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Pressurized liquids extraction as an alternative process to readily obtain bioactive compounds from passion fruit rinds

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ABSTRACT

Bioactive compounds from passion fruit industry by-products (rinds) were obtained using ethanolic-water pressurized liquid extraction (PLE), a green and intensified extraction process. The PLE method was compared with Soxhlet and maceration. Ethanol, water, and their mixtures were used as solvents. PLE and maceration conditions were: temperature 30–60 °C, ethanol concentration 70–100% (v/v), pressure 10 MPa (PLE) and atmospheric pressure (maceration). The extraction processes were evaluated in terms of global yield, total phenolic content, phenolic composition, and antioxidant activity. PLE achieved extracts with higher global yield, phenolic content, and antioxidant activity than the conventional methods. Based on these results and the fast extraction time, PLE at 60 °C using 70% ethanol was the best method to recover bioactive compounds from passion fruit rinds. Five phenolic compounds were identified and quantified in the extracts: isoorientin, vicenin, vitexin, orientin, and isovitexin. The antioxidant capacity and phenolic content exhibited high and positive correlation expressed by the Pearson's coefficient. Extraction kinetics were performed and showed that target compounds could be recovered in short extraction times at the best PLE condition. The passion fruit rinds have therefore proved to be a rich source of phenolic compounds and PLE showed high efficiency to recover such compounds.

1. Introduction

According to FAO (2014), approximately one-third of all food produced is wasted worldwide corresponding to approximately 1.6 billion tons per year. Among this volume, 54% are lost in production steps, postharvest handling, and storage, and other 46% are spoiled in steps downstream of entry in the industry, i.e., processing, distribution and consumption. In weight basis, approximately 40–50% of fruits and vegetables are lost. By-products from passion fruit processing strongly contribute to this scenario. During the processing of passion fruit pulp, 40–60% of the mass are wasted (Zeraik and Yariwake, 2012). According to USAID (2014), the world production of fresh passion fruit was approximately

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1.4 million Mt in 2013. Brazil was the largest producer with 834,749 Mt, representing 59% of the global output. Indonesia and India were the next largest producers with 141,190 Mt (10% of output) and 122,630 Mt (9% of output), respectively.

Earlier studies have pointed some characteristics of passion fruit rinds that enforce the importance of searching novel ways to use this by-product. Zeraik et al. (2011) found a relationship between antioxidant activity and the isoorientin content in extracts from passion fruit rinds. Moreover, several works have described the presence of flavonoids, mainly C-glycosylflavones, as the major constituents of *Passiflora edulis* (Ignat et al., 2011; Silva et al., 2013; Zucolotto et al., 2012). According to Ignat et al. (2011), flavonoids are especially important antioxidants due to their high redox potential, which makes them reducing agents, hydrogen donors, and singlet oxygen quenchers, and they have metal chelating potential. Indeed, C-glycosyl flavonoids of passion fruit have been related to some health benefits like neuroprotective effect (Santos et al., 2016), reduction of lipid peroxidation (Silva et al., 2014), and therapeutic effects such as immunomodulation, anticarcinogenic and antioxidant activities (Silva et al., 2014). However, there are no reports about the extraction of such compounds from passion fruit rinds.

Extraction techniques have been investigated in order to develop faster, more efficient, cheaper and "greener" methods. In this regard, pressurized liquid extraction (PLE) has been widely compared to other extraction techniques (e.g., maceration, ultrasound extraction, solvent partitioning and Soxhlet extraction) in terms of recovery of bioactive compounds, and has shown promising results. The main advantages of PLE are higher extraction efficiencies requiring significantly lower amounts of solvents, reduced extraction time, possibility of process automation and the use of solvents classified as Generally Recognized as Safe (GRAS) (e.g., ethanol, water or its mixtures) (Herrero et al., 2013; Duba et al., 2015; Sousa et al., 2016). In addition, PLE has been appropriately applied to obtain bioactive compounds from several vegetal sources (Duba et al., 2015; Cardenas-Toro et al., 2015; M'hiri et al., 2015; Viganó et al., 2016a).

Taking the mentioned issues into account, the aim of the present work was to apply PLE to obtain bioactive compounds from passion fruit rinds using ethanol and water, two environmentally and safe food solvents, and to compare PLE with conventional extraction methods.

2. Material and methods

2.1. Chemicals

Absolute ethyl alcohol P.A. (Synth, SP, Brazil) and distilled water were used as extraction solvents. For the evaluation of the antioxidant potential, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-etramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) 2,2′-azobis(2-methylpropionamidine) dihydrochloride (APPH) and fluorescein were purchased from Sigma–Aldrich (SP, Brazil). For the determination of the total phenolic content, the Folin–Ciocalteu reagent and gallic acid were purchased from Sigma–Aldrich (SP, Brazil). Glacial acetic acid (Dinâmica Química Contemporânea Ltda., SP, Brazil), acetonitrile (J.T. Baker, Phillipsburg, NJ, USA) and methanol (J.T. Baker, Phillipsburg, NJ, USA) were used for the UPLC–MS analyses. Vicenin, vitexin, isovitexin, orientin and isoorientin (Sigma–Aldrich, SP, Brazil) were used as standards.

2.2. Sample preparation

Passion fruit (*Passiflora edulis* sp.) rinds were provided by the company Sítio do Bello (Paraibuna, SP, southeastern Brazil). The rinds were dried in an air circulation oven (Fanem, 320-SE, São Paulo, Brazil) for 30 h at 60 ± 2 °C. After drying, the rinds were ground in a knife mill (Marconi, MA340, Piracicaba, SP, Brazil), in order to be homogenized and reduce the resistance to mass transfer during the extraction process. The dried and milled raw material was stored in the absence of light at −18 °C until the extraction procedures, and presented the following composition: moisture by the method 931.04 (AOAC, 1997) – 0.032 ± 0.001 kg/kg (dry basis – d.b.); lipids by the method 963.15 (AOAC, 1997) – 0.0080 ± 0.0001 kg/kg (d.b.); proteins by the method 970.22 (AOAC, 1997) – 0.086 ± 0.001 kg/kg (d.b.); ash by the method 972.15 (AOAC, 1997) – 0.0187 ± 0.0003 kg/kg (d.b.); mean particle diameter was obtained by sieving and calculated accordingly to Levenspiel (1984) – 0.315 ± 0.015 mm; real density using helium gas pycnometer – 1.55 ± 0.01 g/cm^3; bulk density was calculated as the ratio between the mass of material necessary to fill a known volume glass tube (Rahman, 2005) – 0.42 ± 0.01 g/cm^3; and porosity was calculated according to Rahman (2005) – 0.73.

2.3. Pressurized liquid extraction

The dynamic extraction method was used in PLE with ethanol and/or water as solvents. This method consists of the continuous solvent flow through a fixed solid bed of particles placed inside the extraction column. Approximately 3.0 g of sample were used, forming a fixed bed inside a 10.9 cm^3 stainless steel column. The sample was put into the column, and a cylindrical glass stick was used to press and obtain a uniform fixed bed. The experiments were performed based on a factorial design with two variables and three levels in duplicate ($2 \times 3^2 = 18$ experiments). The evaluated factors were temperature and ethanol percentage in the solvent. The temperatures were 30, 45 and 60 °C, and ethanol percentages were 70, 85 and 100% (v/v). Temperature levels were selected above the room temperature (25 °C) and the upper limit was 60 °C to prevent the thermal degradation of some components of the extract. The chosen ethanol percentages were above 70% because water content above 30% in the solvent lead to the gelatinization of starch in the raw material, causing interruption of the solvent flow. According to Mustafa and Turner (2011), using high pressure during extraction can affect the matrix and result in disruption, thus enhancing the mass transfer of the solutes from the sample to the solvent. However, the effect of pressure on the recovery of most substances is usually negligible. Therefore, pressure was kept constant at 10.0 ± 0.5 MPa. The solvent flow rates were 3.0, 2.8 and 2.7 mL/min for 100, 85 and 70% ethanol, respectively, in order to keep the mass flow rate constant (2.4 g/min) and achieve a solvent-to-feed (S/F) ratio comparable with those reported for PLE in literature (Machado et al., 2015; Osorio-Tobón et al., 2014). The extraction time (30 min) was defined from preliminary tests, in which it had been verified that all the easily accessible solute was extracted. The extracts were collected in glass flasks and stored under freezing (−18 °C) in the absence of light, for further analyses.

Fig. 1 shows the PLE unit, which was assembled in the Laboratory of High Pressure in Food Engineering (LAPEA, FEA, UNICAMP, Brazil) and prepared to work with pressurized liquids, supercritical fluids and/or supercritical fluids with co-solvents. In PLE the solvent is pumped from the reservoir by a HPLC pump (Jasco, PU-2080, Tokyo, Japan), passes through a coil in a heating bath (Marconi, MA-126, Piracicaba, SP, Brazil) to reach the process temperature. Next, it follows to the jacketed extraction column (Autic, Campinas, SP, Brazil), flows through the sample bed where the extractable compounds are dissolved, leaves the bed and is collected after depressur-