



The combined extraction of polyphenols from grape marc: Ultrasound assisted extraction followed by supercritical CO₂ extraction of ultrasound-raffinate



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ABSTRACT

A combined process of ultrasound assisted extraction (UAE) and supercritical carbon dioxide (SC-CO₂) extraction of the correspondent UAE-Raffinate for the recovery of polyphenols from defatted grape marc was developed on pilot-plant scale. The extraction of polyphenols using UAE at different extraction time (4 and 10 min) and temperature (20 and 80 °C) was investigated. The overall extraction curves of UAE-Raffinates extracted by SC-CO₂ described and critically evaluated. The performance of the combined process was checked by total polyphenols yield, proanthocyanidins content and antioxidant activity. Compared to other previous studies, the polyphenols extraction yield obtained by the combined process was significantly enhanced (3493 mg GAE/100 g DM) as well as the antioxidant activity (7503 mg α -tocopherol/100 g DM).

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1. Introduction

Grape marc is the solid waste of the winemaking process. It consists of skins, seeds, and very small amounts of stems. Since about 70% of grape phenolic compounds remain in the grape marc (Mazza, 1995) after winemaking, it is a rich and inexpensive source for recovering these bioactive components (Kammerer & Carle, 2008; Kammerer, Claus, Carle, & Schieber, 2004; Lu & Foo, 1999) possessing many biological activities, such as antioxidant, cardio-protective, anti-cancer, anti-inflammation, anti-aging and antimicrobial properties (Xia, Deng, Guo, & Li, 2010). The applications of the grape marc extract have been increasing in pharmaceuticals, medical, cosmetic and food industry (Arvanitoyannis, Ladas, & Mavromatis, 2006).

In the literature few papers deal with grape marc polyphenols extraction using non-conventional techniques such as enzyme-assisted extraction (Kammerer, Claus, Schieber, & Carle, 2006), extraction using high voltage electrical discharges (Boussetta, Lanoisellé, Bedel-Cloutour, & Vorobiev, 2009) or high hydrostatic pressure or pulsed electric fields (PEF) (Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008), microwave assisted extraction (MAE) and

ultrasound assisted extraction (UAE) (Ghafoor, Choi, Jeon, & Jo, 2009; Novak, Janeiro, Seruga, & Oliveira-Brett, 2008; Palma & Barroso, 2002; Pan, Niu, & Liu, 2003), and supercritical fluid extraction (SFE) with supercritical carbon dioxide (SC-CO₂) added of a co-solvent (Casas et al., 2010; Da Porto, Decorti, & Natolino, 2014a; Da Porto, Natolino, & Decorti, 2014b; Farias-Campomanes, Rostagno, & Meireles, 2013; Ghafoor, Park, & Choi, 2010; Murga, Ruiz, Beltran, & Cabezas, 2000; Pinelo et al., 2007).

Ultrasound assisted extraction (UAE) is much faster than traditional methods, because when solid–liquid extraction is assisted by ultrasounds the possible benefits of their application are an intensification of mass transfer, improved solvent penetration into the plant tissue and capillary effects. This is due to cavitation collapse, i.e. to the formation, growth and violent collapse of liquid microbubbles that may be present inside the raw material (Mason, 2011; Pingret, Fabiano-Tixier, & Chemat, 2013; Toma, Vinatoru, Paniwnyk, & Mason 2001).

Supercritical fluid extraction (SFE) is an environment-friendly technology, which offers several advantages over classical solvent extraction methods. Supercritical carbon dioxide (SC-CO₂) is the most commonly used solvent in SFE. It is inert, non-toxic, and allows extraction at lower temperature and relatively low pressure. Furthermore, the extracts obtained by SFE are of high quality (Brunner, 1994; Pereira, Prado, & Meireles, 2013).

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Although several authors studied the operating conditions to be applied in ultrasound assisted extraction (UAE) and supercritical carbon dioxide extraction (SC-CO₂) for the recovery of polyphenols from grape marc, to the best of our knowledge, there has been no work, thus far, on the use of ultrasound coupled to SC-CO₂ method to extract polyphenols from grape marc.

The aim of the paper is to focus on the application of 'green' innovative extraction technologies (UAE + SFE) to recover from low-cost raw materials as grape marc is, valuable compounds such as polyphenols having a high commercial value for the cosmetics, food and pharmaceutical industries. This in order to solve, with innovative approaches, the problem arose with the European reform of the wine sector (EC Regulation 479/2008), which promotes the gradual withdrawal of distillation subsidies and consequently revokes the compulsory distillation, and the EU legislation on waste (Directive 2006/12/EC), which provides that Member States shall take the necessary measures to ensure that waste is disposed of or recovered without endangering human health and without using processes or methods harmful to the environment.

In this study, the extraction of polyphenols from grape marc using the combination of ultrasound-assisted extraction followed by re-extraction of the obtained UAE-Raffinate with supercritical CO₂ was investigated. Effects of UAE time and temperature were investigated by a full factorial design. The overall extraction curves of the UAE-Raffinates extracted by SFE were described and evaluated. The performance of the combined process was checked by comparison of total polyphenols yield, proanthocyanidins content and antioxidant activity with both UAE and SFE.

2. Materials and methods

2.1. Chemicals

Carbon dioxide (mass fraction purity 0.999 in the liquid phase) was supplied by Sapio s.r.l (Udine, Italy). Free stable DPPH radical (DPPH•), Folin–Ciocalteu reagent, gallic acid, (±)-catechin, (+)- α -tocopherol, vanillin 99% were purchased from Sigma–Aldrich (Milan, Italy). Sep-Pak Plus tC18 cartridge WAT 036810 and WAT 036800 were purchased from Waters (Milan, Italy). Other reagents were of analytical grade or higher available purity.

2.2. Grape marc and pre-treatment

Grape marc from red grape (*Vitis vinifera* L.) varieties was collected during September 2013 in Friuli Venezia-Giulia region (Italy), air dried at room temperature for 24 h (moisture 14.3% \pm 0.3 w/w) and stored at 4 °C until use. It was ground on a domestic mill, and particles characterized by size classification in a standard sifter with several mesh sizes (<0.5, 0.8–1.0, 1.0–1.25, 1.25–1.50, 1.50–1.75, 1.75–2.0 > 2.0 mm). An average particle diameter $d_p = 0.83 \pm 0.05$ mm was adopted, being calculated by Sauter's equation (Povh, Marques, & Meireles, 2001) to a set of fractions within the previous mesh sized:

$$d_p = m_t \left/ \sum_{i=1}^k m_i / d_{pi} \right. \quad (1)$$

where m_i is the mass of particles retained below mesh size d_{pi} , m_t is the total mass of milled seeds and k is the number of mesh sized.

Ground grape marc was defatted by SC-CO₂ extraction using the SFE-pilot plan following reported in detail (2.4). As suggested by Sovová, Zarevucka, Vacek, and Stransky (2001) pressure was 28 MPa and temperature 45 °C, while CO₂ flow rate was 10 kg/h and 3 h the total extraction time (Da Porto et al., 2014b).

2.3. Ultrasound-assisted extraction

For the ultrasound-assisted extraction (UAE) experiments an ultrasonic sonifier (Sonoplus model HD 2200, Bandelin, Berlin) equipped with a titanium alloy flat tip probe (13 mm diameter) (TT13, Bandelin, Berlin) was used.

Ground grape marc (100 g) previously defatted by supercritical carbon dioxide (SC-CO₂) was mixed with 400 mL ethanol-water solution (ethanol concentration 449.73 g/L) at 20, 50 and 80 °C in a 800 mL beaker. The choice of ethanol-water solution with ethanol concentration of 449.73 g/L as extracting solvent was carried out in relation with the co-solvent used for the following extraction of polyphenols by supercritical fluid extraction (SFE) (Da Porto et al., 2014b). The beaker and its contents were immersed into a water bath coupled to a temperature controller (Frigiterm, J.P. Selecta, Barcelona, Spain). The probe, submerged about 4 cm under the surface of the mixture, worked at 20 kHz frequency and 80 W (set and displayed in % on the scale of 10–100) for 4, 7 and 10 min. Then the samples were filtered under vacuum through Whatman No. 1 paper to separate sonicated grape marc (UAE-Raffinates) from the solvent mixtures, which were subsequently removed with a rotary vacuum evaporator at 50 °C to obtain the correspondent extracts (UAE-Extract). Each extraction was performed in triplicate using three different samples.

2.4. Supercritical CO₂ extraction

SFE pilot-plant (SCF100 serie 3 PLC-GR-DLMP, Separeco S.r.l, Pinerolo, Italy) equipped with 1 L extraction vessel (E₁), two 0.3 L separators in series (S₁, S₂), and a tank (B₁) where CO₂ is stored and recycled was used. The solvent used was carbon dioxide. The flow sheet of SFE pilot-plant is given in Fig. 1.

The extractor was filled with 0.1 kg of UAE-Raffinate distributed in glass beads (0.005 m). The extractions of polyphenols from UAE-Raffinates were carried out using the best operating conditions found in a previous work (Da Porto et al., 2014b), that is to say at pressure of 8 MPa, temperature of 40 °C, solvent flow rate of 6 kg/h CO₂ modified with 10% ethanol-water solution with ethanol concentration of 449.73 g/L.

Aliquots of SC-CO₂ grape extract were collected during extractions in volumetric flask at intervals of about 30 min, to assess several data points for the overall extraction curves (OECs). The ethanol aqueous mixture was then removed from the SC-CO₂ extracts with rotary evaporator (Buchi, B465, Switzerland) at 45 °C. After removal of solvent the extracts were analyzed.

2.5. Scheme of the combined process

The scheme of the combined process of ultrasound assisted extraction (UAE) and supercritical carbon dioxide (SC-CO₂) extraction of the correspondent UAE-Raffinate for the recovery of polyphenols from defatted grape marc is given in Fig. 2.

2.6. Analytical methods

Total polyphenols, fractionation of proanthocyanidins and antioxidant activity analyses were performed both on the extracts obtained by UAE (UAE-Extracts) and by SFE of UAE-Raffinates (SC-CO₂-Extracts).

2.6.1. Total polyphenols (TPC)

Purification by C₁₈ cartridge was carried out for the samples to eliminate the interference of sugars, non volatile acids and amino acids in total phenols determination. Total polyphenols (TPC) were determined using the Folin–Ciocalteu reagent, according to Yu,

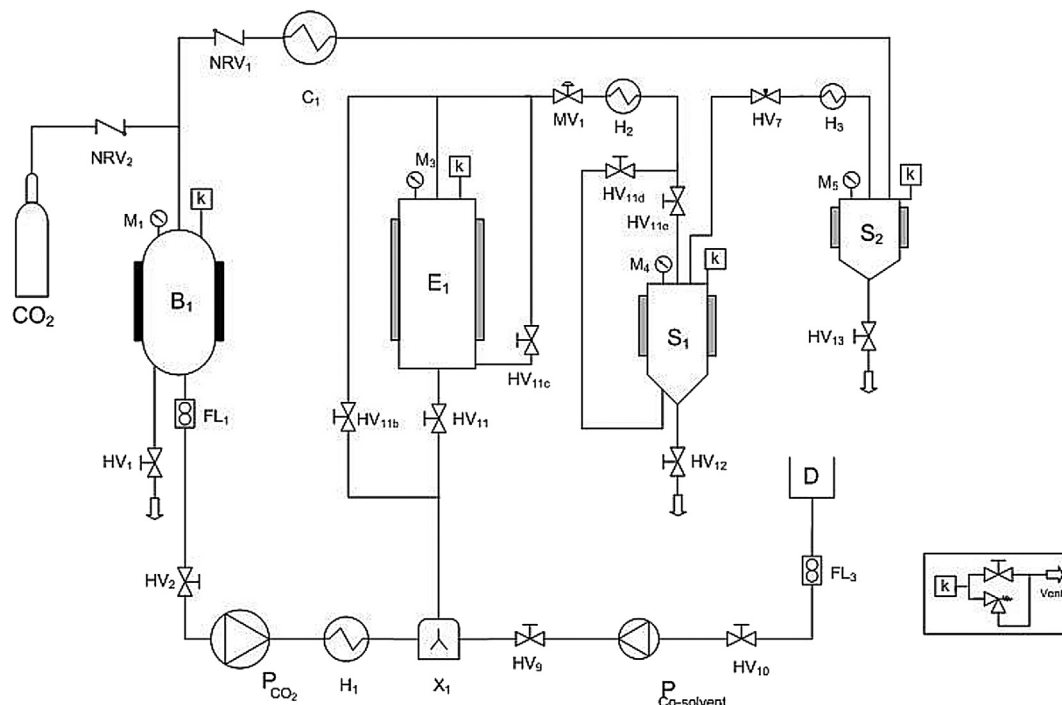


Fig. 1. SFE pilot-plant flow sheet. (B₁) storage tank; (E₁) Extraction vessel; (S₁, S₂) Separators; (H#) Heater exchangers; (C₁) Condenser; (HV#) Hand valves; (MV₁) membrane valve; (NVR#) No return valves; (P) Diaphragm pumps; (F₁) Flowmeter; (M#) Manometers; (k) Safety devices; (FL₁) Coriolis mass flowmeter; (D) Co-solvent storage tank; (X#) Mixer.

Perret, Harris, Wilson, and Haley (2003). Briefly, the reaction mixture contained 100 μL of extract or solvent, 500 μL of the Folin–Ciocateau reagent, 1.5 mL of a sodium carbonate–water solution (200 g/L), and 1.5 mL of pure water. After 2 h of reaction at ambient temperature, absorbance was read at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV 1650, Italy) to calculate TPC. Gallic acid was employed as the standard. A calibration curve was made with standard solutions of gallic acid in the range 0.2–10 mg mL^{-1} and measures were carried out at 765 nm ($R^2 = 0.99$). All analyses were performed in triplicate. Results were expressed as milligrams of equivalent gallic acid per 100 g of dried matter (mg GAE/100 g DM).

2.6.2. Fractionation of proanthocyanidins

Grape marc extracts were fractionated as reported by Sun, Belchior, Ricardo-da-Silva, and Spranger (1999). Briefly, 5 mL of grape marc extracts was concentrated to dryness in a rotary evaporator at $<30^\circ\text{C}$. The residue was dissolved in 20 mL of 67 mmol/L phosphate buffer, pH 7.0. The pH of the resulting solution was adjusted to 7.0 with NaOH or HCl. Two C₁₈ Sep-Pak cartridges were assembled (WAT 36800 on the top and WAT 36810 at the bottom) and conditioned sequentially with 10 mL of methanol, 20 mL of deionized water and 10 mL of phosphate buffer, pH 7.0. Samples were passed through the cartridges at flow rate not higher than 2 mL/min, and phenolic acids were then eliminated by elution with 10 mL of 67 mmol/L phosphate buffer at pH 7.0. The cartridges were dried with nitrogen flow and eluted sequentially with 25 mL of ethyl acetate (fraction FI + FII, containing monomeric and oligomeric flavan-3-ols) and with 15 mL of methanol (fraction FIII, containing polymeric proanthocyanidins). The ethyl acetate eluate was taken to dryness under vacuum using a rotary evaporator (Rotavapor R210, Buchi, Flawil, Switzerland), redissolved in 3 mL of phosphate buffer at pH 7.0 and reloaded onto the same series of cartridges, that had been conditioned as described above. The cartridges were dried with nitrogen flow and eluted sequentially with 25 mL of diethyl ether (fraction FI, containing monomers) and 15 mL of methanol (fraction FII, containing oligomers). The fractions FI, FII and FIII were evaporated to dryness under vacuum in 3 mL of methanol. Sample fractionation was performed in duplicate. The total flavan-3-ol content of each fraction was determined by vanillin assay according to the method described by Sun et al. (1999). Results were expressed as milligrams of catechin per 100 g of dry matter (mg catechin/100 g DM).

2.6.3. Antioxidant activity

The antioxidant activity of phenolic extracts was evaluated by the total free radical scavenger capacity (RSC) following the methodology described by Espin, Soler-Rivas, and Wichers (2000)

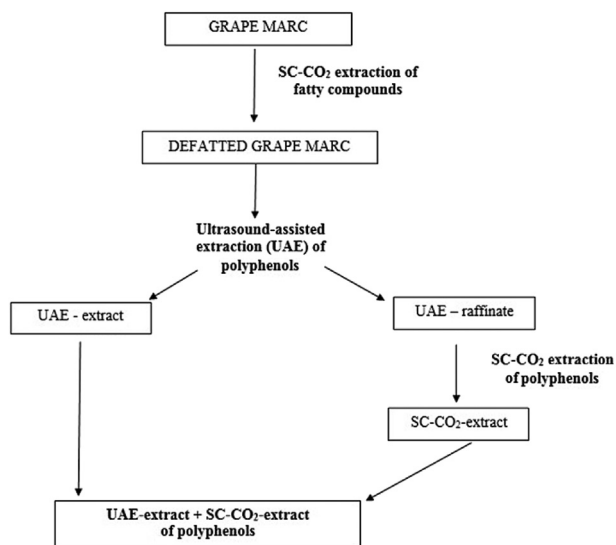


Fig. 2. Scheme of the combined process.

with slight modification. In brief, 10 µl of methanolic extract, previously diluted 1:10, was added with 1990 µl of fresh methanolic DPPH solution (0.093 mmol/L). Then the mixture was shaken vigorously and left in darkness for 60 min. Finally, the absorbance of the mixture was measured against pure methanol (blank) at 515 nm using a UV–Vis spectrophotometer, (Shimadzu UV 1650, Italy). The RSC is the difference of the concentration of DPPH free radical ($C_{\text{DPPH}, i}$) previously dissolved in methanol, after 60 min of reaction with the samples ($C_{\text{DPPH}, f}$). The antioxidant activity of the samples was expressed as milligrams of α -tocopherol per 100 g of dried matter (mg α -tocopherol/100 g DM). A calibration curve was made with standard solutions of α -tocopherol in the range 0.058–2.3 mmol/L ($R^2 = 0.98$). All analyses were performed in triplicate.

2.7. Statistical analysis

A 2^2 factorial design was used to identify the relationship between the response function and the process variables of ultrasound assisted extraction (UAE). The response factor considered was total polyphenols (TPC). The two independent variables or factors were extraction temperature (X_1 , varying between 20 and 80 °C) and time (X_2 , varying between 4 and 10 min). Each variable was coded at two levels, -1 and 1. The design involved four random experiments carried out in triplicate. A second-order polynomial equation was used to express the total polyphenols extracted as a function of independent variables:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 \quad (2)$$

where Y represents the response variable, b_0 is a constant, b_1 and b_{ij} are the linear and interactive coefficients, respectively. The coefficients of the response surface equation were determined by using Minitab 16 software (Minitab Inc.). The goodness of fit was evaluated by the coefficient of determination R^2 and the analysis of variance (ANOVA).

The statistical significances of the differences between means were determined using Tukey's test.

3. Results and discussion

3.1. Ultrasound-assisted extraction (UAE) of total polyphenols: effect of extraction temperature and time

Total phenolic contents (TPC) of defatted grape marc extracts obtained by UAE are shown in Table 1. Estimated coefficients are given in Table 2. Obtained second-order polynomial equation (Eq. (3)) was found well to represent the experimental data ($R^2 = 0.994$).

Eq. (3) shows the relationship between T and time for TPC extraction:

$$\begin{aligned} \text{Total polyphenols (mg GAE/100 g DM)} \\ = 755.16 + 20.9619X_1 + 0.9264X_2 - 0.3133X_1X_2 \end{aligned} \quad (3)$$

Table 1

Total polyphenols of defatted grape marc extracts obtained by ultrasound assisted extraction (UAE) experiments following the 2^2 factorial design.

Exp. no.	T (°C)	t (min)	Total polyphenols (mg GAE 100 g DM ⁻¹)
1	20	10	1120 ± 24
2	80	10	2191 ± 41
3	20	4	1153 ± 11
4	80	4	2336 ± 7

Each data represent the mean of three replicates ± standard deviation.

Table 2

Estimation and significance degree of coefficients for total polyphenols extracted by ultrasound-assisted extraction (UAE).

Coefficient	Coefficients estimated
	Total polyphenols
b_0	755.1600
b_1	20.9619***
b_2	0.9264***
b_{12}	-0.3133***
R^2	0.9940

***Significant at $p < 0.001$.

In Eq. (3), X_1 is the extraction temperature and X_2 is the extraction time. The equation was validated by the comparison of the estimated result of TPC (1810 mg GAE/100 g DM) which matched well with the experimental result of TPC (1811 mg GAE/100 g DM) obtained using UAE conditions of 50 °C and 7 min.

The large coefficient of X_1 indicates that under these operating conditions, temperature is the most decisive working condition and time is the least influential factor.

Unlike reported by Carrera, Rodriguez, Palma, and Barroso (2012) on UAE of phenolic compounds from grapes, by increasing temperature from 20 to 80 °C, an increase of total polyphenols was found. This could be due to grape seeds, which represent about 25% w/w of grape marc composition. Grape seeds are rich source of polyphenol compounds, especially phenolic acids, flavan-3-ols, such as catechins and their isomers, and proanthocyanidins (Lu & Foo, 1999). As Kim et al. (2006) reported that heat treatment of grape seeds liberated phenolic compounds, and thus increased the amount of active compounds, the same effect could be attributed to the high extraction temperature used. In addition, at higher temperature, the solvent viscosity and density decreased, and thus resulting in increased mass transfer. Furthermore, as a result of decreased solvent viscosity, cavitation bubbles within the fluid occurred more easily as the cohesive force, and thus the tensile strength of the liquid was reduced (Toma et al., 2001; Wu, Lin, & Chau, 2001).

The best way of expressing the effect of any parameter on the yield of polyphenols within the experimental space under investigation was to generate response surface plots of the equation. Contour plot showing the effects of temperature and time on total polyphenols is given in Fig. 3. The highest concentration of TPC (2336 mg GAE/100 g DM) was found after 4 min of extraction at 80 °C.

3.2. Supercritical CO₂ extraction of residual total polyphenols from UAE-Raffinates

The UAE-Raffinates namely UAE-R1 (4'-20 °C), UAE-R2 (10'-20 °C), UAE-R3 (4'-80 °C) and UAE-R4 (10'-80 °C) obtained from the UAE experiments were extracted by supercritical carbon dioxide extraction (SC-CO₂). The operating conditions used were the best found in a previous work (Da Porto et al., 2014b), that is to say pressure of 8 MPa, temperature of 40 °C, solvent flow rate of 6 kg/h CO₂ modified with 10% ethanol-water solution with ethanol concentration of 449.73 g/L.

In Fig. 4, the overall extraction curves (OECs) (TPC content vs. time) obtained for the UAE-Raffinates extracted by SC-CO₂ are plotted. The OEC of red defatted grape marc extracted by SFE is reported for comparison. It exhibits a constant-extraction rate period (CER) of 300 min, and a diffusion-controlled period (DC) follows. An intermediary falling extraction rate (FER) period cannot be observed (Pereira & Meireles, 2010). The initial linear period (CER) corresponds about the 82% of the final extracted phenols

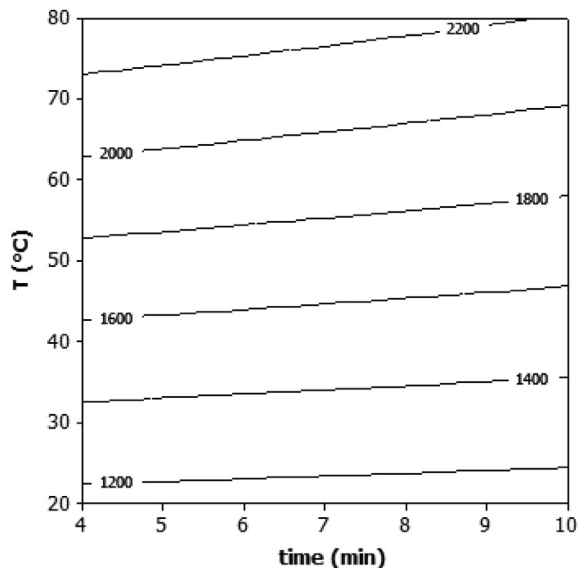


Fig. 3. Contour plot of total polyphenols extracted by ultrasound assisted extraction (UAE) from defatted grape marc using varying durations and temperatures of extraction.

(2736 mg GAE/100 g DM). TPC yield by SFE results higher to that (2200 mg GAE/100 g DM) reported by [Farias-Campomanes et al. \(2013\)](#). The OACs obtained from UAE-Raffinates indicate that shorter is the ultrasound extraction time of grape marc both at temperature of 20 and 80 °C, higher is the recovery of the residual TPC by SC-CO₂ from the correspondent UAE-Raffinates, UAE-R1 (1358 mg GAE/100 mg DM) and UAE-R2 (1134 mg GAE/100 g DM).

A comparison of total TPC recovery obtained combining UAE-Extracts of grape marc with SC-CO₂ extraction of UAE-Raffinates is shown in [Table 3](#). The TPC recovery obtained by SFE (2736 mg GAE/100 g DM) is significantly higher than TPC yields obtained by UAE under different operating conditions tested. However, when UAE of grape marc is carried out for 4 min at 80 °C and it is

Table 3

Comparison of total polyphenols recovery obtained by combining ultrasound assisted extraction (UAE) of defatted grape marc to supercritical fluid extraction (SFE) of the correspondent UAE-Raffinates.

Extraction methods	Total polyphenol (mg GAE 100 g DM ⁻¹)		
	UAE-extract	SFE-[UAE-Raffinate]	Combined extraction methods
SFE	—	2736 ± 11 a	2736 ± 11 c
UAE-E1 + SFE-[UAE-R1]	1153 ± 16 c	1358 ± 8 b	2511 ± 9 d
UAE-E2 + SFE-[UAE-R2]	2336 ± 10 a	1134 ± 8 c	3493 ± 61 a
UAE-E3 + SFE-[UAE-R3]	1121 ± 31 c	754 ± 1 d	1875 ± 29 e
UAE-E4 + SFE-[UAE-R4]	2191 ± 37 b	822 ± 28 d	3013 ± 42 b

Each data represent the mean of three replicates ± standard deviation.

Values with different letter within column indicate significant differences ($p < 0.05$).

combined to SC-CO₂ extraction of the correspondent UAE-Raffinate, the highest TPC recovery (3493 mg GAE/100 g DM) is obtained. Phenolic yield resulted similar to that (3169 mg GAE/100 g DM) reported by [Aliakbarian, Fathi, Perago, and Dehghani \(2012\)](#) using subcritical water, and much higher than 2300 mg GAE/100 g DM and 2527 mg GAE/100 g DM reported by [Farias-Campomanes et al. \(2013\)](#) and [Da Porto et al. \(2014b\)](#) respectively, using supercritical carbon dioxide extraction.

Grape marc polyphenols include flavonoids and non-flavonoids ([Souquet, Cheynier, Brossaud, & Moutounet, 1996](#)). Proanthocyanidins (PAs) are monomeric, oligomeric and polymeric flavonoids reported to have potential health beneficial in human diet. The healthy properties of PAs largely depend on their structure and especially on their degree of polymerization. [Cos et al. \(2004\)](#) reported that at least monomers and smaller oligomeric proanthocyanidins are absorbed.

The chemical composition of defatted grape marc extracts obtained by SFE, UAE carried out for 4 min at 80 °C and SFE of the correspondent UAE-Raffinate (UAE-R1) is reported in [Table 4](#). The lowest level of total proanthocyanidins (PAs) is obtained by UAE (70.1 mg catechin/100 g DM) and the highest by SFE (810.5 mg catechin/100 g DM). It is interesting to note that UAE gives a

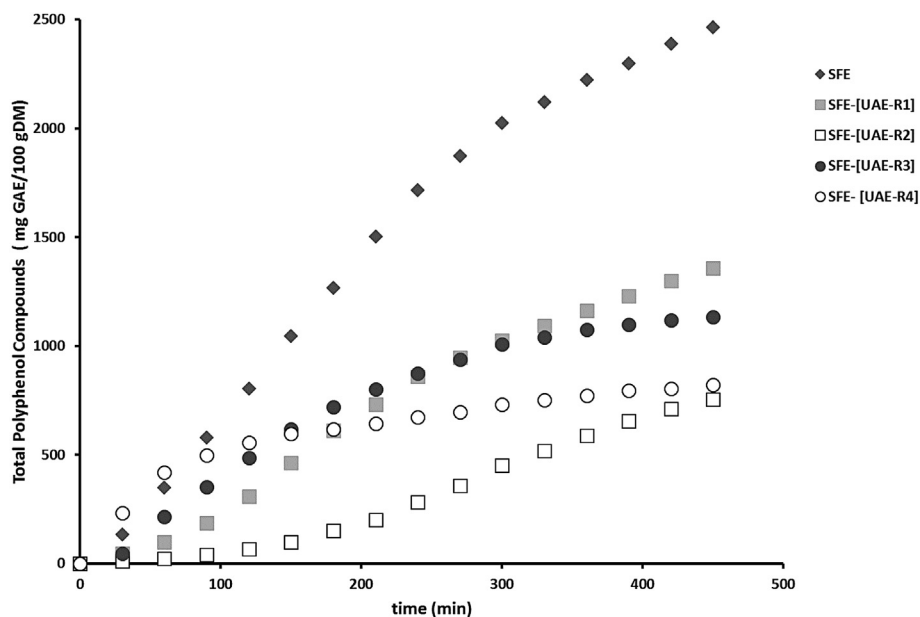


Fig. 4. Comparison of overall extraction curves (OECs) of total polyphenols extracted by supercritical fluid extraction of defatted grape marc (SFE) and by SFE of ultrasound-assisted raffinates (SFE-[UAE-R1], SFE-[UAE-R2], SFE-[UAE-R3], SFE-[UAE-R4]).

Table 4

Chemical composition of defatted grape marc extracts obtained by supercritical fluid extraction (SFE), ultrasound assisted extraction (UAE-E2) at optimized time (4 min) and temperature (80 °C), and supercritical fluid extraction of the correspondent ultrasound assisted raffinate (UAE-R2).

	Extraction methods		
	SFE	UAE-E2 (4'-80 °C)	SFE-[UAE-R2(4'-80 °C)]
Total polyphenols (mg GAE 100 g DM ⁻¹)	2736 ± 11 a	2336 ± 10 b	1134 ± 8 c
Total antioxidant activity (mg α-tocopherol 100 g DM ⁻¹)	4664 ± 16 a	4478 ± 14 b	3025 ± 12 c
<i>Proanthocyanidins</i> (mg catechin 100 g DM ⁻¹)			
Monomeric fraction (FI)	282.8 ± 0.2 a	2.0 ± 3.8 c	57.3 ± 2.4 b
Oligomeric fraction (FII)	167.4 ± 0.1 a	18.0 ± 5.8 c	81.3 ± 2.8 b
Polymeric fraction (FIII)	360.3 ± 0.4 a	50.1 ± 2.6 c	96.3 ± 3.5 b
<i>Proanthocyanidins (%)</i>			
Monomeric fraction (FI)	35	3	24
Oligomeric fraction (FII)	21	26	35
Polymeric fraction (FIII)	44	71	41

Each data represent the mean of three replicates ± standard deviation. Values with different letter within line indicate significant differences ($p < 0.05$).

percentage of monomeric fraction of PAs lower about 10-folds (3%) than SFE (35%) and a percentage of polymeric fraction higher about 2-folds (71%) than SFE (44%). This suggests that SFE is more selective in extracting proanthocyanidins fractions beneficial for human health than UAE. The level of PAs and their percentages in the extract obtained by SFE of UAE-R1 highlight the existing advantages on the use of SFE. Finally, it is interesting to note a 62% increase of total antioxidant activity when the coupled process UAE followed by SFE of UAE-Raffinate, is compared to SFE.

4. Conclusions

A combined process of UAE and SFE of the correspondent UAE-Raffinate for the recovery of polyphenols from grape marc was developed on pilot-plant scale. Compared to other previous studies, the high extraction yield obtained was significantly enhanced as well as the antioxidant activity. The combined extraction procedure described in this study was optimized. It is expected that this combined process can also be applied to recover phenolics from by-products coming from other sectors of agro-food industry.

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