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Effect of ultrasound on the supercritical CO_2 extraction of bioactive compounds from dedo de moça pepper (*Capsicum baccatum* L. var. *pendulum*)

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ABSTRACT

Extracts with bioactive compounds were obtained from the red pepper variety "dedo de moça" (*Capsicum baccatum* L. var. *pendulum*) through supercritical fluid extraction with carbon dioxide assisted by ultrasound (SFE-US). The process was tested at pressures of 15, 20 and 25 MPa; temperatures of 40, 50 and 60 °C, and ultrasonic powers of 200, 400 and 600 W applied during 40, 60 and 80 min of extraction. The CO_2 mass flow rate was fixed at 1.7569×10^{-4} kg/s. Global yield, phenolic content, antioxidant capacity and capsaicinoid concentration were evaluated in the extracts. The application of ultrasound raised the global extraction yield of SFE up to 45%. The phenolic content of the extract increased with the application of higher ultrasound power and radiation time. The capsaicinoid yield was also enhanced with ultrasound up to 12%. However, the antioxidant capacity did not increase with the ultrasound application. The BET-based model and the broken and intact cell model fitted well to the kinetic SFE curves. The BET-based model with three adjustable parameters resulted in the best fits to the experimental data. Field emission scanning electron microscopy (FESEM) images showed that SFE disturbed the vegetable matrix, releasing particles from the inner region of the plant cells to their surface. When the ultrasound was applied this effect was more pronounced. On the other hand, cracks, fissures or any sign of rupture were not identified on the sample surface.

1. Introduction

Hot or spicy peppers (*Capsicum* sp.) are widely known as sources of several nutrients, such as phenolics, flavonoids, carotenoids, antioxidants, and capsaicinoids [1–5]. Antioxidant compounds, such as phenolics [2] and capsaicinoids [6], have been identified as secondary metabolites in *Capsicum* peppers.

Phenolic compounds have been studied in *Capsicum* peppers [1–5], as well as their medical properties, such as cancer and atherosclerosis prevention [7], and anti-inflammatory activity [8]. Capsaicinoids are responsible for the sensory attributes of flavor, taste and pungency of *Capsicum* fruits. Currently, many studies proved the beneficial properties of capsaicin to cancer prevention, pain relief and weight reduction [9].

The recovery of bioactive compounds from vegetal raw materials is typically carried out through conventional extraction methods using organic solvents at high temperatures. These methods are often hazardous to consumers and environment due to the use of toxic and pollutant solvents. Moreover, many extraction processes at high temperatures generate oxidative substances, and result in the loss of thermally sensible components. Recent regulatory laws require the use of environmentally friendly extraction technologies to replace traditional methods [10]. In this context, supercritical fluid extraction (SFE) appears as a new and clean technology for pharmaceutical and food products [11].

The main advantages of SFE over conventional techniques are the use of moderate temperatures, reduced energy costs and production of extracts with high purity. One of the most used supercritical solvents is carbon dioxide (SC-CO_2). The density of

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SC-CO_2 is close to those of liquids, which enhances the solvation power, whereas its viscosity and diffusivity are near those of gases, improving its mass transfer ability. Moreover, the selectivity of a SFE process can be adjusted for each substrate by changing the process temperature and pressure. Finally, SC-CO_2 is non-toxic, non-flammable, non-polluting and low cost solvent, which is relatively inert and can be totally recovered [12–14].

The application of ultrasonic waves in SFE processes has been investigated as a strategy to increase the extraction yield and rate. The use of ultrasound is an efficient way to improve mass transfer mechanisms, such as convection and diffusion. Riera et al. [15] studied the influence of ultrasound in SFE of almond oil (*Prunus amygdalus*) and obtained an extraction yield up to 20% higher than SFE without ultrasound. Reátegui et al. [16] carried out SFE-US to extract antioxidant compounds from blackberry (*Rubus* sp.) bagasse and observed an increased yield up to 14%. Barrales et al. [17] reported a 29% increment in SFE-US when compared to SFE in the extracts of passion fruit (*Passiflora edulis* sp.) seed oil. Santos et al. [4] obtained extracts from malagueta peppers (*Capsicum frutescens* L.) using SFE-US. The authors observed a yield increase up to 30%, but ultrasonic waves did not influence significantly the phenolic content and the capsaicinoid profile in the extracts. Besides, the authors did not evaluate the influence of the pressure and the antioxidant capacity of the extracts.

This work focuses on the effects of a SFE-US process of a widely commercialized Brazilian pepper (*Capsicum bacccatum* L. var. *pendulum*), known as "dedo de moça". The phenolic and the capsaicinoids contents and the antioxidant capacity of the pepper extracts were evaluated. Two mathematical models, the BET-based model [18] and the broken-intact cell model [19], were adjusted to the kinetic curves, and the main mass transfer processes were identified. Furthermore, the morphology of the vegetable matrix was analyzed through field emission scanning electron microscopy (FESEM).

2. Material and methods

The raw material used was the pepper variety "dedo de moça" (*Capsicum baccatum* L. var. *pendulum*), purchased in a local market in Campinas, southeastern Brazil.

2.1. Sample preparation

The sample preparation was made according to the methodology for *Capsicum* peppers developed by Aguiar et al. [1]. First, the fruits were selected according to their physical integrity and immersed in a sanitization sodium hypochlorite solution (10 mL/L) for 20 min. Then the samples were washed with running water and oven-dried at 70 ± 2 °C (Fanem, model 320SE, São Paulo, Brazil) for 24 h, in order to remove the excess of the sanitization solution. In the following step, the samples were knife milled (Marconi, model MA 340, Piracicaba, Brazil), to homogenize the particles and enhance mass transfer during the extractions, and stored under refrigeration (−18 °C). The equilibrium moisture content was calculated after 24 h of drying at 70 ± 2 °C.

2.2. Characterization of the sample and extraction bed

The dried and ground peppers were classified according to their particle size in a vibratory sieve system with sequential openings from 14 to 80 Mesh (Tyler, Wheeling, USA). The mass retained on each sieve was measured in an analytical balance (Radwag, model AS 220/C/2, São Paulo, Brazil), separated and stored in glass flasks under refrigeration (−18 °C). The mean particle diameter was calculated through the A.S.A.E. model [20], according to Eq. (1).

$$d_{mg} = \exp\left\{\frac{\sum_{i=1}^{n}\left[\sqrt{w_i \cdot \log(d_i \cdot d_{i+1})}\right]}{\sum_{i=1}^{n} w_i}\right\} \quad (1)$$

where d_{mg} is the mean particle diameter (mm); d_i is the diameter of the sieve opening i (mm); d_{i+1} is the diameter of the sieve opening above sieve i (mm); w_i is the retained mass (g); n is the total number of fractions.

The bulk density (ρ_a) was defined as the ratio between the sample mass used in the extraction and the volume of the extraction bed. The density of the particles (ρ_r) was measured by helium pichnometry (Quantachrome Instruments, Ultrapyc 1200e, Boynton Beach, USA). The bed porosity (ε) was calculated from the bulk and particle densities, with Eq. (2).

$$\varepsilon = 1 - (\rho_a/\rho_r) \quad (2)$$

Total lipids were determined through extraction by Soxhlet using hexane (Êxodo Científica, Hortolândia, Brazil) as solvent, according to the AOAC method 963.15 [21].

2.3. Supercritical fluid extraction assisted by ultrasound (SFE-US)

2.3.1. SFE-US unit

The SFE-US unit used in the experiments is composed of a 300 mL stainless steel cell that supports pressures up to 45 MPa, a cooling bath (Marconi, MA184, Piracicaba, Brazil) to establish the CO_2 temperature at the pump (PP 111-VE MBR, Maximator, Nordhausen, Germany) inlet, a heating bath (Marconi, model MA126, Piracicaba, Brazil), a heating electric jacket that control the extractor temperature, pressure gauges (Zürich LTDA, Z.10.B$^+$, Água Rasa, Brazil), thermocouples (Pyrotec, sheath 1/8, Campinas, Brazil) and a flow totalizer (LAO, G 0.6 ± 0.001 m^3, São Paulo, Brazil). The ultrasonic power (*Unique Group*, DES500, Campinas, Brazil) is controlled with a 13 mm titanium probe, coupled to a transducer installed on the upper end of the extraction cell, and operated through a generator of ultrasound, which works from 20% to 99% of its total power (800 W). The solvent used was CO_2 (White Martins, Campinas, Brazil) with 99% purity. Fig. 1 shows the diagram of the SFE-US unit with its main components, detailing the extraction bed at the upper right. To prepare the extraction bed for SFE, a glass wool layer was put in the base of the cell, closing this extremity and acting as a filter to avoid the passage of small particles that could obstruct the extraction line. Approximately 5 grams of dried and sieved peppers were placed inside the cell between two layers of glass beads used to complete the cell volume.

2.3.2. Supercritical fluid extraction (SFE)

SFE was performed with and without application of ultrasound. Preliminary SFE tests were carried out in order to calibrate the equipment and to determine the extraction time. Then, the influences of pressure, ultrasonic power and time of ultrasound application on the extraction global yield were evaluated. Other process conditions, such as bed height and the mass of raw material were kept constant. The mass ratio between the solvent and the raw material (S/F) was fixed at 483.63 ± 20.07 kg CO_2/kg feed. This value was assured by keeping constant the CO_2 flow rate at 1.7569×10^{-4} kg/s. This (S/F) value is considered high when compared to those used by Daood et al. [22], Perva-Uzunalic et al. [23], and Duarte et al. [24], which were 30, 120 and 170 kg CO_2/kg red pepper, respectively. Thus, the used (S/F) ratio obtained was enough to achieve the solute exhaustion of the vegetable matrix.

About 5 g of sample were placed inside the extraction cell. The SFE experiments to determine the global yield were composed by an initial static extraction time of 20 min, followed by a dynamic extraction time of 120 min. The application of ultrasound was performed only during the dynamic extraction time. Barrales