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Supercritical extraction and separation of antioxidants from residues of the wine industry

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Abstract

The main objective of this research is to evaluate the possibility to use residues of the wine industry to produce antioxidant concentrates, more specifically, concentrates of gallic acid, catechin, epicatechin and resveratrol. For this purpose, Supercritical Antisolvent Fractionation (SAF) has been used. This technology allows to concentrate ina dry form the biologically active principles of interest for their direct application, providing simultaneously a uniform and suitable size of the product. The most abundant compounds recovered are cathechin (48.5 mg/Kg, epicathechin (36.3 mg/kg), gallic acid (108 mg/Kg) and resveratrol (170 mg/Kg).

Keywords: Grape residues, antioxidants, supercritical antisolvent

1. Introduction

Antioxidants are compounds that inactivate or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidation chain reactions. The natural antioxidants are a large family of compounds including polyphenols and vitamins [1]; their usefulness spreads not only to pharmacological and food (including the so called functional food) applications; but, also to cosmetics, fats and plastic industries. Supercritical carbon dioxide (SC-CO_2) has been proposed to substitute the organic solvents in

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several processes [2,3]. However, supercritical CO_2 has affinity mainly for lypopholic compounds and this characteristics becomes an inconvenience when polar compounds have to be extracted; therefore, organic solvent mixtures-CO_2 under pressure have also been used. Supercritical CO_2 can be also used as antisolvent in the technique called Supercritical Antisolvent Fractionation (SAF) [4,5]. It allows the fractionation of extracts from an organic solution containing the compounds of interest. In the precipitator, the polar compounds that have not been swept away by CO_2 are recovered, whereas, in the separator compounds extracted by the mixture SC-CO_2 + organic solvent can be recovered. The quality of the recovered extracts can be investigated by analyzing the extracts to determine the proportion of the valuable compounds.

2. Materials and methods

2.1 *Material treatment*

Grape seeds were crushed in an electric grinder for 5 minutes, making various stops along the way. To avoid thermal degradation. Subsequently, the material was sieved to select an average particle diameter of 500 -250μm. Later, this material was macerated in absolute ethanol (10gr/100ml) for 24h to obtain the solution feed for the SAF experiments**.**

2.2 *Supercritical antisolvent fractionation (SAF) process*

The apparatus is formed of three main parts: a pumping section, a supercritical fluid extraction section and a separation section.The SAF precipitator is a cylindrical vessel, made in stainless steel (AISI 316). The vessel temperature control is achieved by means of resistances, a thermocouple and a controller. On the bottom part of the vessel a metallic filter collects precipitates. In a separator downstream the precipitator is used to collect the solvent and the compounds that are still dissolved in the mixture CO2-solvent. The separator pressure is measured by a manometer and is controlled by a back pressure valve. In a typical run, first pressure and temperature (in the vessel and in the separator) are stabilized. Then, the liquid flow is stabilized by injecting solvent for five minutes; then the organic solution containing the compounds that will be fractionated is injected. Once the experiment has finished, the solid on the filter inside the extractor vessel is recovered and also the liquid in the separator is collected. Both the solid recovered on the filter, as the solid obtained from evaporating the liquid in the separator are analyzed.The apparatus is schematically reported in Fig.1[6].

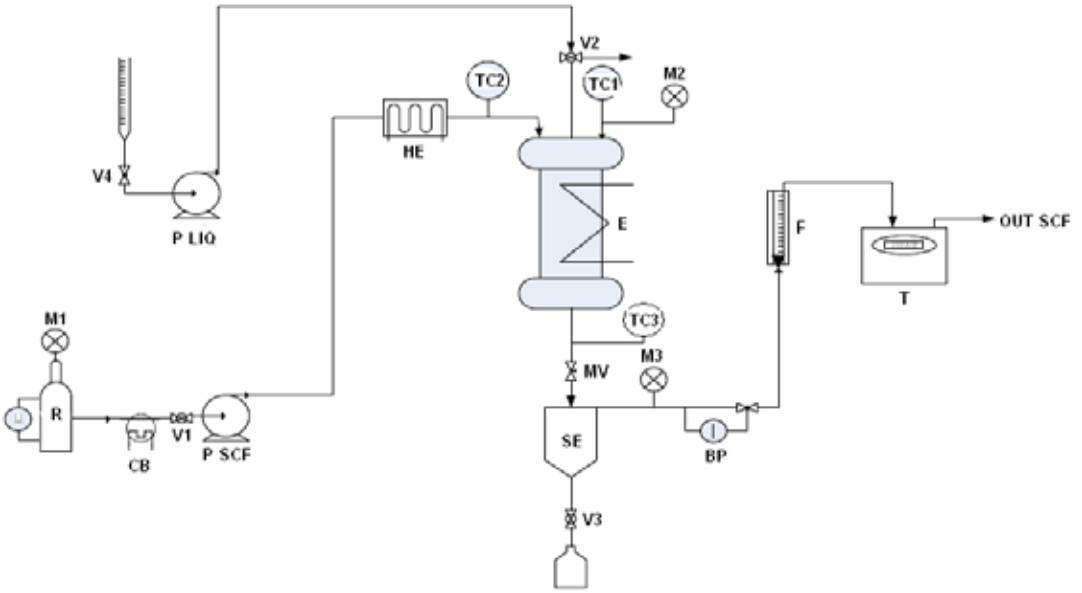

Fig. 1. Scheme of the pilot plant. (P-SCF) CO_2 Pump; (P-LIQ) Liquid pump; (R) CO_2 reservoir; (E) Extractor vessel (CB) Cooling bath; (HE) Heat exchanger; (F) Flowmeter; (T) Totalimeter; (BP) Back pressure; (S) Separator; (MV) Micrometrical valve; (V-) Valves; (M-) Manometers; (TC-) Temperature controls

2.3 *HPLC analysis*

The HPLC analyses were carried out in an HP 1200 System HPLC –DAD (Agilent Technologies), and the standards of gallic acid, catechin, epicatechin and resveratrol were obtained from SIGMA-Aldrich. The used column was a Luna C18 (2), from Phenomenex, with particle size of 5 μm, 25 mm length and 4.6 mm width. The mobile phase flow, 0.3 mL/min, consisted of two solvents: water–acetic acid (2%) (A) and water–acetic acid–acetonitrile (48/2/50) (B). Separations were performed at room temperature by solvent gradient : 0–30% B in 18min, 30% B during 12 min, 30–100% B in 15 min, 100% B during 2min and, then, return to the initial conditions in (0% B) in 2min to re-equilibrate the column. The injection volume was 10 μL.

2.4 *Microscopy analysis*

The scanning electron microscope (SEM) was used to observe the morphology of the extracts obtained by supercritical antisolvent fractionation. To that extent, a LEO 420 version V2.04, from Assing was used.

2.5 Determination of total phenolic content

The total amount of phenolics in extracts was determined using the Folin- Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE)[7]. Concentration of 0.1 and 1mg/ml of extract were also prepared in ethanol. All determinations were performed in triplicate. The Folin-Ciocalteu reagent is sensitive to reducing compounds including

polyphenols; thereby, producing a blue colour upon reaction. This blue colour is measured spectrophotometrically (760 nm). Thus, total phenolic content can be determineted. The amount of total phenol was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: y = 0.001x + 0.03. The total phenol varied from 120.95± 3.1 to 150.4±0.14 mg/gL in the extracts

3. Results and discussion

SAF is based on the complete miscibility between the selected liquid solvent and the supercritical fluid, at the process conditions; whereas, the solutes have to be not soluble in the supercritical medium. Therefore, when the liquid solution (solvent plus solutes) is atomized in the precipitator, the liquid is rapidly extracted by the supercritical antisolvent and the solute precipitates as a powder at the bottom of the vessel. Several SAF experiments were performed by systematically changing the working temperature (40-50º C) and pressure (80-150 bar). The flow rates used were 2.4 kg/h for CO_2 and 1mL/min for the ethanol solution. The best results were obtained operating at the following conditions: temperature 40 °C, pressure 120 bar, CO_2 mole fraction 0.98, and CO_2 density 716.14 Kg/m^3. The data reported in Fig 3 show the comparison between the content of compounds of interest in the starting ethanolic solution and their content in the precipitate demonstrating that the large majority of these compounds was successfully recovered in the precipitator. Fig 4 shows the macro and micro images of precipitate. The macroscopic image shows an orange non cohesive powder covering all the bottom of the precipitator. The SEM image indicates that the powder is formed by nanoparticles.

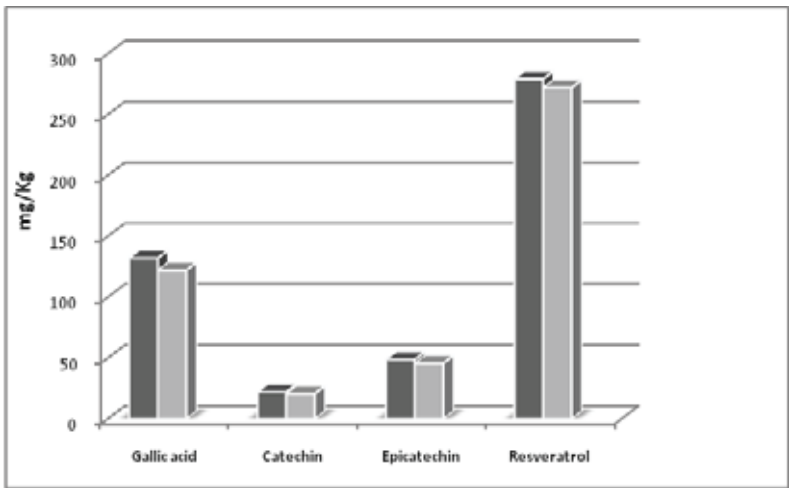

Fig. 3. (■) content in the starting solution, (■) content in the precipitate

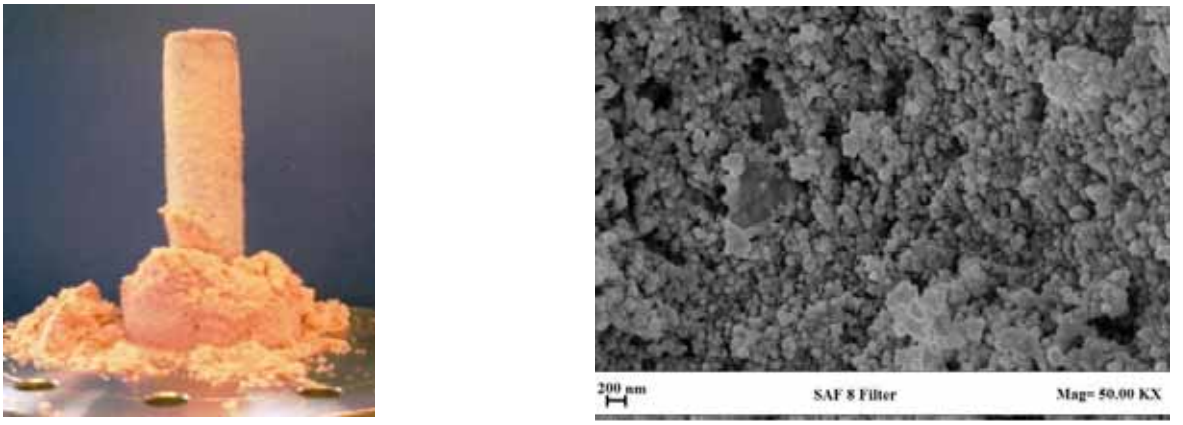

Fig. 4. (a) Dry extract in the precipitator; (b) SEM image of dry solid

4. Conclusions

SAF process has been developed to extract and recover the polyphenols from residues of grape seeds. In this manner, in a single step, it was possible to recovery powderous, solventless precipitate that contains practically the same quantity of polyphenols that is present in the ethanol extracts. The overall content of polyphenols recovered is 18.1 g/kg of treated seeds.

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