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# The combined extraction of polyphenols from grape marc: Ultrasound assisted extraction followed by supercritical $CO_2$ extraction of ultrasound-raffinate

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ABSTRACT

A combined process of ultrasound assisted extraction (UAE) and supercritical carbon dioxide (SC-$CO_2$) 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-$CO_2$ 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).



## 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, cardioprotective, 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-$CO_2$) 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 cavitational 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-$CO_2$) 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-$CO_2$) 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-$CO_2$ 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 $CO_2$ 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–Ciocalteau reagent, gallic acid, (±)-catechin, (+)-α-tocopherol, vanillin 99% were purchased from Sigma–Aldrich (Milan, Italy). Sep-Pak Plus tC18cartridge 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% ± 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:

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

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-$CO_2$ 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 $CO_2$ 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-$CO_2$) 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 $CO_2$ extraction

SFE pilot-plant (SCF100 serie 3 PLC-GR-DLMP, Separeco S.r.l, Pinerolo, Italy) equipped with 1 L extraction vessel ($E_1$), two 0.3 L separators in series ($S_1$, $S_2$), and a tank ($B_1$) where $CO_2$ 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 $CO_2$ modified with 10% ethanol-water solution with ethanol concentration of 449.73 g/L.

Aliquots of SC-$CO_2$ 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-$CO_2$ 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-$CO_2$) 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-$CO_2$-Extracts).

#### 2.6.1. Total polyphenols (TPC)

Purification by $C_{18}$ 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–Ciocalteau reagent, according to Yu,