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Analytical Methods

Recovery of anthocyanins from residues of *Rubus fruticosus*, *Vaccinium myrtillus* and *Eugenia brasiliensis* by ultrasound assisted extraction, pressurized liquid extraction and their combination

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ABSTRACT

This work investigated the extraction efficiency of polyphenols (anthocyanins) from blackberry, blueberry and grumixama residues using combined ultrasonic assisted extraction (UAE) and pressurized liquid extraction (PLE) (UAE + PLE). The performance of UAE + PLE was compared to those achieved by the isolated PLE and UAE methods and conventional Soxhlet extraction. The effects of the extraction methods and solvents (acidified water pH 2.0, ethanol + water 50% v/v and ethanol + water 70% ethanol v/v) on total phenolics content, anthocyanin composition and antioxidant capacity of extracts were investigated by a full factorial design. The extraction efficiency for total phenolics and antioxidant capacity in decreasing order was: UAE + PLE > PLE ≈ Soxhlet > UAE, and for anthocyanins it was: Soxhlet ≈ UAE > UAE + PLE > PLE, using hydroethanolic mixtures as solvents. Extractions with acidified water and ultrasound were not effective to recover phenolics. Two, four and fourteen anthocyanins were identified in the extracts from grumixama, blackberry and blueberry, respectively.

1. Introduction

Many recent researches have shown that fruit processing residues (seeds, peels, stems and bagasse) are rich sources of compounds with benefic effects to human health, such as phenolics (Balasundram, Sundram, & Samman, 2006; Galanakis, 2012). However, these residues are often discarded (Wijngaard, Ballay, & Brunton, 2012). With the increase of food manufacturing, the concern of producers and government organizations with the treatment and disposal of their processing wastes has grown, since it represents a cost increase and has negative environmental impact (Galanakis, 2012). In this sense, environmental regulations are getting more severe (Wijngaard et al., 2012). Therefore, the interest in valorization of food residues is focused on reducing their direct disposal on nature by sustainable and safe means.

The bagasses of small fruits, such as berries, have gained attention as residues from juice and other processes, since they contain significant amounts of micronutrients and antioxidant compounds, such as polyphenols (Balasundram et al., 2006; Galanakis, 2012; Paredes-Lopez, Cervantes-Ceja, Vigna-Perez, & Hernandez-Perez, 2010). About 60–70% of the phenolics of small berries are found in their peel and seeds, being anthocyanins (cyanidin-3-*O*-glycoside, cyanidin-3-*O*-rutinoside and delfinidin-3-*O*-glicosyde) the major ones, which belong to the flavonoid group and are responsible for the pigmentation of these fruits (Paredes-Lopez et al., 2010). Among berries, the processing residues of grumixama (*Eugenia brasiliensis*), blueberry (*Vaccinium myrtillus)* and blackberry (*Rubus fruticosus*), as well as these fresh fruits, appear as rich sources of anthocyanins (Infante, Rosalen, Lazarini, Franchin, & Alencar, 2016; Machado, Pasquel-Reátegui, Barbero, & Martínez, 2015; Teixeira, Bertoldi, Lajolo, & Hassimotto, 2015).

The antioxidant capacities of phenolics and anthocyanins, as well as their contribution in human diet and several biologic effects, have been widely demonstrated. Polyphenols act as antioxidants due to their ability to donate hydrogen or electrons for neutralizing free radicals, and also to their intermediate radicals, which avoid oxidation of other compounds, like fatty acids. They also chelate transition metals as Fe^{2+} and Cu^{+}, besides modifying

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the redox potential of media (Balasundram et al., 2006). Thus, polyphenols find many industrial applications, such as natural dyes and aroma, food preservatives, dietary and fortification supplements or even in the formulation of ink, paper, cosmetics and pharmaceuticals (Jackman, Yada, Tung, & Speers, 1987; Paredes-Lopez et al., 2010).

Polyphenols are conventionally extracted with methanol and acetone (Mustafa & Turner, 2011). However, considering the requirements of food and pharmaceutical industries and environmental and economic impacts, there is a growing interest in using green solvents like ethanol, water and mixtures of both (classified as GRAS – *Generally Recognized as Safe* – solvents) to achieve a safe and environmentally friendly product.

The recovery of phytochemicals from vegetal sources has been usually performed through conventional extraction techniques such as Soxhlet, maceration and stirring. These methods require long times, high solvent and energy consumption, and provide extracts with limited quality. The high solvent consumption results in additional costs for their removal, besides environmental hazards. Moreover, many natural products, such as anthocyanins, are thermally unstable and can be degraded during long extractions at high temperatures (Barba, Zhu, Koubaa, Sant'Ana, & Orlien, 2016; Cai et al., 2016). To overcome these disadvantages, new extraction techniques have been investigated.

Ultrasound Assisted Extraction (UAE) is a rapid and efficient method to recover phenolics. The increased UAE yield is attributed to acoustic cavitation, which consists in the formation, growth and collapse of microbubbles on the solid surface, leading to its corrosion and erosion, and finally the rupture of cell walls, thus allowing the penetration of the solvent into the solid and enhancing mass transfer. In addition, ultrasound can help releasing intracellular material and desorbing compounds from the solid surface, resulting in higher extraction rates and yields. Finally, the solubility of compounds can also increase due to high local temperatures near the collapse region (Chemat, Zill-E-Huma, & Khan, 2011).

Pressurized Liquid Extraction (PLE) uses liquid solvents at high temperatures and pressures. The use of high temperatures can increase the solubility of extractable compounds, brake solute-solid bonds, increase diffusion and reduce the solvent's viscosity and interfacial tension, thus enhancing mass transfer. Temperature also affects the dielectric constant of the solvent, thus affecting its selectivity. High pressures increase the solvent penetration in the solid and allow keeping the solvent in the liquid state at temperatures above their normal boiling point (Mustafa & Turner, 2011).

Although many works have investigated the extraction of phenolics through UAE and PLE, there are no reports of the combined UAE + PLE methods to extract phenolics and anthocyanins from fruit residues. Given this scenario, this work aimed: (i) to valorize food residues through the extraction of phenolics and anthocyanins; and (ii) to investigate the extraction efficiency of polyphenols (anthocyanins) from blackberry, blueberry and grumixama residues using the combined UAE + PLE method. The performance of the UAE + PLE was evaluated by comparing the results of global yield, total phenolic content, monomeric anthocyanins, individual anthocyanins content, and antioxidant capacity with those obtained by the isolated PLE and UAE techniques and by the conventional Soxhlet method.

2. Materials and methods

Most experimental work was performed in the Laboratory of High Pressure in Food Engineering (LAPEA – DEA/FEA/UNICAMP, Campinas, Brazil). The chromatographic analyses were performed in the Department of Analytical Chemistry of the University of Cádiz (Puerto Real, Spain).

2.1. Reagents and solvents

All the chemicals were of analytical grade or the highest available purity. The anthocyanin standard (cyanidin chloride), the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and the reagents 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), potassium persulphate and gallic acid monohydrate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Citric, chloridric and glacial acetic acids, trihydrated sodium acetate and sodium carbonate were purchased from Synth (São Paulo, SP, Brazil), and ethanol was acquired at Êxodo Científica (Campinas, SP, Brazil). The Folin-Ciocalteu reagent and hexahydrated ferric chloride were from Dinâmica (São Paulo, SP, Brazil). For the UHPLC analyses, HPLC grade methanol and formic acid were purchased from Merck (Darmstadt, Germany) and Panreac (Barcelona, Spain), respectively. Ultrapure water was prepared in a Milli-Q purification system (Millipore, Bedford, USA).

2.2. Fruit processing residues

Residues from the industrial processing of blueberry (*Vaccinium myrtillus)* juice were purchased from the company Orgânicos Pérola da Terra, located at Antônio Prado (RS), southern Brazil (28°51′30″S, 51°16′58″W). The residues from the processing of blackberry (*Rubus fruticosus*) and grumixama (*Eugenia brasiliensis*) pulps were acquired at Sítio do Bello, Paraibuna (SP), southeastern Brazil (23°23′10″S, 45°39′44″W).

The residues were collected immediately after the end of the production of the mentioned juice and pulps and sealed in plastic bags. These bags were stored in a dark environment containing solid carbon dioxide (−78 °C), in which they remained around 3 days, until being sent to LAPEA. The material placed in styrofoam boxes also containing solid carbon dioxide, in order to avoid chemical and microbiological degradation. The samples were identified and stored under freezing (−18 °C) until the experiments. In order to avoid variations, a unique lot of each residue was used. The moisture content and Brix of the residues were 29.86%, 31.62% and 17.02%, and 8.6%; 20.4% and 13.5% for blackberry, blueberry and grumixama, respectively. The Brix of the samples was periodically analyzed as a method of controlling the physical-chemical state of the samples.

2.3. Extractions

The residues described in Section 2.2 were subjected to extraction with hydroethanolic mixtures (50% and 70% ethanol (v/v) in water) and acidified water at pH 2.0, excepting Soxhlet, in which pure ethanol was the solvent. The solvent choice was based on previous works (Garcia-Mendoza et al., 2017; Machado et al., 2015; Paes, Dotta, Barbero, & Martínez, 2014), which found hydroethanolic mixtures are interesting solvents for the extraction of phenolics, besides being classified as GRAS (Generally Recognized as Safe) solvents. All the extractions were performed at 80 °C.

2.3.1. Pressurized liquid extraction (PLE)

The PLE unit (Supplementary Material 1) and its operation procedure are described by Pereira, Garcia, Rodrigues, and Martínez (2016) and Machado et al. (2015), with few modifications. To prepare the extraction bed, approximately 5 g of fresh residue were added between two glass sphere layers in a 100 mL stainless steel cell. Glass wool was added to avoid the passage of solid particles and blocking of the piping line. The PLE conditions were as follows: pressure of 10.0 MPa, temperature of 80 °C, time of 30 min and solvent to feed mass ratio (S/F) of 18 kg solvent/kg fresh residue.