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Water and ethanol as co-solvent in supercritical fluid extraction of proanthocyanidins from grape marc: A comparison and a proposal

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ABSTRACT

Supercritical carbon dioxide (SC-CO_2) extraction of grape marc was studied 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. The overall extraction curves were determined and suggested 10 MPa and 313.15 K as the best operating conditions for SC-CO_2 + 15%W extraction, and 10 MPa and 333.15 K for SC-CO_2 + 15% EtOH. The phenolic yields obtained were 63.4 g/kg of extract for SC-CO_2 + 15% W and 38.8 g/kg of extract for SC-CO_2 + 15% EtOH. An alternative method combining Sc-CO_2 + 15% W extraction, followed by SC-CO_2 + 15% EtOH was tested. This procedure provided the best results allowing to obtain 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 α-tocopherol/100 g DM). SC-CO_2 methods were compared with methanol extraction.

1. Introduction

Word wine production in 2012 has been estimated at 250.9 million hectoliters by the International Organization of Vine and Wine (OIV) and in Europe at 131.7 million hectoliters. The wine production industries generate a large amount of solid waste, including grape marc and wine lees. Grape marc constitutes 20–25% of the weight of the grapes, and the seed and skin contents, on a wet basis are 25 and 50% respectively, although the exact distribution of these components depends on several factors [1]. In 2008, the Europe Community has revoked the compulsory distillation of the by-products of winemaking (EC Regulation 479/2008 and 555/2008) and this has created a great problem on winery waste handling and disposal. Grape marc has a heavy environmental impact for the high content of phenols that considerably increase chemical and biochemical oxygen demands. This biomass could be disposed and valorized first by extraction of added-value bioactive compounds and then by production of bio-fuels. One of the higher value options is the recovery of polyphenols, which could be used in pharmaceutical, cosmetics and food industry.

Grape's polyphenols include flavonoids and non-flavonoids [2]. Proanthocyanidins (PAs), also known as condensed tannins, are oligomeric and polymeric flavonoids of high complexity, with molecular weight over 500. They are composed by flavan-3-ol subunits connected by C—C bonds. Their sizes get higher as the grape gets mature, and it has being reported that their bioactive properties are determinate by molecular composition and size [3]. PAs subunits are differenced by their substitutions and the stereochemistry of their structures. The most common monomers are (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate and (−)-epigallocatechin [4]. The amount, structure and degree of polymerization of grape PAs differ, depending on their localization in the grape tissues. The seeds contain higher concentration of monomeric, oligomeric and polymeric flavan-3-ols than the skins [4]. However, the skin tannins have a much higher degree of polymerization than that from the seeds [5]. Recent research on the role of PAs as plant-based health-beneficial components in the human diet reported potential health beneficial effects including antioxidant, anti-diabetic, anti-carcinogenic, and anti-inflammatory activities. The healthy properties of PAs largely depend on their structure and especially on their degree of polymerization. Cos et al. [6] reported that at least monomers and smaller oligomeric procyanidins are absorbed.

Extensive studies are being carried out for the development of extraction process which are novel and applicable to a variety of bioactive compounds from plant materials. Supercritical fluid extraction (SFE) with supercritical carbon dioxide (SC-CO_2) has been widely used for the extraction from natural products. SFE is an environment-friendly technology that represents an alternative to conventional extraction methods and offers several advantages over classical solvent extraction methods. In fact, supercritical fluids have a high diffusivity, low viscosity and surface tension and small changes in pressure or temperature result in large changes in density. SC-CO_2 is the most commonly used solvent in SFE. It is

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inert, non-toxic, and allows extraction at lower temperature and relatively low pressure. Furthermore, the extracts obtained by SFE are of high quality [7].

SFE with $SC\text{-}CO_2$ has been applied on wine by-products for the recovery of grape seed oil [8–12] and with the addition of a co-solvent, usually ethanol or methanol, for the recovery of phenols [13–17]. However, to the best of our knowledge, there has been no work, thus far, on the extraction of phenols from grape marc using $SC\text{-}CO_2$ plus water as co-solvent.

In this study, the extraction of phenolic compounds from grape marc using $SC\text{-}CO_2$ with 15% water or/and 15% ethanol as co-solvent was performed and an alternative $SC\text{-}CO_2$ method proposed and tested. The effect of different temperature, 313.15, 323.15 and 333.15 K, and pressure 10, 20 MPa on the extraction amount of phenols has been investigated. The performance of $SC\text{-}CO_2$ methods was checked by evaluation of phenolic yield, proanthocyanidins content and antioxidant activity.

2. Material and methods

2.1. Materials and reagents

Grape marc from white grape (*Vitis vinifera* L.) varieties was collected during September 2012 in Friuli Venezia-Giulia region (Italy).

Carbon dioxide (mass fraction purity 0.999 in the liquid phase) was supplied by Sapio s.r.l (Udine, Italy). Free stable DPPH radical ($DPPH^\bullet$), Folin–Ciocalteau reagent, gallic acid, (±)-catechin, (+)-α-tocopherol and 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 preparation

Grape marc was air dried at room temperature (moisture 14.3% ± 0.3 w/w) and stored at 277.15 K until use. Grinding of grape marc was carried out 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 $d_p = 0.83 \pm 0.02$ mm was adopted, being calculated by Sauter's equation [18] to a set of fractions within the previous mesh sized:

$$d_p = \frac{m_t}{\sum_{i=1}^{k} m_i / d_{pi}}$$

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.

2.3. Classical organic solvent extraction

Ground grape marc was continuously extracted with *n*-hexane for 6 h at a maximum temperature of 343.15 K in a Soxhlet apparatus to extract lipids. Subsequently, 1 g of defatted grape marc with 5 mL methanol were mixed and shaken at room temperature for 90 min to extract phenolic compounds [19].

2.4. Supercritical fluid extraction (SFE)

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 (Sapio s.r.l, Udine, Italy). The flow sheet of SFE pilot plant is given in Fig. 1.

Ground grape marc was defatted by $SC\text{-}CO_2$ extraction. The extractor was filled with 0.480 kg of grape marc (density 600 kg m^{-3}). As suggested by Sovova et al. [9] pressure was 28 MPa and temperature 318.25 K, while CO_2 flow rate was 10 kg/h and 3 h the total extraction time.

Subsequently, phenols were extracted by SFE. Due to the fact that $SC\text{-}CO_2$ is non-polar, modification of $SC\text{-}CO_2$ through co-solvent or modifiers is needed to extract polyphenols. According to Murga et al. [13], de Campos et al. [20] and Yilmaz et al. [21] we used 15% wt. of ethanol (96%) as co-solvent to have an efficient extraction of proanthocyanidins. The water percentage used was the same (15% wt.). The extractor was filled with 0.1 kg of ground

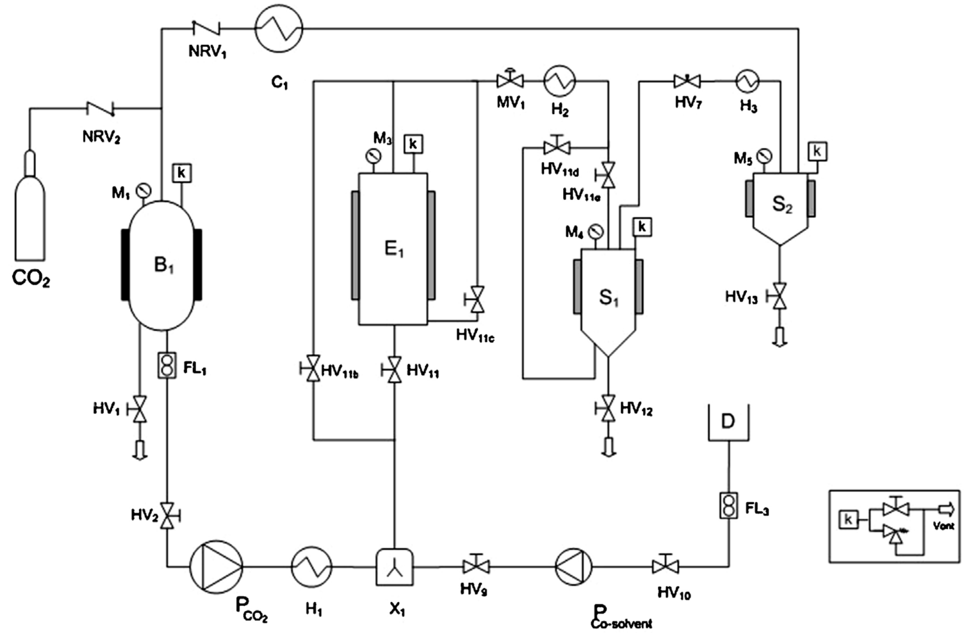

Fig. 1. SFE pilot plant flow sheet. (B_1) storage tank; (E_1) extraction vessel; (S_1, S_2) separators; (H#) heater exchangers; (C_1) condenser; (HV#) hand valves; (MV_1) membrane valve; (NVR#) no return valves; (P) diaphragm pumps; (F_1) flowmeter; (M#) manometers; (k) safety devices; (FL_1) Coriolis mass flowmeter; (D) co-solvent storage tank; (X#) mixer.