, corresponding to dimmers and trimmers in the permeate. Those evidences stand out membrane separation as an attractive alternative for recovery specific phenolic compounds from grape fruits and wastes.

7. Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is a technique that uses supercritical fluids (systems formed by one or more compounds at conditions over their critical values of pressure and temperature) as an extraction solvent in the separating one component (the extractant) from another (the matrix). In this process, the mobile phase is subjected to pressures and temperatures near or above the critical point for the purpose of enhancing the mobile phase-solvating power [50]. The supercritical fluid is used as an alternative to traditional organic liquid solvents. The most widely used supercritical fluids are CO₂ (T_c = 31°C, P_c = 74 bar) and water (as above described) (T_c = 374°C, P_c = 221 bar), but some processes involve the use of supercritical methanol, ethanol, propane, ethane [51, 52]. A basic SFE system consists of the following parts: the delivery system of supercritical fluid is very important because a high purity is required. The pumps employed in supercritical fluid extraction must be able to drive carbon dioxide at high pressures required, maintaining a constant flow. A heater capable of controlling the temperature in the furnace, and a cell or stop able to withstand the pressures generated by the pump is required. The most

important part is the restrictor which controls the flow of the supercritical fluid flowing through the cell and, moreover, is responsible for depressurizing the fluid by passing existing supercritical conditions in the cell extraction atmospheric conditions. Finally, the collection system of solute is responsible for increasing the fluid density and hence its solvent power decreases, achieving the separation of the solute and fluid (usually is achieved by depressurizing the fluid) [53]. A symmetric diagram of typical SFE instrumentation is given in **Figure 4**.

Most of the studies evaluating the potential of SFE to recover valuable compounds from grape by-products have been focused on seed oil and proanthocyanidins recovery. In this line, Oliveira et al. [54] proposed an increase in aggregated value of the huge amount of residues generated by wineries. In their study, it was evaluated the global extraction yield, the antimicrobial activity and the composition profile of Merlot and Syrah grape pomace during the application of supercritical fluids supplemented with a co-solvent (300 bar at 50 and 60°C). Even though the extraction yields were remarkably low, the supercritical fluid extracts presented the highest antimicrobial effectiveness (against four strains of bacteria) compared to the other grape pomace extracts due to the presence of bioactive compounds. Da Porto et al. [7] evaluated supercritical carbon dioxide (SC- CO_2) extraction of grape marc using water (W) and ethanol (EtOH) as co-solvent at 15% (w/w), 100, and 200 MPa, and 313.15, 323.15, and 333.15°K to analyze their influence upon total phenols of the extracts. Supercritical extraction obtained the highest phenolic yield (68.0 g/kg of extract), phenol content (733.6 mg GAE/100 g DM), proanthocyanidins concentration (572.8 mg catechin/100 g DM), and antioxidant activity (2649.6 mg—tocopherol/100 g DM) in comparison with conventional extraction.

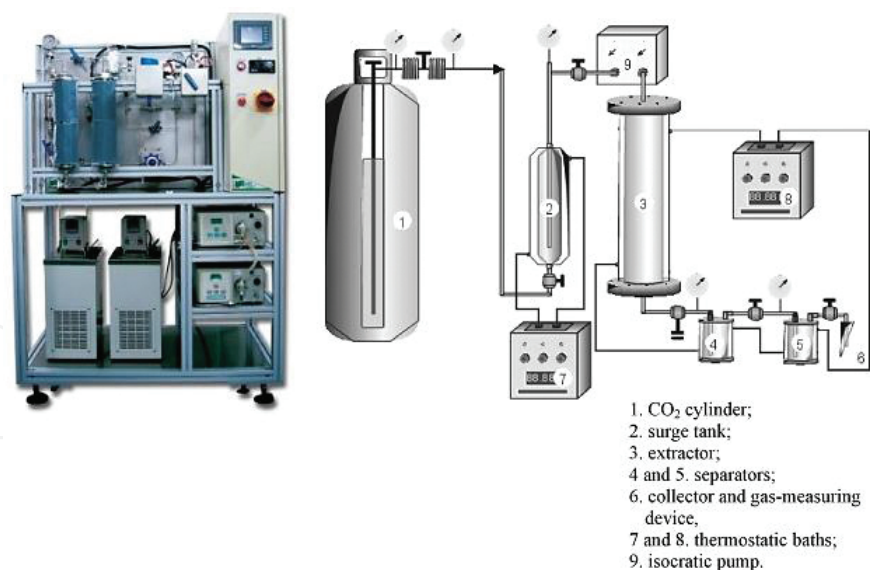


Figure 4. Schematic representation of a supercritical fluid extraction (SFE) system.

Rombaut et al. [55] compared three seed oil extraction methods (screw pressing, extraction by supercritical CO_2 percolation, and the combination of these two processes) and evaluated their efficiency for producing oil rich in phenolic compounds. The results suggested that the processes using supercritical CO_2 permit an increase in the co-extraction of phenolic with oil.

By combining a uniaxial compression with supercritical CO₂, oil yield is enhanced from 0 (hydraulic pressing, without supercritical CO₂) to 35%. On the other hand, Fariás-Campomanes et al. [56] evaluated the economic feasibility of large-scale operations of supercritical fluid extraction (supercritical CO₂ containing 10% ethanol (w/w) at 313°K and 20–35 MPa) for the recovery of phenolics using grape bagasse. The supercritical CO₂/ethanol extraction process produced extracts with higher concentrations of phenolics (23 g/kg) than that extracts produced using conventional techniques with an economic evaluation of the process that estimated a cost of manufacturing of US\$ 133.16/kg. However, more investigation about the effect of this extraction technique on functionality and change of the extracted-phenolics and its application in biotechnological processes is needed.

8. Pulsed-electric field extraction (PEF)

Exposing a plant cell to a high-intensity electric field (kV/cm) in the form of very short pulses (μs to ms) induces the formation of temporary or permanent pores on the cell membrane. This phenomenon, named electroporation, causes the permeabilization of cell membrane and increases its permeability and if the intensity of the treatment is sufficiently high, cell membrane disintegration occurs. During a pulsed-electric field extraction, the material is placed between two electrodes forming a treatment chamber and high-voltage-repetitive pulses are applied across the system in order to achieve membrane breakdown. Pulse amplitude in PEF equipment is ranging from 100 to 300 V/cm to 20–80 kV/cm [57]. Normally, PEF treatment is conducted at ambient temperature or slightly higher than the ambient temperature and for a treatment time less than 1 s (ms or μs) [58]. A PEF system, in general, consists of three basic components: a high voltage pulse generator, a treatment chamber, and a control system for monitoring the process parameters. In recent year, PEF technology has been mostly investigated in the recuperation of winery wastes and grape skin polyphenols. The recovered antioxidative compounds depend on the nature of raw materials, and in particular, the tissue structure of the source and the PEF treatment conditions applied. El Darra et al. [59] evaluated the influence of PEF (0.8–5 kV/cm, 1–100 ms, 42–53 kJ/kg) on the recuperation of phenolic compounds from Cabernet Franc grapes and its relationship with the process fermentation compared with a conventional treatment (50°C for 15 min). The study showed a significant improvement in phenolics extraction (anthocyanin and tannin contents), color intensity, and scavenging activity of the samples during red wine fermentation after applying PEF (51–62%) and thermal treatments (20%). In addition, Delsart et al. [60] determined the presence of phenolic compounds from Merlot grapes and the effect of PEF on the fermentation process of this grape variety and the related wine characteristics. The experiments focused in the application of PEF treatments (500–700 V/cm) with times of incidence of 40–100 ms where the measured responses were color intensity, anthocyanins, and phenolic content during the alcoholic fermentation and seven months during storage. The results suggest that pulsed electric field treatment has the advantage of nonthermal-selective extraction (<5°C) involving no loss of product quality in with respect to the classical process.

Other authors investigated the application of PEF treatment combined with densification to recover phenolic compounds from grape pomace of low moisture content, without any addition of conductive liquid [61]. Moreover, they studied the influence of a supplementary hydro-alcoholic extraction under various temperatures. The results indicate that PEF treatment (1.2 kV/cm, 18 kJ/kg) in grape pomace (1 g/cm³) allows greater recovery of polyphenolic compounds for this matrix. Also, it was determined that this technology allows more selective recovery of anthocyanins after applying the treatment and finding compounds like anthocyanins/total flavan-3-ols at 20°C of 7.1 and 9.0, for control and PEF-treated samples, respectively. With the above aspects in view and the improvement of later biotechnological stages on winemaking, PEF could be considered as a good technique of enhancement of phenolic compounds on wine industry products.

9. Biotechnology applied on phenolic releasing from grape waste

Biotechnological releases of phenolic compounds from plant materials are associated to the degradation of cell-wall polysaccharides for microbial enzymes by use crude enzymatic extracts or purified commercial enzymes, which are able to eliminate this physical barrier and opens up the cell. Biotechnology techniques such as enzyme technology and solid-state fermentation have been successfully applied for phenolic extraction from grape samples with important environmental advantages. In addition, the enzymatic extraction method excludes the use of xenobiotics or toxic reagents, something that must also be taken into account, as it is more environmentally friendly [62].

In this sense, it has been reported that the use of cell-wall hydrolyzing enzymes can significantly increase the release of phenolic compounds from grape skins and seeds in a very short time. In a compressively study, Xu et al. [63] reported that β -glucosidase and pectinase can increase the releasing of total phenolic compounds from grape skins at 12 and 72%, respectively, when compared to the control. Therefore, both enzyme types could be considered to achieve an effective enzyme method for releasing phenolics from grape skins. Those findings are according to Fernández et al. [64], who observed an increment on phenolic compounds of 1.26-, 1.32-, and 1.34-fold when pectinase, cellulose, and tannase were used for describing the enzymatic effect of such enzymes on grape skins and seeds. In addition, due to the complexity of plant cell structure, combinations of those enzymes were evaluated; however, significant a significant effect on phenolic releasing was not observed. According to these studies, pectinase could be the enzyme, which allows the major amounts of phenolic compounds may be due to the high pectin content on this material. More recently, endoprotease mixtures have studied in order to obtain not only phenolic compounds, but also other functional biomolecules with anti-inflammatory and antioxidant capacities such as peptides. From this, flavonoids (flavanols and flavonols) and phenolic acids were observed as the main phenolic compounds present in grape pomace [62]. **Table 3** summarizes some contribution on biotechnological releasing of phenolic compounds from grape wastes. Nevertheless, it is important to observe that just one study regarding to the application on the solid-state fermentation of phenolic recovery from grape by-products was found, which indicates that the application of this process is still a

matter of research for production of bioactive compounds from such agroindustrial material. However, biotechnological tools are very important environmental advantages since it reduces the use of xenobiotics or toxic reagents used in the recovery of phenolics in other techniques.

Biotechnology technique	Grape tissue	Enzyme/ microorganism	Phenolic compounds	Reference
Enzymatic	Whole grape pomace	Trypsin and chymotrypsin mixture	C, EC, Q, PB1, PB2, K, R	[71]
	Whole grape waste	Commercial enzymatic preparation (Novoferm)	GA, Rs, OCA	[72]
	Seeds	Cellulase	Do not identified for this enzyme	[63]
		β -Glucosidase	EA, EAH, ECG, EC and GA	
		Pectinase	Do not identified for this enzyme	
	Skin	Pectinase	EGC-P, EC-P, ECG-P and C	[64]
	Seed		C-P, EC-P, C, ECG-P, EC and ECG	
	Skin	Cellulase	EGC-P, C-P, EC-P, C and ECG-P	[62]
	Seed		C-P, EC-P, C, ECG-P, EC and ECG	
	Skin	Tanasse	EGC-P, C-P, EC-P, C and ECG-P	
Solid-state fermentation	Seed		EC-P, C, ECG-P, EC and ECG	[73]
	Whole grape pomace	Mixture of proteases	GA, CA, CfA, PA, C, EC, PB1 and some glucosides	
	Whole grape waste	<i>Aspergillus niger GH1</i>	GA (as a main phenolic)	

C, catechin; EC, epicatechin; EGC, epigallocatechin; C-P, catechin-phloroglucinol; EC-P, epicatechin-phloroglucinol; ECG-P, epicatechin gallate-phloroglucinol; EGC-P, epigallocatechin-phloroglucinol; EA, ellagic acid; EAH, ellagic acid hexoside; ECG, epicatechin gallate; GA, gallic acid; CA, caffeic acid; CfA, caftaric acid; PA, protocatechuic acid; PB1, procyanidin B1; PB2, procyanidin B2; Q, quercetin; K, kaempferol; R, resveratrol; Rs, resorcinol; and OCA, O-coumaric acid.

Table 3. Biotechnology techniques applied for phenolic compounds recovery from grape by-products.

10. Concluding remarks

As shown, grape fruit and by-products have demonstrated be an excellent source for obtaining phenolic compounds with the use of several conventional and/or emerging technologies. The growing demand to extract high-quality and high-activity extracts rich in phenolic compounds from plant materials encourages researches for found convenient extraction methods to this proposal. Since all the methods above described are based on different mechanism and extraction processes, the possible arrangement and development of hybrid procedures must be investigated to select the appropriate extracting-technique considering the target phenolic compounds, the physicochemical characteristic of the source, and the economic and environmental advantages of those methodologies.

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