J. of Supercritical Fluids 118 (2016) 1–10

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Supercritical antisolvent precipitation of polyphenols from grape marc extract

Andrea Natolino[a,*], Carla Da Porto[a], Soraya Rodríguez-Rojo[b], Teresa Moreno[b], M. José Cocero[b]

[a] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via Sondrio 2/A, 33100 Udine, Italy
[b] Departamento de Ingenieria Quimica y Tecnologia del Medio Ambiente University of Valladolid, Valladolid, Spain

ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form 18 July 2016
Accepted 19 July 2016
Available online 27 July 2016

Keywords:
Supercritical anti-solvent process (SAS)
Grape marc extract
Polyphenols
Antioxidant activity
Proanthocyanidins

ABSTRACT

Supercritical anti-solvent (SAS) conditions to obtain the maximum enrichment of total polyphenols from grape marc extract was studied by 2^3 factorial design. Operating at 12 MPa, 45 °C and 0.99 CO_2 molar fraction, a relative enrichment of 350% of total polyphenols as well as a relative enrichment of proanthocyanidins fractions between 300 and 450% was achieved. Under these process conditions, microparticles in the order of 5 μm size were produced. The content of the main phenolic acid (gallic acid) and flavonoids (catechin, epicatechin, quercetin, malvidin and procyanidin B2) for the different precipitates was determined using high performance liquid chromatography. Starting from 100 g of grape marc, the amount of total polyphenols obtained by SAS ranged from 60 to 300 mg $_{GAE(GallicAcidEquivalent)}$/100 g $_{DGM(DriedGrapeMarc)}$.

1. Introduction

Wine production industries generate a large amount of solid waste, including grape marc. 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 is the solid waste of 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 [1] after winemaking, it is a rich and inexpensive source of added-value bioactive compounds [2–4], which can be useful in pharmaceutical, cosmetics and food industries.

Grape's polyphenols include flavonoids and non-flavonoids [5]. Proanthocyanidins (PAs), also known as condensed tannins, are oligomeric and polymeric flavonoids of high complexity. Recent research on the role of PAs as plant-based health-beneficial components in the human diet reported potential health beneficial effects depending on their structure and especially on their degree of polymerization. Cos et al. [6] reported that at least monomers and smaller oligomeric proanthocyanidins are absorbed by human body.

Despite the literature reports several papers regarding grape marc polyphenols extraction using supercritical carbon dioxide (SC-CO_2) as pure solvent or with the addition of a co-solvent [7–13], only a relatively small number of supercritical anti-solvent (SAS) works have been focused on the precipitation and purification of polyphenols from winemaking by-products [14,15].

The Supercritical Anti-Solvent (SAS) process [16,17] is a very flexible technique for the micronization of pharmaceutical [18–25] and natural compounds [26–29]. In pharmaceutical and cosmetic products, particle size, PSD and morphology influence their bioavailability. Generally, a small size implies a greater percentage of drugs absorbed by the human body and a reduction of the doses number [30]. Particle size and PSD affect the delivery route of the drugs: particles should be around 0.1–0.3 μm for intravenously delivery, 1–5 μm for inhalation delivery and 0.1–100 μm for oral delivery [31]. The process consists of dissolving the solutes of interest in a conventional solvent and, subsequently, the mixture is sprayed continuously co-currently with the supercritical carbon dioxide (SC-CO_2) into a precipitator vessel. The SC-CO_2 acts as an anti-solvent, decreasing the solubilities of the solutes in the mixture. Therefore, a fast supersaturation takes place, leading to nucleation and formation of nano- and microparticles. Afterwards, SC-CO_2 efficiently extracts the organic solvent, allowing obtaining completely solvent-free products.

The aim of this work is to precipitate polyphenols from ethanolic grape marc extract using supercritical antisolvent process (SAS).

* Corresponding author.
E-mail address: natolino.andrea@spes.uniud.it (A. Natolino).

http://dx.doi.org/10.1016/j.supflu.2016.07.015
0896-8446/

A 2^3 full factorial design has been applied to study the effect of the main SAS process parameters (pressure, temperature and CO_2 molar fraction) on yield, total polyphenol compound (TPC) and antioxidant activity. Fractionation of proanthocyanidins as well as HPLC analysis of more common polyphenols and scanning electronic microscopy (SEM) analysis have been performed on the precipitates obtained under the best SAS experimental conditions found.

Great interest has been focused on the extraction of proanthocyanidin oligomers, and in particular of procyanidins A-type and B-type dimers. The grape residues can be a good source of a B-type procyanidins. Procyanidin B2, and generally the B-type procyanidins, shows several beneficial effects on human health, such as antioxidative properties, inhibition of LDL (Low-density lipoproteins) oxidation [32], antiviral activity [33], and anti-cancer properties [34,35].

2. Materials and methods

2.1. Materials and reagents

Grape marc, from red grape (*Vitis vinifera* L.) varieties, was collected during September 2014 in Friuli Venezia-Giulia region (Italy).

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

2.2. Grape marc preparation

Grape marc was air dried at room temperature for 24 h (moisture 9.46 ± 0.21% w/w) and stored at 4 °C until use. It was grounded 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 [36], showed in Eq. (1), to set of fractions within the previous mesh sized:

$$d_P = \frac{mt}{\sum_{i=1}^{k} m_i/d_{pi}} \quad (1)$$

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

Ground grape marc was defatted by SC-CO_2 extraction. The plant used was a custom-made SFE setup, made of a diaphragm pump, an extraction vessel (4 L), a cyclonic separator (2.5 L), a Coriolis gas flowmeter for CO_2. The operating conditions were monitored by *PicoLog* data acquisition software. The extractor was filled with 1.0 kg of raw material. As suggested by Sovova et al. [37] pressure was 28 MPa, temperature 45 °C, CO_2 flow rate 10.0 kg/h and 3 h the total extraction time.

2.3. Extraction of phenolic compounds

Defatted ground grape marc (50 g) was mixed with 250 mL of pure ethanol (solid-to-liquid ratio 1:5) and shaken for 90 min at room temperature to extract phenolic compounds as reported by Pinelo et al. [38]. Exhausted the extraction, the liquid extract was recovered with a filter paper. The obtained extract represents the feed solution for the SAS experiments. An aliquot of 5 mL of extract was dried using a rotary evaporator to remove the solvent at 45 °C under reduced pressure. The dried extract obtained was weighted and the extraction yield calculated. All the extractions were done in triplicate.

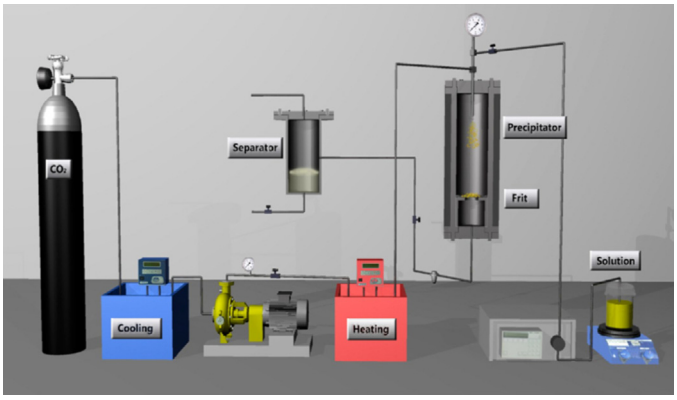

Fig. 1. Schematic diagram of SAS pilot plant.

2.4. Supercritical antisolvent process

Supercritical antisolvent process (SAS) pilot plant equipped with precipitation vessel, CO_2 pump, HPLC pump, Coriolis flow meter, flash separator and security valves, was used. The schematic diagram of SAS pilot plant is given in Fig. 1. The CO_2 was taken from a gas cylinder and cooled down before being pressurized with a diaphragm pump, and is heated up to the required operating temperature. When the mass flow of CO_2 is constant and the working pressure and temperature remain stable, the solution is pumped by a chromatographic pump into the precipitator chamber at the desired flow rate. The precipitation chamber is a jacketed AISI 316 stainless steel vessel of 1.5 L volume. The precipitator chamber is equipped with a Pt-100 thermo resistance with an accuracy of 0.1 °C and a membrane digital pressure meter with an accuracy of 0.5 bar to measure operating conditions. The inlet of the fluids is made through a concentric tube nozzle placed at the center top of the precipitation vessel; the nozzle consists of a 1/16 in. tube (inner diameter: 1 mm) for the solution, placed inside a ¼ in. tube (3.2 mm i.d.) for the CO_2. At the bottom of the vessel there is a porous metallic frit with a screen size of 1 μm. The pressure in the precipitator is controlled by a back-pressure valve (model BP66, GO, Spartanburg, SC). Additionally, the valve and the outlet tube are electrically heated to prevent freezing or plugging. A vessel is used to achieve the separation of solvent and CO_2 after pressure release. When the desired amount of solution has been injected (50 mL), the liquid pump is stopped and only pure CO_2 is fed for 25 min at a double times higher flow rate and the same operating conditions to ensure the complete removal of organic solvent from the precipitation chamber. Finally, the precipitator is depressurized and the particles are recovered. The precipitate is stored at temperature below 5 °C and protected from light, to avoid the decomposition of the products, before their analysis.

2.5. Modeling by full factorial design

A number of factors influencing the SAS process such as pressure (P), temperature (T) and CO_2 molar fraction (X_{CO2}) were studied by 2^3 full factorial design.

Two levels for each studied process parameters (P—pressure, T—temperature and X_{CO2}—CO_2 molar fraction), affecting SAS process, are studied: 10 and 12 MPa, 40 and 45 °C, 0.97 and 0.99 of CO_2 molar fraction. The experimental SAS operating conditions were selected according to the vapor liquid equilibria (VLE)