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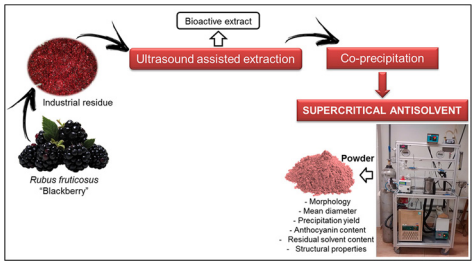
Co-precipitation of anthocyanins of the extract obtained from blackberry residues by pressurized antisolvent process

Ana Paula Da Fonseca Machado[a], Miriam Rueda[b], Gerardo Fernández Barbero[c], Ángel Martín[b], Maria José Cocero[b], Julian Martínez[a,*]

[a] University of Campinas, College of Food Engineering, Department of Food Engineering, 80, Monteiro Lobato Street, 13083-862 Campinas, São Paulo, Brazil
[b] University of Valladolid, Department of Chemical Engineering and Environmental Technology, c/Doctor Mergelina s/n, 47011 Valladolid, Spain
[c] University of Cadiz, Faculty of Sciences, Department of Analytical Chemistry, IVAGRO, República Saharaui Avenue s/n, 11510 Puerto Real, Cadiz, Spain

GRAPHICAL ABSTRACT



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ABSTRACT

An ethanolic anthocyanin-rich extract was obtained from blackberry residues through ultrasound assisted extraction, and extracts were co-precipitated with the biodegradable polymer polyvinylpyrrolidone (PVP) through the supercritical antisolvent process (SAS), using carbon dioxide as antisolvent. The influence of temperature (30–45 °C), PVP concentration in the extract (0.5–4.0%) and solution flow rate (1–10 mL/min) on the SAS process were investigated. The decrease of these three parameters contributed to produce particles with smaller diameter, residual ethanol, humidity and water activity, although with higher hygroscopicity. Moreover, the increase of these parameters intensified the agglomeration of the particles. SEM images revealed co-precipitates with irregular shapes obtained at high temperatures, flow rates and PVP concentrations. FTIR analysis showed that neither extract nor PVP properties were modified in the SAS process. Good precipitation yields were achieved, indicating that the SAS process is effective and selective in the co-precipitation of anthocyanins.

1. Introduction

The market of functional food, ingredients and nutraceuticals is increasing as consequence of the interest of consumers in preventing diseases and improving their health through the use of natural sources [1]. In fact, polyphenols have shown anticancer, antioxidant, anti-inflammatory and antiviral activities. Large amounts of liquid and solid residues containing polyphenols are currently discarded by food industries [2]. Therefore, their conversion into high added-value bioproducts, such as biofuels and food ingredients is a growing trend [3]. Moreover, the recovery of these residues has become a sustainable opportunity to reduce the pollution caused by their direct disposal on nature [4].

By-products from blackberry processing have been recognized as rich sources of bioactive phenolics compounds [5,6]. They are mainly composed by peel, seeds and stems, which represents around 20% of

* Corresponding author.
E-mail address: julian@unicamp.br (J. Martínez).

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the whole fruit's mass [3,7]. Flavonoids (such as anthocyanins, kaempferol and quercetin), phenolic acids (gallic, ellagic, caffeic, ferulic and coumaric), ellagitannins and proanthocyanidins are among the main components of blackberry residues, being anthocyanins their main pigments that give this fruits their typical red color [7,8]. Research with different varieties revealed that cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside are the major anthocyanins in blackberries [8]. The health benefits of polyphenols from blackberry and its products have been investigated due to proven effects against chronic diseases, such as cancer, neurodegeneration, cardiovascular diseases, oxidative stress and block of oxidant signalization paths [9].

Despite the abundance of phenolic compounds in blackberry residues and other vegetable sources, their application is often hampered by their low stability at certain environmental and process conditions, human digestion and limited solubility in aqueous media [10,11]. In this sense, encapsulation and micronization are attractive techniques to improve the stability, bioavailability and bioaccessibility of such compounds, besides controlling their release rate in the target medium. Many natural or synthetic polymers can be used as encapsulation agents or core material of bioactive phenolics, once they are biocompatible and biodegradable [12]. Polyvinylpyrrolidone (PVP) stands out for its vast pharmaceutic applicability and for being soluble in water and many organic solvents [13,14].

In the last two decades, encapsulation, micronization and co-precipitation techniques using pressurized fluids have gained attention, due to their advantages over conventional methods, such as spray-drying, freeze-drying and coacervation [12]. More specifically, the supercritical antisolvent (SAS) process is a promising technique that can be applied to produce particles in nano- and micrometric scale with controlled size and distribution, and without residual solvent [15]. The most used supercritical antisolvent is carbon dioxide (CO_2), because of its particular characteristics, such as being chemically inert, non-toxic, non-flammable, abundant in nature, environmentally accepted, cheap and for not leaving toxic residues in the product. Its critical properties are moderate – critical temperature (Tc) = 31.1 °C, critical pressure (Pc) = 7.38 MPa [12,16]. The moderate critical temperature of CO_2 makes it adequate to process thermally sensible compounds, such as anthocyanins.

Another advantage of SAS for the production of microparticles rich in bioactive compounds is the possibility of improving the process selectivity and yield, since the operation parameters (temperature, pressure, injection nozzle size, antisolvent and solution flow rates) are tunable [17–19].

In the SAS process the target compounds are first dissolved in a liquid solvent. This solution is continuously injected into a precipitation chamber through nozzle, together with supercritical CO_2. CO_2 acts as antisolvent, reducing the solubility of the target compounds in the liquid solvent. Thus, supersaturation is achieved, leading to the nucleation of nano- or microparticles [11]. It is also possible to produce polymer co-precipitates or microcapsules in a single step, using a polymer soluble in the same solvent as the target compounds. Although the precipitation of many biomaterials by SAS have been reported [20–22], this technique has not been extensively explored in the processing of chemically complex mixtures, such as vegetal ethanolic extracts + polymer.

Given this context, the present work investigated the effects of temperature, polymer concentration in the liquid solution and solution flow rate on the co-precipitation/encapsulation of an extract rich in anthocyanins obtained from blackberry residues through SAS technique using PVP as encapsulating agent.

2. Materials and methods

Most of this work was performed in the High Pressure Process Group (HPPG) of the Department of Chemical Engineering and Environmental Technology of the University of Valladolid (UVA) – Valladolid/Spain. The chromatographic analyses were done in the Department of Analytical Chemistry of the University of Cadiz (Puerto Real, Spain).

2.1. Materials

Blackberry (*Rubus fruticosus*) residues were obtained from a pulp processing industry located in Paraibuna, south-eastern Brazil (23° 23′ 10″ S, 45° 39′ 44″ W). The residues were freeze-dried, milled, packed in plastic bags and stored under freezing until the extraction.

Carbon dioxide (99.9% pure) was acquired from Carburos Metálicos S.A. (Valladolid, Spain). Absolute ethanol (96% pure), Folin-Ciocalteu reagent, potassium chloride, sodium acetate, sodium carbonate, monosodium phosphate and disodium phosphate were purchased from PanReac Química (AppliChem ITW Reagents, Barcelona, Spain). The anthocyanin standard (cyanidin chloride), the reagents 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), fluorescein sodium salt, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), gallic acid and PVP (mean molecular mass = 10,000 g/mol) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). For the UHPLC analyses, methanol and formic acid, both HPLC grade, were acquired at Merck (Darmstadt, Germany) and PanReac (Barcelona, Spain), respectively. These solvents were degassed and filtered through a 0.20 µm membrane (Fluoropore™, Millipore, Molsheim, France) before being used. Ultrapure water was prepared in a Milli-Q purification system (Millipore® S.A., Molsheim, France).

2.2. Preparation of the extract

Ultrasound assisted extraction (UAE) was used to extract anthocyanins from the blackberry residues. This method was based on successful previous works on extraction of anthocyanin and phenolics [6,23–25]. UAE was performed in a bench scale ultrasonic bath (Selecta®, Model 3000513, Barcelona, Spain) at fixed frequency (37 kHz) and power (150 W). Absolute ethanol was used as solvent, since it is GRAS (*Generally Recognized as Safe*) and has good affinity with supercritical CO_2, envisaging the efficiency of the SAS process. For the extraction, 15.0 g of dried and milled blackberry residue were mixed with 200 mL ethanol in 250 mL beakers and sonicated for 10 min at room pressure and temperature. The residue and solvent amounts were defined in order to achieve an extract with 1.50% total solids (w/w). The extracts were filtered under vacuum, collected in a single 10 L vessel and stored at −18 °C until SAS process.

2.3. Extract characterization

2.3.1. Total solids

The total solid content (TS) of the extract was determined gravimetrically. Around 6.0 mL of the extract were dried in air circulation stove (Memmert, Model. UN, Barcelona, Spain) at 70 °C, until reaching constant mass. TS was expressed in percentage (% – g total solids/100 mL dry extract).

2.3.2. Anthocyanin quantification by UHPLC-UV–vis

The identified anthocyanins in the extract were quantified in an Ultra-Performance Liquid Chromatography (UHPLC) system LaChrom (VWR Hitachi, Tokyo, Japan) using the method described by Machado et al. (2015) [5]. Calibration curves were prepared for each anthocyanin with concentration from 0 to 55 mg/L. Results were expressed as mg anthocyanin/g dry extract (de).

2.3.3. Monomeric anthocyanins

Total monomeric anthocyanins (MA) were determined through the differential pH method described by Giusti and Wrolstad [26]. Extracts were dissolved in two buffer solutions, potassium chloride pH 1.0 (0.025 M) and sodium acetate pH 4.5 (0.40 M), both adjusted with concentrated HCl. The solutions were measured at 510 and 700 nm in a