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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

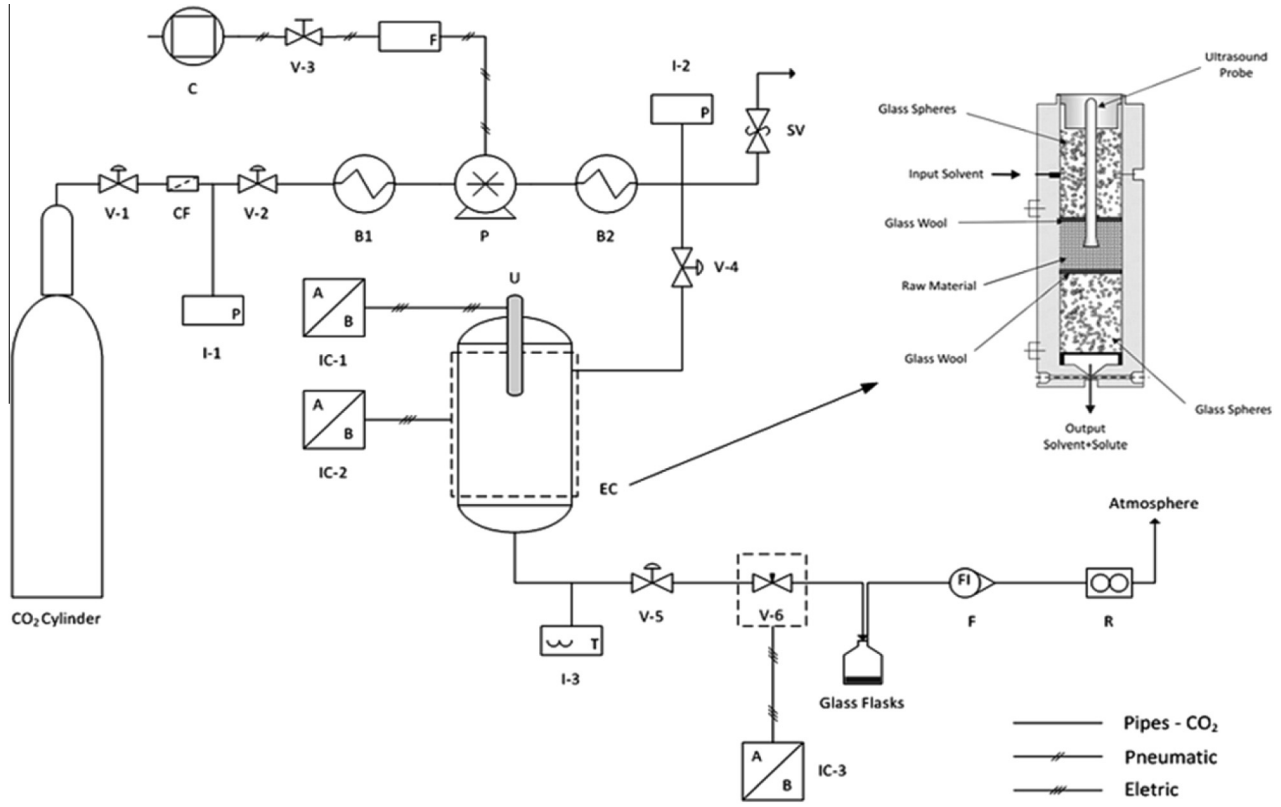


Fig. 1. Diagram of the SFE-US unit: V-1, V-2, V-3, V-4 and V-5 – control valves; V-6 – micrometer valve; SV – safety valve; C – compressor; F – compressed air filter; CF – CO_2 filter; B1 – cooling bath; P – pump; B2 – heating bath; I-1 and I-2 – pressure indicators; I-3 – temperature indicator; IC-1, IC-2 and IC-3 – indicators and controllers of ultrasonic power, temperature of extraction column and temperature of micrometer valve, respectively; U – ultrasound probe; R – flow totalizer; F – flow meter; EC – extraction column and internal configuration of the extraction bed of 300 mL for SFE-US used in the experiments.

et al. [17] observed that in SFE-US experiments an initial static time is needed to enhance the contact between the solvent and the extractable solute before releasing the CO_2 flow.

The SFE experiments with or without ultrasound application were performed in order to investigate the effects of pressure, temperature, ultrasonic power and time of ultrasound application on the global yield, antioxidant capacity, phenolics and capsaicinoids contents of the extracts from the selected peppers.

The first SFE plan was a full factorial of the two independent variables (pressure and temperature) on two levels and in triplicate. In addition, three central points were added, totaling 15 experiments, an experimental design type $2^{(2-0)}$. The influence of pressure, ultrasonic power and its application time during SFE was investigated in the second experimental plan (SFE-US). In SFE-US a full factorial design of the three independent variables (pressure, ultrasound power and radiation time) was adopted on two levels and in triplicate. Also, three central points were added, achieving a total of 27 experiments, an experimental design type $2^{(3-0)}$. In SFE-US planning, the temperature was fixed at 40 °C, which was the optimum found by Aguiar et al. [1] for SFE from malagueta pepper (*Capsicum frutescens* L.). The global yield (X_o) was calculated as the ratio between the extraction mass (m_E) and the sample mass (F) in dry basis, according to Eq. (3).

$$X_o = (m_{ext}/F) \times 100 \quad (3)$$

Table 1 shows the experimental plans performed to analyze the effects of the mentioned process parameters in SFE and SFE-US.

Table 1
Experimental planning matrix in SFE and SFE-US experiments from dedo de moça pepper (*Capsicum baccatum* L var. *pendulum*).

Method	Temperature (°C)	Pressure (MPa)	US power (W)	US time (min)
SFE	40	15	–	–
	40	25	–	–
	60	15	–	–
	60	25	–	–
	50	20	–	–
SFE-US	40	15	200	40
		15	200	80
		15	600	40
		15	600	80
		25	200	40
		25	200	80
		25	600	40
		25	600	80
		20	400	60

SFE – Supercritical fluid extraction; US – Ultrasound.

2.3.3. Total phenolic content

The total phenolic content of the extracts was determined by UV–vis spectrophotometry through the Folin–Ciocalteu method, according to the methodology proposed by Singleton et al. [25] with modifications. Briefly, 2.5 mL of the Folin–Ciocalteu reagent (Dinâmica, Diadema, Brazil) were diluted in distillated water (1:10 v/v) and added to 0.5 mL of an extract solution diluted in methanol (Analytical Standard, Synth, Diadema, Brazil). After 5 min, 2.0 mL of sodium carbonate (7.5%) were added and the mixture was kept in the dark for 2 h. Absorbance was measured at 760 nm. Gallic acid (Sigma–Aldrich/St. Louis, USA) was used as standard and the results expressed in milligrams of gallic acid equivalent per gram of raw material (mg GAE/g RM).

2.3.4. Antioxidant capacity

Two methods were used to determine the total antioxidant capacity of the extracts from dedo de moça peppers: DPPH and FRAP.

2.3.5. DPPH (2,2-Diphenyl-1-picryl-hidrazil) method

The determination of the *in vitro* antioxidant capacity through DPPH (2,2-Diphenyl-1-picryl-hidrazil) was based on the methodology presented by Rufino et al. [26] with some modifications. Summarizing, the extracts were diluted in ethanol at 1.0 mg/mL. Next, a solution of DPPH (60 μM) was prepared dissolving 4.8 mg of the DPPH reagent in 200 mL of ethanol. A Trolox solution (2000 μM) was prepared dissolving 25 mg of Trolox reagent in 50 mL of ethanol. From that solution, 5 mL volumetric balloons were used to prepare solutions with concentrations of 50, 100, 200, 400, 600, 800 and 1000 μM to plot the Trolox calibration curve.

In the dark, 0.1 mL of Trolox solution, ethanol (control) or diluted sample were transferred to test tubes. 3.9 mL of the DPPH reagent solution were added, with manual stirring for 30 s, and all tubes were let reacting for 40 min in the dark at room temperature (25 °C). Finally, absorbance was measured at 516 nm. Trolox (Sigma–Aldrich/St. Louis, USA) was used as standard and the results were expressed in micromol of Trolox per gram of raw material (μmol Trolox/g RM).

2.3.6. FRAP (Ferric Reducing Antioxidant Power) method

The determination of the antioxidant capacity by FRAP (Ferric Reducing Antioxidant Power) followed the methodology of Benzie and Strain [27], with modifications. Solutions of ferric chloride (20 mM), acetate buffer (0.3 M), standard solution of ferrous sulfate (2 mM) and hydrochloric acid (40 mM), all diluted in distillated water, were prepared. The TPTZ (2,4,6-Tripyridyl-S-Triazine) solution (Sigma–Aldrich/St. Louis, USA) (10 mM) was prepared by the dissolution of 3.12 g of TPTZ in approximately 5 mL of HCl (40 mM) and the volume completed to 1 L in a volumetric balloon. The FRAP reagent was obtained from the combination of 25 mL of acetate buffer (0.3 M), 2.5 mL of TPTZ solution (10 mM) and 2.5 mL of aqueous solution of ferric chloride (20 mM).

The calibration curve was obtained with standard solutions of ferrous sulfate with concentrations varying from 500 to 2000 μM. An aliquot of 90 μL of each solution of ferrous sulfate (500 μM, 750 μM, 1000 μM, 1250 μM, 1500 μM, 1750 μM and 2000 μM) was transferred to test tubes, added to 270 μL of distilled water, mixed with 2.7 mL of FRAP reagent, homogenized in a tube stirrer and kept at 37 °C. The absorbance at 595 nm was measured and the reaction time was 30 min. The FRAP reagent was used as blank to calibrate the spectrophotometer. TPTZ was used as standard and the results expressed in grams of ferrous sulfate per gram of raw material (g $FeSO_4$/g RM).

2.3.7. Analysis of capsaicinoids

The total capsaicinoid content of the extracts was determined according to Barbero et al. [28] by ultra high performance liquid chromatography linked to diode arrangement disposition (UHPLC-DAD). Capsaicin (97%) and dihydrocapsaicin (90%) standards were acquired from Sigma–Aldrich (St. Louis, USA). Water was obtained from a deionization system Milli-Q (Millipore, Bedford, USA). Methanol and glacial acetic acid used in chromatography separation were acquired from Fischer Scientific (Loughborough, UK).

The UHPLC analyses were conducted in an Acquity UPLC system (Waters, Milford, USA) equipped with a photodiode detector array (Model 2996 PDA). The software Empower 2 (Waters) was used to control the equipment and to data acquisition. The capsaicinoids were analyzed in a chromatography column (Waters – BEH C18 column; 50 mm × 2.1 mm I.D., particle size 1.7 μm). Chromatographic separation was achieved using the gradient of two solvents: acidified water (0.1% of acetic acid, solvent A) and acidified methanol (0.1% of acetic acid, solvent B) as follow (min, % of solvent B): 0.85 min, 55%; 1.60 min, 55%; 1.95 min, 60%; 2.45 min, 63%; 2.80 min, 70%; 3.00 min, 70%; 4.00 min, 100%. The capsaicinoids were measured at 280 nm and the chromatographic separation temperature was 50 °C.

The described method was used to prepare the calibration curves of capsaicin (C) ($y = 2660.63x + 139.36$) and dihydrocapsaicin (DHC) ($y = 2865.97x + 89.83$), which are the two commercially available capsaicinoid standards. The correlation coefficients (r^2) for C and DHC were 0.9997 and 0.9998, respectively. The limits of detection were 0.242 mg/L for C and 0.137 mg/L for DHC, and it was calculated as the analyte concentration giving a signal to noise ratio (S/N) of 3:1 [1]. These values were calculated using the ALAMIN software [29].

The five major identified capsaicinoids in the extracts from dedo de moça pepper were nordihydrocapsaicin (n-DHC), capsaicin (C), dihydrocapsaicin (DHC), homocapsaicin (h-C) and homodihydrocapsaicin (h-DHC). C and DHC were quantified from the calibration curves obtained from the respective standard solutions. Since there are not commercial standards of n-DHC, h-C and h-DHC, these compounds were quantified from the calibration curves of DHC (for n-DHC and h-DHC) and C (for h-C), based on the structural similarities between these molecules and also considering their molecular weights [1,4]. All analyses were acquired in triplicate.

2.4. Mathematical modeling

Two mathematical models based on thermodynamic and mass transfer phenomena during the extraction process were adjusted to the experimental SFE curves in order to evaluate the influence of the process conditions, mainly those related to ultrasound, in the extraction kinetics.

2.4.1. BET-based model

The BET-based model was developed by Pardo et al. [18], based on the Brunauer–Emmett–Teller (BET) theory of adsorption. This model expresses the extraction yield as a function of time using one single equation with two or three adjustable parameters, all with clear physical meaning. The model with three adjustable parameters is shown in Eq. (4).

$$t = \frac{m_0}{2Q_{CO_2}y^*}\left\{x'_0 - x' + (2-K)\left[x - x_m \ln\left(\frac{\alpha}{\beta}\right)\right] + Kx_m \ln\left[\frac{\alpha'}{\beta'(1-x)^2}\right]\right\} \quad (4)$$

where:

$$x'_0 = \sqrt{a+b+c} \quad (5)$$

$$x' = \sqrt{a(1-x)^2 + b(1-x) + c} \quad (6)$$

$$a = K^2 \quad (7)$$

$$b = 2(2-K)Kx_m \quad (8)$$

$$c = (Kx_m)^2 \quad (9)$$

$$\alpha = x' + K(1-x) + (2-K)x_m \quad (10)$$

$$\alpha' = x' + (2-K)(1-x) + Kx_m \quad (11)$$

$$\beta = x'_0 + K + (2-K)x_m \quad (12)$$

$$\beta' = x_0' + (2 - K) + Kx_m \tag{13}$$

$$y^* = y_{sat}\left[1 - \exp\left(-\frac{kL}{u\varepsilon}\right)\right] \tag{14}$$

$$k = (1 - \varepsilon)a_{sf}k_{sf} \tag{15}$$

where: x is the mass ratio between the solute extracted at time t and the extractable solute initially present in the SFE bed (kg/kg); m_0 is the initial extractable mass of solute in the SFE bed (kg); Q_{CO_2} is the CO_2 mass flow rate (kg/s); y_{sat} is the solute mass fraction in the saturated SC-CO_2 phase (kg/kg); L is the extractor length (m); u is the interstitial solvent velocity (m/s); ε is the SFE bed porosity; a_{sf} is the effective solid–fluid contact area for mass transfer (m^{-1}); k_{sf} is the mass transfer coefficient for the transport of solute through the external fluid film around the solid particles (m/s); y^*, x_m and K are the adjustable parameters of the model, which are the solubility of the solute in SC-CO_2 corrected by diffusion limitations (kg/kg), the solute mass fraction in the first monolayer (kg/kg), and the ratio between the adsorption equilibrium constants of the solute in the first monolayer and that in subsequent layers (kg/kg), respectively.

When the equilibrium constants between the first and the subsequent layers are approximately equal, $K \approx 1$, Eq. (4) can be reduced to a simpler model with two adjustable parameters, as shown in Eq. (16).

$$t = \left(\frac{m_o}{Q_{CO_2}y^*}\right)[(x - x_m)\ln(1 - x)] \tag{16}$$

In Eq. (16) y^* and x_m are the only adjustable parameters, therefore this equation can be used as a first approximation for the experimental data. The model proposed by Pardo et al. [18] was adjusted to the experimental SFE curves using a minimum of constrained nonlinear multivariable function algorithm from the MATLAB software (2014Ra, MathWorks, Natick, MA, USA).

2.4.2. Broken and intact cell model

The broken and intact cell model proposed by Sovová [19] assumes that the solvent is free of solute at the extractor inlet. The solid particle size and the initial distribution of the solute are constant. Part of the extractable solute is directly exposed to the solvent due to cell breaking on pretreatment. The other part of the solute remains inside the intact cells, where the solvent needs to penetrate through diffusion. Based on these considerations, the SFE process can be divided in three steps: the first is controlled by the convection in the fluid phase, with constant extraction rate where the readily available solute is removed; in the second, either convection or diffusion are important; and the third is controlled by the diffusion in the solid phase, where the remaining solute inside the intact cells is extracted.

The routine of Powell [30] was used to fit the model to experimental data. This routine is an iterative adjustment method that works with a range of values of the parameters defined by the user in a limited number of iterations. Some process data are need to apply the model of Sovová [19], such as global yield (X_o), extraction bed dimensions (L – height and d – diameter), solid sample mass (m_0), solvent and solid densities (ρ_s and ρ_a, respectively), extract solubility (y^*), solvent flow rate (Q_{CO_2}), accumulated extract mass (m_{ext}) and time (t). The adopted solubility was 0.0032 (kg of solute/kg of solvent), which was determined by Silva et al. [31] in the mathematical modeling of malagueta pepper (*Capsicum frutescens* L.) using the dynamic method proposed by Rodrigues et al. [32], assuming that the SFE bed content is a pseudo-ternary system formed by solvent, extract and solid matrix. Three model parameters were adjusted: the intact solid ratio (X_k), the fluid phase mass transfer coefficient (k_{Y_A}), which is convective, and the solid phase mass transfer coefficient (k_{X_A}), which is rather diffusive.

For both models the objective function was defined as the average absolute relative deviation (AARD) referred to yield (broken-intact cell models) or time (BET-based model) according to Eq. (17).

$$AARD\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{x_{i,exp} - x_{i,cal}}{x_{i,exp}}\right| \tag{17}$$

where: $AARD$ is the average absolute relative deviation (%), n is the number of experimental data, and $x_{i,exp}$ and $x_{i,cal}$ refer to experimental and calculated yields/times for the data i, respectively.

2.5. Field emission scanning electron microscopy (FESEM)

The morphology of the particles of the pepper pericarp was evaluated before and after SFE through FESEM images. The pericarp particles were previously separated from peduncles and seeds using tweezers. Different areas of the sample particles were analyzed, including the dry raw material, the particles that underwent SFE (25 MPa and 40 °C) and SFE-US (25 MPa, 40 °C, 600 W and 80 min). A minimum of 20 images was obtained in each sample.

FESEM images were obtained in a scanning electron microscope equipped with a field emission gun (Quanta 650, FEI, Hillsboro, Oregon, USA). Prior to analysis, the samples were coated with gold in a SCD 050 splutter coater (Oerlikon-Balzers, Balzers, Liechtenstein). Both equipment were available at the Brazilian National Laboratory of Nanotechnology (LNNano), located in Campinas, Brazil. The analysis on the surface samples was performed under vacuum, using an acceleration tension of 5 kV.

2.6. Statistical analysis

Global yield, total phenolics, antioxidant capacities (DPPH and FRAP) and total capsaicinoids were evaluated by the Tukey's test at level of 5% ($p < 0.05$), using the software Statistica 7.0 (Statsoft Inc., USA), in order to detect significant differences in the results of SFE.

3. Results and discussion

3.1. Sample characterization

The physical and chemical characteristics of the dried and milled peppers used in the extraction experiments are shown on Table 2. Santos et al. [4] obtained total lipid content of malagueta pepper (*Capsicum frutescens* L.) by Soxhlet with hexane of 9.70 ± 0.10% and Aguiar et al. [33] obtained 2.60 ± 0.03% for biquinho pepper (*Capsicum chinense*) using the same technique. However, some factors, such as the possibility of cross-species and a large number of pepper genotypes may explain the differences in chemical compositions of *Capsicum* peppers [34–36].

Table 3 shows the global yields, total phenolic contents, and antioxidant capacities (DPPH and FRAP) of the extracts obtained by SFE and SFE-US.

Table 2
Physical and chemical characteristics from dried and milled peppers.

Characteristic	Dedo de moça pepper
Mean particle diameter	0.68 ± 0.03 mm
Real density	1.41 ± 0.01 g/cm^3
Porosity	0.69
Moisture content	4.32 ± 0.10%
Total lipid	5.11 ± 0.36%

Table 3
Global yields, total phenolic contents and antioxidant capacities (DPPH and FRAP) of the extracts from dedo de moça pepper obtained by SFE and SFE-US.

Method	Temperature (°C)	Pressure (MPa)	US power (W)	US time (min)	CO_2 density[1] (kg/m^3)	US energy (kJ/cm^2)	X_o (%)*	TPC*	DPPH*	FRAP*
SFE	40	15	–	–	789.58	–	1.58 ± 0.02^{bc}	0.07 ± 0.00^{c}	0.82 ± 0.22^{b}	0.03 ± 0.00^{a}
	40	25	–	–	889.77	–	1.94 ± 0.20^{ab}	0.13 ± 0.01^{bc}	0.77 ± 0.20^{b}	0.03 ± 0.00^{a}
	60	15	–	–	607.72	–	1.42 ± 0.11^{c}	0.07 ± 0.02^{c}	0.59 ± 0.06^{b}	0.02 ± 0.00^{a}
	60	25	–	–	794.36	–	2.33 ± 0.26^{a}	0.23 ± 0.02^{a}	1.12 ± 0.08^{b}	0.04 ± 0.00^{a}
	50	20	–	–	792.90	–	2.07 ± 0.17^{a}	0.14 ± 0.04^{b}	2.57 ± 0.47^{a}	0.03 ± 0.00^{a}
SFE-US	40	15	200	40	789.58	361.64	1.85 ± 0.29^{bc}	0.13 ± 0.02^{e}	0.12 ± 0.08^{b}	0.01 ± 0.00^{b}
		15	200	80	789.58	723.27	2.17 ± 0.38^{abc}	0.62 ± 0.11^{a}	0.10 ± 0.10^{b}	0.03 ± 0.01^{a}
		15	600	40	789.58	1084.91	1.49 ± 0.26^{c}	0.26 ± 0.07^{de}	0.32 ± 0.23^{ab}	0.01 ± 0.00^{ab}
		15	600	80	789.58	2169.82	1.59 ± 0.06^{c}	0.28 ± 0.01^{cd}	0.46 ± 0.12^{ab}	0.01 ± 0.01^{ab}
		25	200	40	889.77	361.64	2.31 ± 0.39^{abc}	0.42 ± 0.03^{bc}	0.93 ± 0.36^{a}	0.01 ± 0.00^{ab}
		25	200	80	889.77	723.27	2.39 ± 0.13^{abc}	0.49 ± 0.01^{ab}	0.34 ± 0.31^{ab}	0.01 ± 0.00^{ab}
		25	600	40	889.77	1084.91	2.61 ± 0.41^{ab}	0.19 ± 0.06^{de}	0.21 ± 0.20^{b}	0.02 ± 0.01^{ab}
		25	600	80	889.77	2169.82	2.82 ± 0.55^{a}	0.22 ± 0.06^{de}	0.24 ± 0.10^{b}	0.02 ± 0.00^{a}
		20	400	60	849.90	1084.91	1.71 ± 0.17^{bc}	0.32 ± 0.03^{cd}	0.70 ± 0.23^{ab}	0.01 ± 0.00^{ab}

Results are expressed as mean ± standard deviation of the analyses. US – Ultrasound.
[1] CO_2 density calculated using the Angus, Armstrong and Reuck equation [37]; TPC – Total phenolic content expressed as mg EAG/g RM; DPPH – antioxidant capacity expressed as μmol Trolox/g RM; FRAP – antioxidant capacity expressed as g $FeSO_4$/g RM.
* Equal letters in the same column indicate that there is no significant difference at the level of 5% by the Tukey's test.

3.2. Global yield (X_o)

In the SFE experiments, the statistical analysis showed that the effects of pressure and the interaction between pressure and temperature were statistically significant according to the Tukey's test at level of 5% (p-value < 0.05), for the results of global yield. According to Castro et al. [38], the raise in pressure decreases the mean distance between the molecules, enhancing the solute/solvent interactions.

In SFE-US only the effects of pressure and the interaction between pressure and ultrasound power, were statistically significant according to the Tukey's test at level of 5% (p-value < 0.05). On the other hand, the time of ultrasound applied was not significant (p-value > 0.05). Santos et al. [4] observed that the effects of ultrasonic power and radiation time resulted in the highest global yield of the extracts from malagueta pepper (*Capsicum frutescens* L.). Balachadran et al. [39] evaluated the extracts from ginger (*Zingiber officinale roscoe*) in the SFE-US and observed that ultrasonic waves increased the number of broken cells of the vegetable matrix, enhancing the contact between solvent and solute. According to Riera et al. [15], the increase of global yield is caused by the raise of mass transfer coefficients with ultrasound during the extraction. Hu et al. [40] observed that the effects of higher ultrasonic powers imply in stronger vibrations in the interface between the solvent and the solid matrix, thus a large quantity of solute becomes available to be extracted.

According to the information on Table 3, one can observe that the use of ultrasound (SFE-US) increased the global yield in comparison to SFE experiments. At higher ultrasound power, SFE-US achieved a yield up to 45% higher than SFE. The increase in global yield may be due to the disruption of the cells caused by the ultrasonic waves, enhancing the access of the solvent to the internal region of the vegetable matrix [39].

3.3. Total phenolics content (TPC)

The statistical analysis of the SFE experiments showed that the effects of pressure, temperature and the interaction between them, were statistically significant according to the Tukey's test at level of 5% (p-value < 0.05), for determination of phenolic content. The results presented on Table 3 show that the effect of high pressures and temperatures enhance the solubilization of phenolics in SC-CO_2. Murga et al. [41] studied the solubility of phenolic compounds in SFE from grapes seeds and also observed that the solubility of these compounds increased with pressure and temperature. The authors also noticed that higher CO_2 densities increased the solubility of these compounds and their amounts in the extracts.

For SFE-US, the statistical analysis showed that the effects of ultrasound power and radiation time were significant according to the Tukey's test at level of 5% (p-value < 0.05). From Table 3 it is possible to notice an increase in the concentration of phenolics when the ultrasound was applied. According to Carrera et al. [42], the energy provided by ultrasound releases the phenolic compounds from the vegetable matrix, increasing their amounts in the extract. On the other hand, the use of higher ultrasonic power values diminished the phenolic recoveries. Carrera et al. [42] also reported that ultrasound accelerates the degradation of phenolics and possibly promotes the formation of free radicals, increasing oxidation reactions.

3.4. Antioxidant capacity

For the determination of antioxidant capacity by DPPH method in SFE, the statistical analysis showed that the effect of the interaction between pressure and temperature was statistically significant at the level of 5% according to the Tukey's test (p-value < 0.05). Passos et al. [43] extracted grape seed oil through SFE and observed that pressure and temperature affect the solubility in SC-CO_2, due to density changes. SC-CO_2 density increases with pressure and thus enhances the solubility of antioxidant compounds. On the other hand, the increase in temperature decreases the SC-CO_2 density and reduced the antioxidant capacity of the extracts. For SFE-US, the effects of interaction between power and pressure, and power and radiation time, were statistically significant at the level of 5% (p-value < 0.05). From Table 3 one can see that the increment in the ultrasound power decreased the antioxidant concentration in the extracts. Higher ultrasonic power increases the degradation of antioxidant compounds due to the aggression to the vegetable matrix, or because of the raise in the temperature of the extraction cell, which may reduce their solubility in SC-CO_2.

The results obtained by FRAP for SFE-US show that the effect of ultrasound power did not change statistically the antioxidant capacity (p-value > 0.05), while in SFE the effect of none variable (pressure, temperature or their interaction) was statistically significant (p-value > 0.05).

Summarizing, the extracts obtained from SFE and SFE-US experiments were able to sequester the DPPH radical and to reduce Fe^{+3}.

Table 4
Capsaicinoids yield of oleoresin from dedo de moça pepper obtained by SFE and SFE-US.

Method	Temperature (°C)	Pressure (MPa)	US power (W)	US time (min)	CO_2 density[†] (kg/m^3)	Energy (kJ/cm^2)	n-DHC[*]	C[*]	DHC[*]	h-C[*]	h-DHC[*]	Total capsaicinoids[*]
SFE	40	15	–	–	789.58	–	0.04 ± 0.00^b	0.68 ± 0.06^b	0.28 ± 0.02^b	0.03 ± 0.00^b	0.01 ± 0.00^b	1.05 ± 0.09^b
	40	25	–	–	889.77	–	0.06 ± 0.01^{ab}	0.88 ± 0.11^{ab}	0.37 ± 0.04^{ab}	0.04 ± 0.00^{ab}	0.01 ± 0.00^{ab}	1.36 ± 0.17^{ab}
	60	15	–	–	607.72	–	0.04 ± 0.00^b	0.69 ± 0.02^b	0.29 ± 0.01^b	0.03 ± 0.00^b	0.01 ± 0.00^b	1.06 ± 0.03^b
	60	25	–	–	794.36	–	0.07 ± 0.00^a	1.06 ± 0.08^a	0.44 ± 0.03^a	0.05 ± 0.00^a	0.01 ± 0.00^a	1.63 ± 0.12^a
	50	20	–	–	792.90	–	0.06 ± 0.01^a	0.99 ± 0.10^a	0.41 ± 0.04^a	0.05 ± 0.00^a	0.01 ± 0.00^a	1.53 ± 0.16^a
SFE-US	40	15	200	40	789.58	361.64	0.06 ± 0.00^a	0.76 ± 0.05^a	0.32 ± 0.02^a	0.04 ± 0.00^a	0.01 ± 0.00^a	1.17 ± 0.09^a
		15	200	80	789.58	723.27	0.05 ± 0.00^a	0.85 ± 0.03^a	0.36 ± 0.02^a	0.04 ± 0.00^a	0.01 ± 0.00^a	1.31 ± 0.06^a
		15	600	40	789.58	1084.91	0.04 ± 0.01^a	0.66 ± 0.16^a	0.28 ± 0.07^a	0.03 ± 0.00^a	0.01 ± 0.00^a	1.02 ± 0.24^a
		15	600	80	789.58	2169.82	0.05 ± 0.00^a	0.74 ± 0.04^a	0.31 ± 0.02^a	0.03 ± 0.00^a	0.01 ± 0.00^a	1.14 ± 0.06^a
		25	200	40	889.77	361.64	0.06 ± 0.01^a	0.99 ± 0.21^a	0.41 ± 0.09^a	0.05 ± 0.01^a	0.01 ± 0.00^a	1.52 ± 0.33^a
		25	200	80	889.77	723.27	0.06 ± 0.01^a	0.94 ± 0.13^a	0.40 ± 0.05^a	0.04 ± 0.00^a	0.01 ± 0.00^a	1.46 ± 0.20^a
		25	600	40	889.77	1084.91	0.06 ± 0.01^a	0.99 ± 0.10^a	0.41 ± 0.04^a	0.05 ± 0.00^a	0.01 ± 0.00^a	1.52 ± 0.16^a
		25	600	80	889.77	2169.82	0.06 ± 0.00^a	0.94 ± 0.09^a	0.39 ± 0.04^a	0.04 ± 0.00^a	0.01 ± 0.00^a	1.45 ± 0.13^a
		20	400	60	849.90	1084.91	0.05 ± 0.01^a	0.81 ± 0.16^a	0.34 ± 0.07^a	0.04 ± 0.01^a	0.01 ± 0.00^a	1.25 ± 0.25^a

Results are expressed as mean ± standard deviation of the analyses.
Equal letters in the same column indicate no significant difference between the results, at the level of 5%.
[†] CO_2 density calculated using the Angus, Armstrong and Reuck equation [37]. n-DHC – nordihydrocapsaicin, C – capsaicin, DHC – dihydrocapsaicin, h-C – homocapsaicin and h-DHC – homodihydrocapsaicin. All capsaicinoids expressed as mg capsaicinoids/g raw material.
[*] Equal letters in the same column indicate that there is no significant difference at the level of 5% by the Tukey's test.

The antioxidant capacities might have been influenced by the bioactive compounds, such as phenolics, and by reaction mechanisms of each method. The DPPH assay detects compounds that can act by direct reduction, via electron transfer and hydrogen atom transfer reactions. On the other hand, FRAP reaction detects compounds with redox potentials of <0.7 V (potential redox of Fe^{+3}-TPTZ) [44].

3.5. Analysis of capsaicinoids

Table 4 presents the concentrations of the capsaicinoids n-DHC, C, DHC, h-C and h-DHC found in the extracts obtained by SFE and SFE-US. The higher capsaicinoid recoveries by SFE were obtained with CO_2 densities varying from 792.90 to 889.77 kg/m^3. Extractions with lower CO_2 densities (from 607.72 to 789.58 kg/m^3) resulted in lower concentrations of these compounds. Peusch et al. [45] and Perva-Unzunalic et al. [23] also observed that the capsaicinoids yield in SFE increases with density.

In both SFE and SFE-US experiments, the statistical analysis showed that only the effect of pressure was significant at level of 5% by the Tukey's test (p-value < 0.05). From Table 4 one can also see that the application of ultrasound (SFE-US) provided extracts with higher concentrations of total capsaicinoids in comparison to SFE experiments. The solvent accessibility to the pepper seeds and placenta must have been enhanced due to the action of ultrasound, disrupting the vegetal matrix and allowing the penetration of CO_2. Govindrajan et al. [46] observed that, in the drying process, the wax layers of the peppers are more prone to degradation and the application of ultrasound accelerates the process of shrinkage/-collapse of the cells, enhancing the absorption of the extraction solvent.

3.6. Mathematical modeling

Table 5 presents the data needed to apply the BET-based [18] and the broken and intact cell [19] models to the SFE and SFE-US curves, the values of the adjusted parameters, the objective function (AARD) of each model and its correlation coefficient (r^2). Fig. 2 shows the kinetics of experimental and modeled data obtained at 15 MPa through SFE (15 MPa and 40 °C) and SFE-US (15 MPa, 40 °C, 200 W and 40 min) using the broken and intact cell [19] and the BET-based [18] models.

Analyzing the SFE kinetics at 15 MPa without ultrasound, one can notice that the CER (constant extraction rate), FER (falling extraction rate) and DC (diffusion controlled) periods are not clearly distinguishable when compared to the kinetics with ultrasound. A possible reason is that the extraction time applied was not enough to achieve the DC period. At 15 MPa, the broken and intact cell model [19] provided a constant extraction rate period (t_{cer}) of approximately 19 min without ultrasound and 11 min with ultrasound. Moreover, both models indicated that ultrasound at 15 MPa reduced the extraction time of the readily available solute, resulting in a higher extraction yield.

Fig. 3 shows the kinetics of the experimental and modeled data obtained at 25 MPa through SFE (25 MPa and 40 °C) and SFE-US (25 MPa, 40 °C, 600 W and 80 min) using the broken and intact cell [19] and the BET-based [18] models, where the three periods (CER, FER and DC) can be identified. The broken and intact cell model [19] provided a CER period of approximately 23 min without ultrasound and 37 min with ultrasound, a difference of 55%. The results of both models show that ultrasound at 25 MPa increased the extraction time of oleoresin in the CER period. Besides, SFE without ultrasound achieved a yield 15% higher than that obtained with ultrasound in the CER period. The same trend was observed by Riera et al. [15]. A possible explanation to this behavior is that high pressures and ultrasonic powers may cause compression of the

Table 5
Experimental data needed to apply the BET-based [18] and the broken and intact cell [19] models to the SFE and SFE-US curves, the values of the adjusted parameters, the objective function (AARD) of each model and its correlation coefficient. Input data: temperature (T = 313.15 K); cell diameter (d = (6.8 ± 0.03) × 10^{-3} m); extraction density (ρ_{ext} = (1.41 ± 0.01) × 10^3 kg/m^3); CO_2 density (ρ_{CO_2} = 7.89 × 10^2 kg/m^3); bed height (H_b = 0.00489 m); bed diameter (d_b = 0.0544 m); feed sample (F = 0.050 ± 0,001 kg); solubility (y^* = 0.0032 kg extract/kg solvent).

Parameter	SFE condition			
	15 MPa	15 MPa + US	25 MPa	25 MPa + US
X_o (kg extract/kg RM)	0.0163 ± 0.0004	0.0172 ± 0.002	0.0181 ± 0.0006	0.0243 ± 0.0008
$Q_{CO_2} \times 10^{-4}$ (kg/s)	1.757	1.748	1.754	1.745
m_o (kg extract/kg RM)	0.016 ± 0.022	0.0185 ± 0.003	0.0194 ± 0.002	0.0282 ± 0.005

Condition	Adjusted parameters							
	Sovová			Pardo (2P)		Pardo (3P)		
	$k_{Y_A} \times 10^{-3}$	$k_{X_A} \times 10^{-4}$	X_K	x_m	$y^* \times 10^{-5}$	x_m	$y^* \times 10^{-5}$	K
15 MPa without US	0.566	2.337	0.013	0.299	2.46	0.161	2.15	1.778
15 MPa with US	1.181	2.718	0.013	36.81	170.0	0.886	13.40	5.099
25 MPa without US	1.241	0.999	0.007	25.90	140.0	0.885	19.90	6.441
25 MPa with US	1.067	0.999	0.009	1.734	113.0	0.628	7.82	2.684

Condition	AARD (%)					
	Sovová	r^2 (%)	Pardo (2P)	r^2 (%)	Pardo (3P)	r^2 (%)
15 MPa without US	11.325	99.4	8.715	99.8	8.796	99.8
15 MPa with US	19.418	99.4	14.260	99.6	10.537	97.8
25 MPa without US	11.356	94.9	10.349	99.1	4.071	98.4
25 MPa with US	13.007	95.5	8.028	99.4	8.342	98.8

(2P) and (3P) – Two and three adjustable parameters in the BET-based model [18]. r^2 – Correlation coefficient.

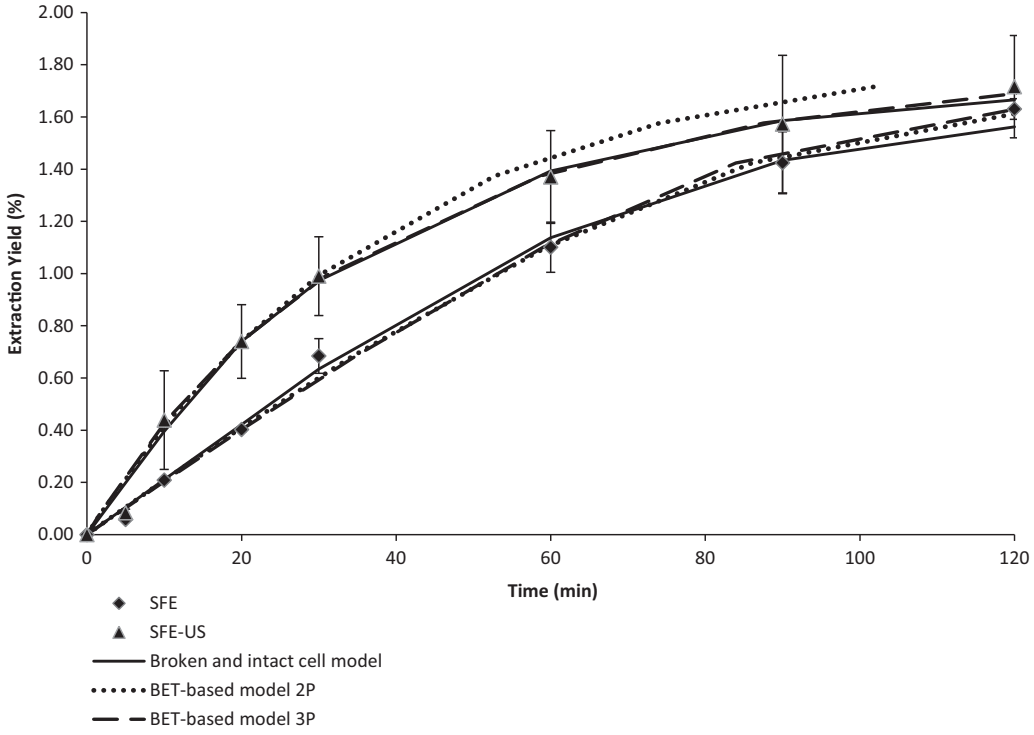


Fig. 2. Kinetics of the experimental and modeled data obtained at 15 MPa through SFE (15 MPa and 40 °C) and SFE-US (15 MPa, 40 °C, 200 W and 40 min) using the broken and intact cell [19] and the BET-based [18] models.

extraction bed, which can obstruct the solvent flow or decrease the contact extract/solvent in the beginning of the extraction. However, in the FER and DC periods, where diffusion is the main mass transfer mechanism, the application of ultrasound increased the extraction yield.

According to the broken and intact cell model [19], in all the evaluated kinetics the values of the mass transfer coefficient in the fluid phase (k_{Y_A}) were higher than those of the solid phase (k_{X_A}). Similar results were found by Santos et al. [4] and Silva et al. [31] in the SFE from malagueta pepper. One can observe that the solute is hardly dissolved when located internally in the particles and the time needed to cross the solid–fluids interface is higher than when the solute is located in the surface of the particles. Thus, diffusion is less representative than convection.

As can be noted, the values of the parameters y^*, x_m and K adjusted with the BET-based model [18] at 15 MPa increased with

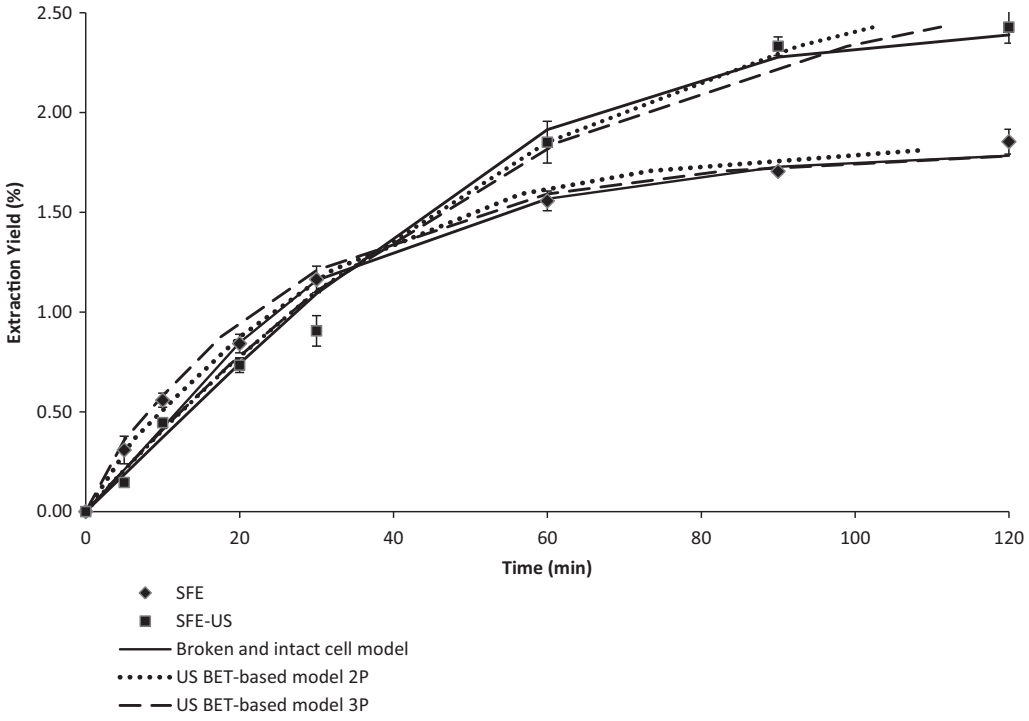


Fig. 3. Kinetics of the experimental and modeled data obtained at 25 MPa through SFE (25 MPa and 40 °C) and SFE-US (25 MPa, 40 °C, 600 W and 80 min) using the broken and intact cell [19] and the BET-based [18] models.

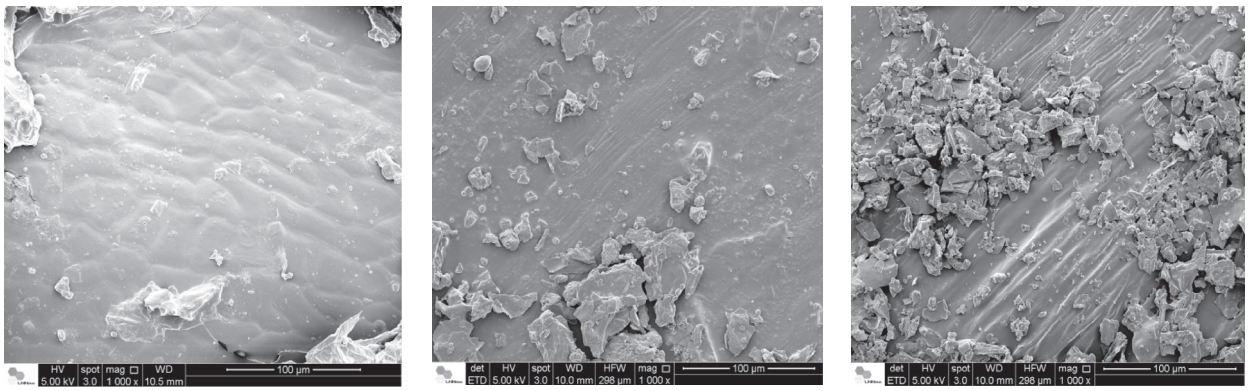


Fig. 4. FESEM images obtained on the pericarp of dedo de moça pepper samples: raw material (a); SFE (25 MPa and 40 °C) (b) and SFE-US (25 MPa, 40 °C, 600 W and 80 min) (c). Scale bar – 100 μm.

ultrasound. So, ultrasound was capable to disrupt the vegetable matrix and raise the extraction of solutes from the first monolayer, increasing the readily available solute in contact with the solvent. Therefore, the use of ultrasound at 15 MPa increased the extraction yield in all SFE periods.

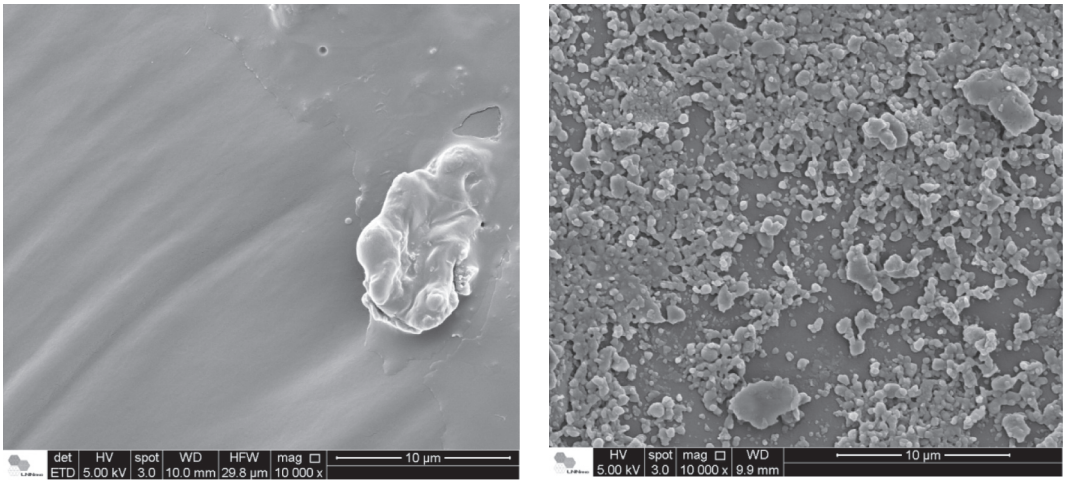
In the kinetics assay at 25 MPa, the BET-based model [18] shows that in the first period (CER) the application of ultrasound reduced the solubility, the mass of solute in the first monolayer and the adsorption equilibrium constant. This behavior may be due to the physical condition inside the extraction cell. Pressure, ultrasonic power and application time decreased the solvation capacity of SC-CO_2 in the CER period [43]. However, the FER and DC periods, controlled by diffusion, had their yields increased when ultrasound was applied. Table 5 also shows that the BET-based model [18] with three adjustable parameters achieved the best adjustments of the kinetics evaluated due to its lower AARD.

To sum up, the broken and intact cell [19] and the BET-based models [18] were capable to describe the main mass transfer phenomena. Comparing both models, the BET-based is simpler, since it uses a single equation to adjust the parameters, besides providing the solubility as an adjustable parameter.

3.7. Field emission scanning electron analysis (FESEM)

The effects of SFE and ultrasound on the morphological structure of the vegetable matrix were evaluated through FESEM. Fig. 4 shows the FESEM images obtained on the surface of the pepper sample pericarp: raw material (a), after SFE (25 MPa and 40 °C) (b) and after SFE-US (25 MPa, 40 °C, 600 W and 80 min) (c).

The samples that underwent SFE (Figs. 4b and c) presented a higher amount of particles deposed on their surface when compared to the raw material (Fig. 4a). In SFE processes, the contact



(a) SFE (b) SFE-US

Fig. 5. FESEM images obtained on the pericarp of dedo de moça samples: SFE (25 MPa and 40 °C) (a) and SFE-US (25 MPa, 40 °C, 600 W and 80 min) (b). Scale bar – 10 µm.

of the supercritical fluid with the solute and mechanical vibration can disrupt the cell wall, releasing particles from the internal part of the vegetable matrix to the surface. When ultrasound is applied, this effect on the surface is enhanced. In this case, ultrasound intensifies the mass transfer process, increasing the access of the solvent to these particles, which results in higher extraction rate from the samples. Pasquel-Reátegui et al. [16] and Santos et al. [4] also observed using FESEM a greater amount of particles present on the sample surface in SFE processes.

Fig. 5 presents a magnified view of the samples after SFE (25 MPa and 40 °C) (a) and SFE-US (25 MPa, 40 °C, 600 W and 80 min) (b). These images show the surface below the particle deposition without cracks, fissures or any sign of disruption, which means that SFE and SFE-US processes did not damage the surface of the samples.

4. Conclusions

The application of ultrasound increased the global yield of SFE from dedo de moça pepper up to 45%. The phenolic content also increased with ultrasound, but high ultrasonic power reduced its content. Ultrasound did not increase the concentration of antioxidant compounds probably because of the increase in the internal temperature of the extraction cell, which could have reduced the solubility of these components in SC-CO_2. However, SFE-US increased the concentration of capsaicinoids when compared to SFE. A direct relation between CO_2 density and the capsaicinoid content was observed.

The kinetic models proposed by Sovová [19] and Pardo et al. [18] were effective to describe the mass transfer phenomena that occurred in SFE and SFE-US. At 15 MPa, ultrasound enhanced the access of the solvent to the extractable solute. On the other hand, at 25 MPa the application of ultrasound reduced the solubility in the initial period (CER). The best adjustment of the objective function (AARD) was achieved by the BET-based model [18] with three adjustable parameters. The FESEM images revealed that SFE changed the structure of the vegetable matrix of dedo de moça pepper samples. In SFE-US a higher quantity of particles was released from the internal region of the cell wall to the surface, which explains the changes observed in the extraction kinetics and yields.

Summarizing, ultrasound was capable to improve the SFE yield from dedo de moça pepper, and the combined SFE-US technique can be used to replace conventional extraction techniques that use organic and toxic solvents. A challenge for future works in this field is the scale-up of SFE-US systems, the variation of particle size and its influence in the mass transfer processes, and the study of the economic viability of the SFE-US process.

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