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Supercritical CO_2 extraction of passion fruit (*Passiflora edulis* sp.) seed oil assisted by ultrasound

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ABSTRACT

This work evaluated the effect of ultrasound, temperature and pressure on the supercritical CO_2 extraction of passion fruit seed oil. The raw material was a by-product of the pulp processing industry (seeds mixed with pulp). Fatty acids, tocopherol and tocotrienol composition and DPPH radical scavenging activity were evaluated. The obtained oil was rich in polyunsaturated fatty acids (about 67%), tocopherol and tocotrienol (between 60 and 90 mg/100 g oil), presented high DPPH radical scavenging activity (between 1.8 and 2.6 mg TE/g oil), which showed correlation with the tocopherol and tocotrienol total content ($r = +0{,}872$). The application of the ultrasound power of 160 W favored the oil extraction, since the SFE global yield improvement achieved 29% (at 40 °C and 16 MPa). The mathematical model of Sovová (1994) was able to describe the extraction kinetics. There were increases of the constant extraction rate time and of the fluid phase mass transfer coefficient, and reduction of the solute ratio inside the cells due to the ultrasound application. The images obtained by field emission scanning electron microscopy showed mechanical damage and smaller particle size when ultrasound was applied.

1. Introduction

Passion fruit is important industrially due to the pulp and juice production [1]. However, the fruit processing industry generates great amount of waste (peel and seeds). In this context, it is economically, scientifically and ecologically interesting to investigate new applications for these by-products [2]. The passion fruit seeds are used nowadays to produce oil, which is destined to the cosmetic and food industries [1], due to its antioxidant activity [3], tocopherol and polyphenol content [4]. Cold press [5] and Soxhlet with different solvents [6] have been used to extract this oil, with similar efficiencies.

Supercritical fluid extraction (SFE) has been used as an alternative technique to extract oil from different vegetal tissues, such as seeds and peels [7]. The moderate critical temperature and pressure of carbon dioxide (CO_2) make this solvent the most commonly used. Ultrasound has been used during SFE in order to improve the extraction efficiency. Ultrasonic waves produce compression and decompression cycles, where there could be formation of bubbles. These bubbles grow as the ultrasonic waves go through the fluid, until they reach a critical size and collapse, in a process known as cavitation. When cavitation happens in a fluid close to a solid surface, the collapse generates a high speed jet of liquid that can break some structures of the surface [8] and release part of the extractable material. SFE enhanced by ultrasound is a promising process to reduce extraction times, and to increase the extraction yields compared to those obtained without ultrasound [9–14].

The objective of this work was to investigate the effects of pressure, temperature and ultrasonic waves on the SFE of passion fruit seed oil (*Passiflora edulis* sp.), as well as to evaluate the fatty acid composition, tocopherol and tocotrienol content and the DPPH radical scavenging activity of the extracts. The mathematical model of Sovová [15] has been widely used for SFE kinetics modeling, because of it considers the three stages of the SFE: (i) CER: constant extraction rate, characterized by the extraction of the easy access material, being convection the predominant mass transfer mechanism; (ii) FER: falling extraction rate, convection and diffusion comes to be important; (iii) DC: diffusion controlled, where diffusion is the predominant mass transfer mechanism. This model was adjusted to the extraction curves, and the influence of ultrasound power on the extraction kinetics was verified. Further, the effect of the ultrasonic waves on the passion fruit seed particles was evaluated by field emission scanning electron microscopy (FESEM).

2. Material and methods

By-products of the manufacturing of passion fruit pulp (*Passiflora edulis* sp.) provided by the fruit pulp industry "*Sítio do Belo*",

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located in *Paraibuna – SP*, southeastern Brazil, were used as raw material. This by-product consisted of a mixture of passion fruit seeds and pulp, directly separated from the production line.

2.1. Sample preparation

The industrial by-product was dried in an air circulation oven (model 320 SE, Fanem, SP – Brazil) at 50 °C during 24 h. The dried product was then ground in a 600 W blender (R12008/81, Philips, Brazil) in order to reduce the particle size to increase the contact surface with the extraction solvent. The dried and ground material (in this study referred to as "passion fruit seeds") was kept in absence of light, at a temperature of −18 °C, until its use.

2.2. Particle characterization and proximate composition

2.2.1. Particle characterization

The passion fruit seeds were characterized by size classification in a vertical vibratory sieve shaker (Bertel Metallurgic Ind. Ltd., São Paulo, SP, Brazil), and the mean particle diameter ($\bar{d}$) was calculated using Eq. (1).

$$\bar{d} = \left(\Sigma \frac{\frac{m_i}{\Sigma m_i}}{d_i} \right)^{-1} \quad (1)$$

Where $\bar{d}$: mean particles diameter; d_i: diameter of the sieve i; m_i: mass of the sample retained on the sieve i.The real density (ρ_r) of the passion fruit seed particles was determined by helium gas pichnometry. The apparent density (ρ_a) was obtained dividing the sample mass used to make the particles bed inside the extraction column by the volume of the extraction bed. Then, the porosity (ϵ) of the bed of particles was calculated using Eq. (2).

$$\epsilon = 1 - \left(\frac{\rho_a}{\rho_r} \right) \quad (2)$$

The analyses described in this section were performed in triplicate.

2.2.2. Proximate composition

Analyses of moisture and ash were performed according to AOAC techniques [16] (methods 934.06 and 942.05 respectively). Crude protein was determined by the semi-micro-Kjeldahl procedure, described by AOAC [16] (method 970.22). The total lipids were extracted by the Soxhlet method using petroleum ether as solvent [16] (method 963.15).

2.3. Global yield (X_0)

For each extraction experiment (Soxhlet, SFE with and without ultrasound) the extraction global yield (X_0) was calculated according to Eq. (3), which relates the total extract mass ($m_{extract}$) and the sample mass (F) in dry basis.

$$X_0 = \frac{m_{extract}}{F} \times 100 \quad (3)$$

2.4. Soxhlet extraction

The Soxhlet method was selected as conventional extraction technique, using hexane as solvent, due to its non-polar characteristic, which is adequate for lipid extraction, and its current use in oil extraction by food industries [17]. For each extraction 5 g of dried sample were packed in filter paper and inserted in the Soxhlet extractor. Hexane (0.15 L) was added and the system was heated until boiling. Reflux was kept for 6 h, then the solvent was evaporated under vacuum (at 35 °C), and the recovered extract was weighed and stored under freezing (−18 °C) until further analyses. The Soxhlet extractions were performed in triplicates.

2.5. Supercritical extraction assisted by ultrasound

The SFE experiments were performed in an ultrasound-assisted supercritical fluid extraction (SFE + US) unit, which consists of a 0.3 L extraction column; a pneumatic pump (PP 111-VE MBR, Maximator, Nordhausen, Germany); two thermostatic baths (model MA184, Marconi, Campinas, Brazil) to control the temperature of CO_2 at the pump inlet and SFE temperature; a flow totalizer and manometers. The ultrasound system (model DES500, Unique Group, Campinas, Brazil) is composed by a transducer unit with frequency of 20 kHz and a variable output power controller. The ultrasound probe is installed inside the SFE column. Fig. 1 illustrates the SFE + US unit, with special focus on the SFE + US bed.

The mass of passion fruit seeds used in each extraction was 5 g, defined aiming to obtain enough extract to perform the subsequent analyses. The SFE procedure was composed by an initial static extraction time of 20 min for all of the experiments, and after that, a dynamic extraction time of 100 min for the global yield experiments. The mass ratio between solvent and passion fruit seeds (S/F) was kept constant at 210 ± 2 kg CO_2/kg seeds in the global yield experiments. This ratio was assured by keeping constant the solvent flow rate (1.75×10^{-4} kg/s) and extraction time (100 min without including the static time). The application of ultrasound was implemented only during the static time of 20 min.

The response surface methodology was applied to evaluate the effects of temperature (Z_1), pressure (Z_2) and ultrasound power (Z_3) on the SFE global yield, using a central composite design. The parameter values have been coded using Eq. (4).

$$X_i = \frac{Z_i - Z_r}{\Delta_i}; i = 1, 2, 3 \quad (4)$$

Where X_i is the coded value for the parameter; Z_i is the real value; Z_r is the real value of the parameter in the central point; Δ_I is the step change in the variable Z_i.

The experimental conditions are expressed in Table 1, the experiments were performed in duplicates to ensure reproducibility. Pressure range has been selected based on the previous work of Liu et al. [18], considering values from 12 to 29 MPa. Temperature range has been determined by previous assays with the same raw matter, considering as a working range temperatures from 38 to 53 °C. Regarding the ultrasound power, the maximum reachable range of values with the equipment was used (from 0 to 800 W).

Second order polynomial regression has been applied over the responses obtained from the extraction experiments. Eq. (5) represents the statistical model adjusted.

$$\mathcal{X}_o = b_o + \Sigma_{i=1}^{3} b_i X_i + \Sigma_{i=1}^{3} b_{ii} X_i^2 + \Sigma_{j=1}^{3} \Sigma_{i=1}^{3} b_{ij} X_i X_j \quad (5)$$

Where $\mathcal{X}_o$ represents the values estimated by the model; b_o is a constraint, b_i; b_i; b_{ij} are the linear, quadratic and interaction coefficient, respectively; X_i and X_j are the coded parameter values. To prove the model significance with a confidence level of 95%, analysis of variance (ANOVA) has been performed using the software Statistica 7.

Since the solvent density is an important parameter that influences the solvation power of CO_2, it was calculated using the correlation of Angus [19].

2.6. Evaluation of the extracts

Based on the results of the experimental design described on Table 1 two conditions were selected to evaluate the SFE kinetics, the extracts' composition and the effects on the sample structure.

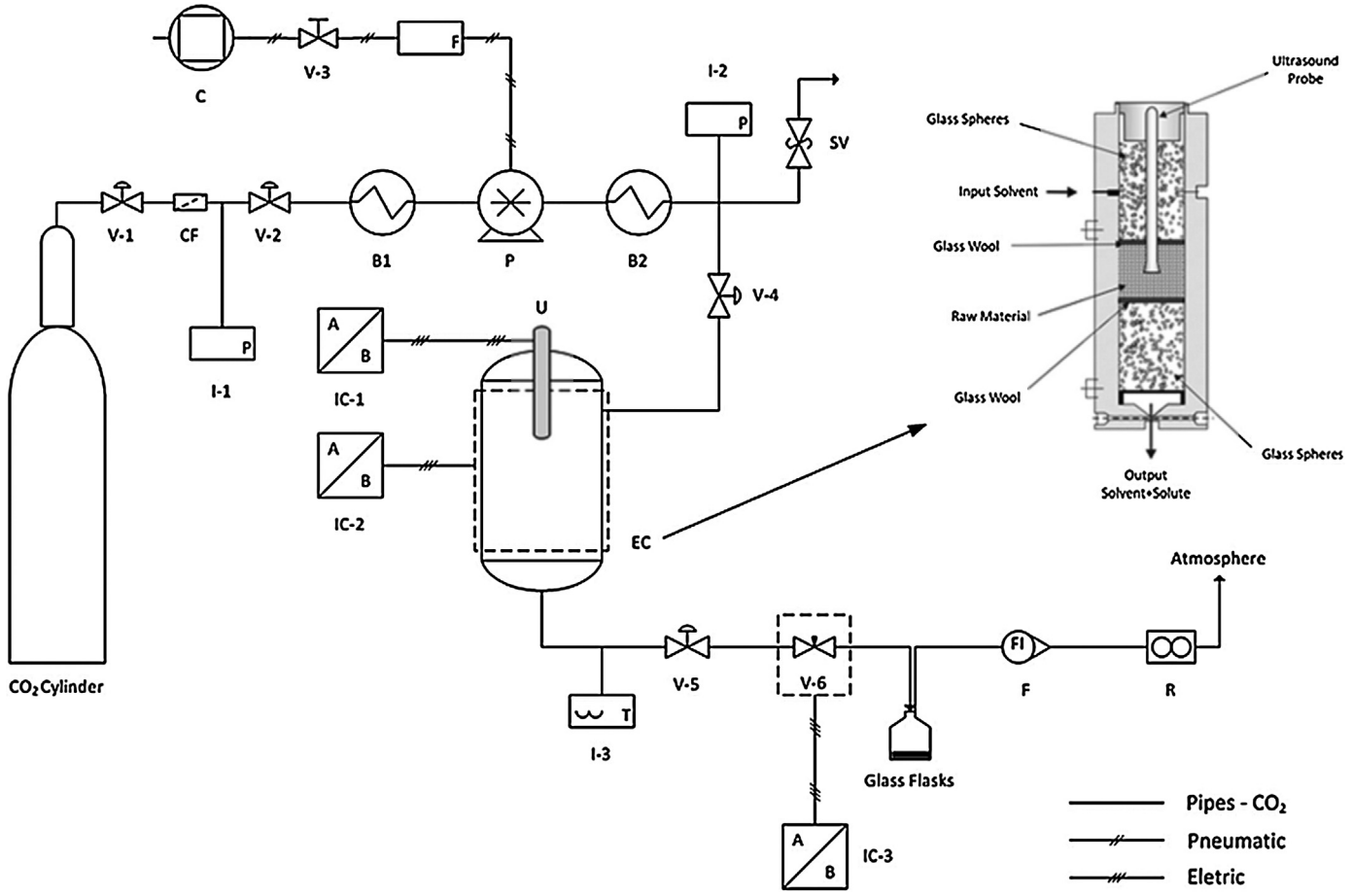

Fig. 1. Diagram of the SFE + US unit: V-1–V-5–control valves; V-6–micrometer valve; SV – safety valve; C – compressor; F – compressed air filter; CF – CO2 filter; B1–cooling bath; P – pump; B2–heating bath; I-1 and I-2–pressure indicators; I-3–temperature indicator; IC-1, IC-2 and IC-3–indicators and controllers of ultrasound 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.

The selected conditions, presented on Table 2, were (i) the condition in which the highest yield was obtained, also considering the minimum energy consumption in terms of temperature and pressure; and (ii) the condition in which the greatest difference in the SFE global yield was observed for different ultrasound powers applied. All the experiments described in this section were analyzed through the Tukey's test, with significance of 5%, to determine if there were significant differences between the different treatments.

2.6.1. Antioxidant activity

The in vitro antioxidant activity of the extracts was determined by the DPPH (2,2-Diphenyl-1-picryl-hidrazil) method [20] with modifications. Solutions of the extracted oils in ethanol were pre-

Table 1
Global yield of SFE passion fruit seed oil without and with ultrasound.

Codified variables			Real variable			X_0 (%)	CO_2 density (kg/m^3)
X_1	X_2	X_3	T (°C)	P (MPa)	U (W)		
−1	−1	−1	40	16	160	15.8 ± 0.3	804.5
−1	−1	1	40	16	640	13.5 ± 0.4	804.5
-1	1	−1	40	26	160	20.9 ± 0.2	896.4
-1	1	1	40	26	640	20.69 ± 0.05	896.4
1	−1	−1	50	16	160	8.9 ± 0.3	729.7
1	−1	1	50	16	640	7.1 ± 0.4	729.7
1	1	−1	50	26	160	20.6 ± 0.3	850.9
1	1	1	50	26	640	19.9 ± 0.4	850.9
0	0	0	45	21	400	19.15 ± 0.09	832.3
0	0	0	45	21	400	19.14 ± 0.07	832.3
−1.6	0	0	38	21	400	19.0 ± 0.7	874.5
0	−1.6	0	45	12	400	2.56 ± 0.02	700.6
0	0	−1.6	45	21	0	19.6 ± 0.4	832.3
1.6	0	0	53	21	400	15.5 ± 0.7	787.5
0	1.6	0	45	29	400	20.10 ± 0.04	893.4
0	0	1.6	45	21	800	18.94 ± 0.03	832.5

X_1: coded temperature; X_2: coded pressure; X_3: coded ultrasound power; X_0: SFE global yield. Values presented as mean ± standard deviation.

Table 2
Extraction yield, tocopherol and tocotrienol composition and DPPH scavenging activity of passion fruit seed oil extracted at 40 °C with different conditions by SFE and by Soxhlet using hexane as solvent.

Experimental condition			γ-Tocopherol (mg/100 g)	γ-Tocotrienol (mg/100 g)	δ-tocotrienol (mg/100 g)	DPPH(mg TE/g oil)	X_0 (%)	% Recovered
	P (MPa)	*U* (W)						
SFE	16	0	10.6 ± 0.9^{F}	36 ± 4^{ABC}	45 ± 3^{A}	2.3 ± 0.3	12.3 ± 0.1^{E}	49.7
	16	160	9.7 ± 0.7^{F}	34 ± 2^{CD}	43 ± 2^{AB}	2.6 ± 0.1	15.8 ± 0.3^{D}	64.0
	16	640	9.4 ± 0.4^{F}	33 ± 1^{CD}	44 ± 1^{A}	2.6 ± 0.3	13.5 ± 0.4^{E}	54.9
	26	0	8.0 ± 0.3^{F}	29 ± 1^{CDE}	35 ± 1^{BCD}	1.7 ± 0.2	18.5 ± 0.2^{C}	75.1
	26	160	7.3 ± 0.2^{F}	27 ± 1^{DE}	34 ± 1^{CD}	1.8 ± 0.3	20.9 ± 0.2^{B}	84.7
	26	640	6.7 ± 0.4^{F}	24 ± 2^{E}	33 ± 1^{CD}	2.1 ± 0.3	20.7 ± 0.1^{B}	83.9
	Soxhlet Hexane		8.7 ± 0.9^{F}	30 ± 1^{CDE}	34 ± 1^{BCD}	0.9 ± 0.1	24.7 ± 0.3^{A}	100.0

P: pressure; *U*: ultrasonic power; TE: Trolox equivalent. Values expressed as mean ± standard deviation.% recovered calculated based on the Soxhlet extraction yield. Means followed by the same letters do not differ significantly according to Tukey's test (0.05%).

pared, in concentrations of 15 mg/mL. A DPPH solution (60 μM) in ethanol and Trolox solutions in ethanol (50, 100, 200, 400, 600, 800, 1000 e 1200 μM) were also prepared. In the dark, a 0.1 mL aliquot of the sample solution, ethanol (control) or the Trolox solution (to plot the standard curve) was added into a test tube, and then 3.9 mL of the DPPH solution were added, with manual stirring for 30 s. The mixtures were let reacting in the dark at room temperature (25 °C) for 40 min. Then, the absorbance of the mixture was measured at a wavelength of 515 nm in a UV–vis spectrophotometer (Hach, DR/4000U, Colorado, USA), using ethanol as blank. Using the values of the absorbance standard curve, the antioxidant activity of the extracts was expressed as equivalent units of Trolox per gram of oil (mg TE/g oil).

2.6.2. Determination of tocopherols and tocotrienols by HPLC

The tocopherol and tocotrienol contents of the extracts were determined according to the AOCS official method Ce-8-89 [21] by High Performance Liquid Chromatography (HPLC). A PerkinElmer Series 200HPLC (Perkin Elmer, Sao Paulo, Brazil) was used with the following analytical conditions: isocratic pump PerkinElmer Series 200, fluorescence detector PerkinElmer Series 200a; wavelength – Excitation 290 nm Emission 330 nm; Analytical Column – Hibar® RT 250 mm × 4 mm Li Chrosorb® Si 60 (5 μm); and mobile phase – hexane/isopropanol (99/1) at 1.0 mL/min flow rate. The identification was performed by comparing the standards' retention time and quantification was performed by external standardization, using as standards α, β, γ, and δ tocopherol and tocotrienol (Sigma–Aldrich, Sao Paulo, Brazil). Each extract was analyzed in replicates and results were expressed as mean and standard deviation.

2.6.3. Determination of fatty acid (FA) composition by gas chromatography (GC)

FA composition of the extracts was determined by GC, according to the official method Ce 1f–96 of AOCS [16]. Prior to chromatographic analysis, the extracts were prepared in the form of fatty acid methyl esters (FAME) according to the method of Hartman and Lago [22]. The chromatographic analyses were performed using a capillary GC system (Agilent, 6850 Series GC System, Santa Clara, CA, USA) under the following experimental conditions: DB-23 capillary column (Agilent, 50% cyanopropyl-methylpolysiloxane, 0.25 μm × 60 m × 0.25 mm i.d., Santa Clara, CA, USA); helium as carrier gas at a flow rate of 0.001 L/min; linear velocity of 24 cm/s; injection temperature of 250 °C; column temperature of 110 °C for 5 min, (110–215) °C (rate of 5 °C/min), 215 °C for 24 min; detection temperature of 280 °C; and injection volume of 1.0 μL. The fatty acid methyl esters were identified by comparison with external standard (GLC-68A, Nu Check Prep, Elysian, MN, USA). Quantification was performed by internal normalization.

2.7. SFE kinetics

The kinetic experiments to obtain the SFE curves were performed by measuring the extract mass or global yield as a function of the extraction time, at the conditions of pressure, temperature and ultrasound power reported on Table 2. The extraction process was performed as described in Section 2.5.

Once the extraction curves were obtained, the mathematical model of Sovová [15] was fitted to the experimental data, and the mass transfer coefficients for the solid (k_{xa}) and fluid (k_{ya}) phases, as well as the mass ratio of solute inside the particles (Xk) were obtained. The comparison between these parameters on the different conditions helps comprehending the influence of pressure and ultrasound power on the SFE kinetics. The modeling process consisted on the individual fitting of each experimental curve. The software Force Fortran Compiler and Editor 2.0 was used, applying the free routine of Powell [23]. The solubility of the extract in CO_2, which is needed to apply the model, was determined according to the dynamic method of Rodrigues et al. [24].

2.8. Field emission scanning electron microscopy (FESEM)

FESEM images were obtained in samples of the unextracted dry raw material, of the material which results from the Soxhlet extraction and of the passion fruit seed particles after SFE, without and with ultrasound at the powers of 160 and 640 W. The FESEM equipment was 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 National Laboratory of Nanotechnology (LNNano), located in Campinas-SP, Brazil. The surface analyses were performed under vacuum, using an acceleration tension of 5 kV.

3. Results and discussion

3.1. Particles characterization and proximate composition

The physical and chemical characteristics of the passion fruit seed particles used as the raw material are shown on Table 3. The proximate composition is similar to that published by Ferrari et al. [2] for the characterization of by-products of the passion fruit processing industry, with minor differences that could be explained by differences in the growing period or in the subspecies evaluated.

3.2. SFE global yield

Table 1 shows the experimental design with the results of SFE global yield. Three statistically equal yields were achieved: (i) 20.9 ± 0.2% obtained at 40 °C, 26 MPa and 160 W of ultrasonic

Table 3
Proximate composition and particles characterization of passion fruit seed particles.

Analysis	Value
Ash (%)	6.6 ± 0.1
Protein (%)	17.7 ± 0.1
Total lipid content (%)	24.2 ± 0.1
Carbohydrate (%)	51.5 ± 0.1
Moisture (%)	3.5 ± 0.1
Particle average diameter (mm)	0.74 ± 0.02
Real density (kg/m^3)	1280 ± 10
Apparent density (kg/m^3)	420 ± 10

Values presented as mean ± standard deviation.

power; (ii) 20.69 ± 0.05 obtained at 40 °C, 26 MPa and 640 W of ultrasonic power; (iii) 20.6 ± 0.3 obtained at 50 °C, 26 MPa and 160 W of ultrasonic power. In these conditions the highest values of CO_2 density were achieved, which may explain the increase of its solvation power. The attained global yield was lower than those reported by Liu et al.[18] and Gholamreza Z. et al. [25]. One reason to explain this difference is the origin of the raw material that might result in variation of the fruit composition. On the other hand, the mentioned researchers used passion fruit seeds without pulp for the extractions; meanwhile, the present work used an industrial by-product which is a mix of seeds and pulp. As pulp has almost no oil on its composition, its presence must reduce the global extraction yield. The polynomial model of Eq. (6) represents statistically the behavior of the SFE global yield as a function of the codified parameters, with a confidence level of 95%.

$$\hat{Y}_o = 19.260 - 0.659X_1^2 - 2.755X_2^2 - 1.475X_1 + 4.856X_2 - 0.446X_3 + 1.541X_1X_2 + 0..396X_2X_3 \quad (6)$$

Where: $\hat{Y}_0$: predicted global yield; X_1: codified temperature; X_2: codified pressure; X_3: codified ultrasound power.

Temperature and pressure demonstrated linear, quadratic and interaction effects. That behavior was expected, since those are the parameters which influence the solvent density, which affects its solvation power [26]. An illustration of this behavior is exhibited on Fig. 2 (a).

Fig. 2 (b) shows the effects of ultrasound power and temperature over the global extraction yield. It is possible to see that a negative effect of ultrasound is more pronounced at low temperatures, probably because the temperature increase of the extraction bed, caused by the ultrasonic waves, reduces the solvent density, thus, decreasing its solvation power. This could explain why the SFE global yield is lower when higher ultrasound power is applied.

Fig. 2 (c) shows the effect of pressure and ultrasound power on the SFE global yield. It is noted that at lower pressure the effect of ultrasound is more marked. Part of the extractable oil is initially located inside the passion fruit seed particles. Therefore, it should diffuse from the particle core to its surface to be dissolved by CO_2. The diffusion rate depends on the oil concentration gradient between the seed particle and the fluid phase, which is a function of temperature and pressure. As mentioned before, one of the effects of ultrasonic waves over the extraction bed is the increase of temperature, which leads to the reduction of the solvent's density and thus of the extract's solubility. As the solubility decreases, the concentration gradient between the seed particle and the fluid phase declines, and then diffusion becomes slower.

Based on the results presented in this section, the application of ultrasound to the SFE process seems to be counterproductive. However, in this experimental design only one experiment without ultrasound has been performed. Therefore, in order to reinforce the evaluation of the effect of the ultrasound on the SFE process, as additional experimental design was conducted, and its results are presented in Section 3.3.

3.3. Effect of ultrasound on SFE

Additional SFE experiments were performed at 40 °C to evaluate the effect of ultrasound on the global yield, DPPH radical scavenging activity, tocopherol and tocotrienol content, fatty acids profile and extraction kinetics. The results of these experiments are expressed on Table 2.

The SFE global yield achieved without ultrasound at 16 MPa and 40 °C was 12.3 ± 0.1%. When SFE was assisted by ultrasound power of 160 W, at the same pressure and temperature, the global yield increased around 29%, similarly to the increase observed by Santos et al. [10] (28%) for SFE from malagueta pepper assisted by ultrasound at 360 W. However, the use of a higher ultrasound power in this work (640 W) resulted in an increase of the global yield of only 10% compared to the case of no ultrasound. The lower effect of ultrasound at high power could be explained by the vibration effect of ultrasound, which besides breaking the passion fruit seed particles, could promote movement of the particles, forming preferential paths for the solvent through the extraction bed, which reduce the process efficiency. Moreover, excessive reduction of particle size may cause obstruction in pipelines of the column outlet.

In the experiments performed at 26 MPa the global yield was affected by the application of ultrasound, although it was not affected by the different ultrasonic powers. These conditions resulted on the highest global yields attained in this work. However, the highest increase of global yield was not achieved at this condition. The increase of global yield due to the ultrasound at 26 MPa was about 13%, similar to that obtained by Pasquel-Reátegui et al. [9] (14%) for the SFE of antioxidant compounds blackberry bagasse.

The possible cavitation produced by the ultrasonic waves in the extraction bed could have been the responsible for the increase in SFE global yield. At 16 MPa, 40 °C and 160 W the cavitation effect seems to be greater. This could be explained by the extraction conditions, where a lower combination of pressure-temperature was used. Thus, CO_2 gas bubbles could have been formed inside the extracting bed, and if they collapse, microjets can hit and break the cell walls releasing internal compounds such as oil.

When ultrasonic power of 640 W was applied, an increase of the system temperature was observed. Thus, the extraction occurs at a temperature higher than planned, which can be sufficient to modify the proprieties of CO_2, decreasing the efficiency of SFE when compared to that obtained at160 W of ultrasound. Moreover, as described by Hu et al. [12] the vibratory effect induced by the high ultrasound power could also lead to the efficiency reduction of SFE, for two reasons: (i) Vibration could cause the movement of particles, leading to the formation of preferential paths for the solvent inside the extraction bed; and (ii) Vibration could drive to an excessive size reduction, thus some particles could penetrate the filters of the SFE unit, resulting in the obstruction of piping.

SFE performed at 26 MPa also presented an increase of global yield due to the ultrasound application, but the effect was lower than at 16 MPa. In this case the higher pressure applied may inhibit the formation of bubbles inside the extraction bed, decreasing the possible impact of cavitation. When 640 W of ultrasonic power were applied, a slight increase of the system temperature was noted. Nevertheless, in this case the effect of temperature was attenuated by the high pressure, so there was no significant difference between the SFE global yields at 160 and 640 W of ultrasound at the pressure of 26 MPa. The small effect of ultrasound at high pressures may occur because in these conditions, SFE without ultrasound is able to extract material that would need ultrasound to be recovered at lower pressures. As a consequence, at 26 MPa there is a smaller ratio of extract that needs the use of ultrasound to be released from the particles and extracted.

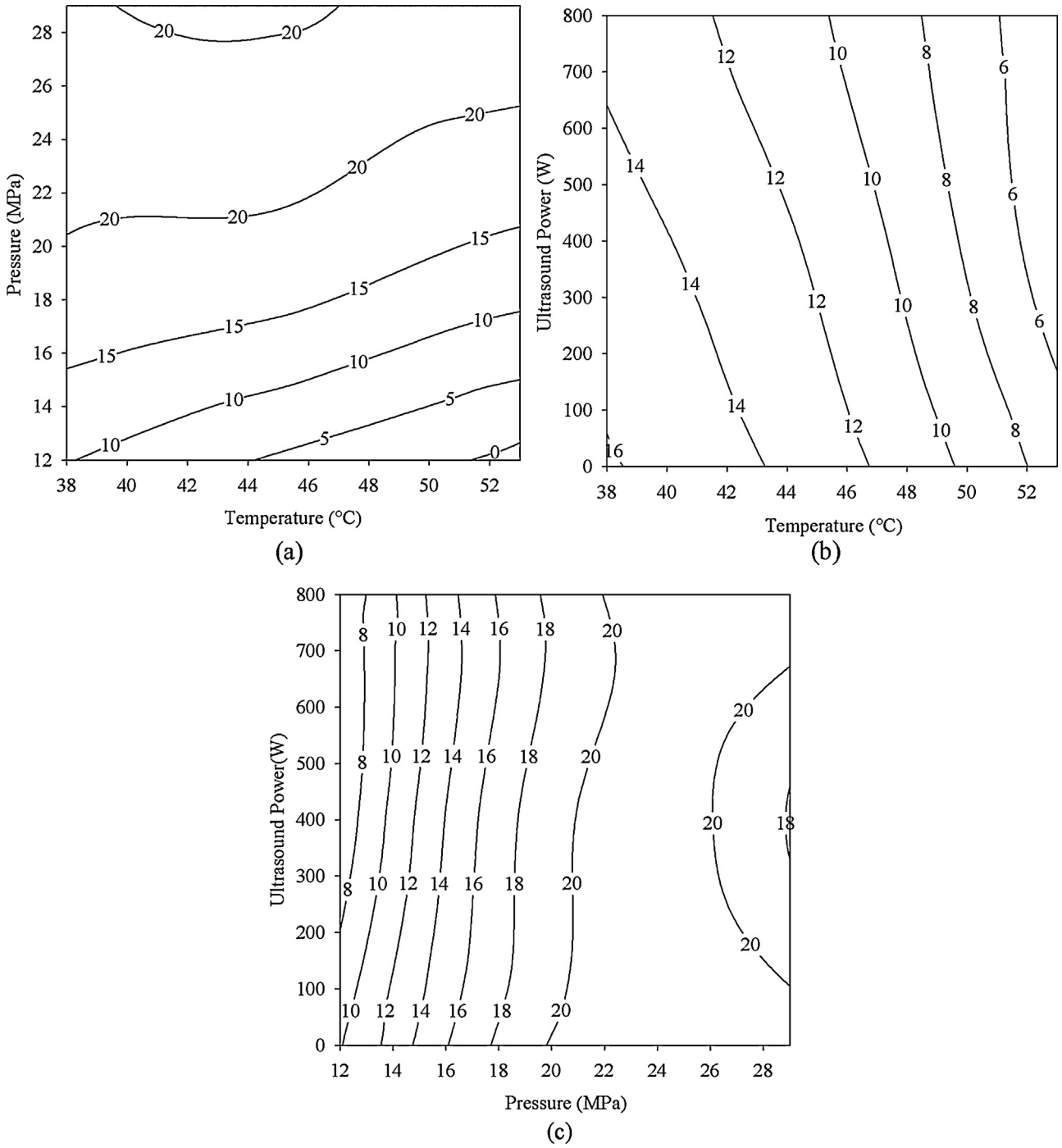

Fig. 2. Response surfaces for SFE global yield represented as contour diagrams of: (a) temperature vs. pressure, with ultrasound power fixed at 160 W; (b) temperature vs. ultrasound power, with pressure fixed at 16 MPa; (c) pressure vs. ultrasound power with temperature fixed at 40 °C.

3.4. Tocopherol and tocotrienol content

Table 2 shows the tocopherol and tocotrienol composition of the passion fruit seed oil. γ-tocopherol, γ-tocotrienol and δ-tocotrienol were identified in all the samples. γ-tocopherol was found at lower concentration, which did not present significant differences between the extraction conditions. However, the mean concentrations are slightly higher for the extracts obtained by SFE at 16 MPa. It is important to remark that the SFE runs carried out at 16 MPa resulted on the lower yields (around 12.3 and 15.8%), when compared to the high yields obtained at 26 MPa (around 18.5 and 20.9%). Therefore, it could be suggested that tocopherols and tocotrienols, due to their nonpolar nature, have great affinity to CO_2 and can be extracted with high yield in almost any condition among those performed. Nevertheless, when the SFE conditions favor the extraction of other components, the increase of global yield dilutes the tocopherols and tocotrienols, decreasing their concentration in the extracted oil. The total tocopherol and tocotrienol content varied from 87 to 91 mg/100 g of oil for the best extraction condition of these compounds, and from 63 to 72 mg/100 g of oil for the worst extraction condition. These values are higher than the 50 mg/100 g of oil reported by Malacrida and Jorge [4] for passion

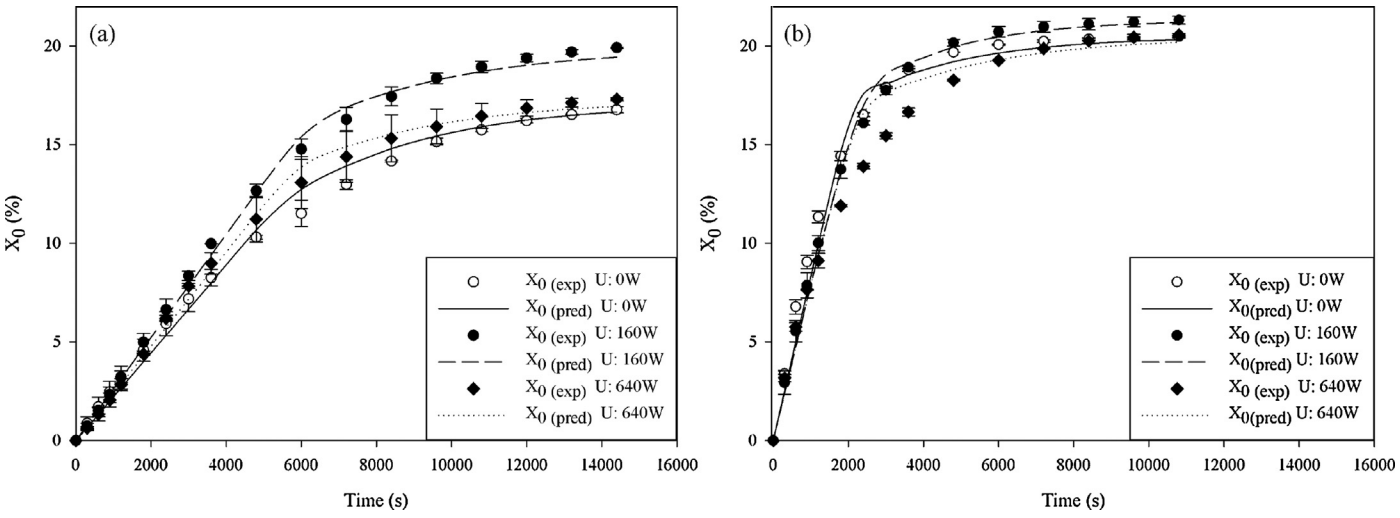

Fig. 3. SFE kinetics of passion fruit seed oil at: (a) 40 °C e 16 MPa, with different ultrasonic powers (0 W, 160 W e 640 W) and (b) 26 MPa and 40 °C, with different ultrasonic powers (0 W, 160 W e 640 W), with the respective kinetic curves adjusted using the model of Sovová [15]. Where: exp: experimental; pred: predicted.

fruit seed oil extracted by Soxhlet. The best conditions in this work to obtain tocopherols and tocotrienols resulted in contents comparable to those of palm oil extract, reported by Monde et al. [27], who obtained from 86 to 112 mg/100 g of oil. Considering that palm oil is one of the richest source of tocopherols and tocotrienols [28], the content achieved in this work makes passion fruit seed a promising source of these compounds.

3.5. DPPH radical scavenging activity and its relation with tocopherols and tocotrienols

The DPPH radical scavenging activity exhibited by the extracts may be attributed to the presence of tocopherols and tocotrienols in the oil. To verify that hypothesis, the correlation between the concentration of tocopherols and tocotrienols, and the DPPH radical scavenging activity was evaluated.

Table 4
Fatty acid profile of passion fruit seed oil extracted by SFE, with and without ultrasound at different conditions, and extracted by Soxhlet with hexane as solvent. All the SFE experiments were performed at 40 °C.

		SFE					
Fatty acid	Soxhlet	*P*: 16 MPa*U*: 160 W	*P*: 16 MPa*U*: 640 W	*P*: 16 MPa*U*: 0 W	*P*: 26 MPa*U*: 160 W	*P*: 26 MPa*U*: 640 W	*P*: 26 MPa*U*: 0 W
14:0	0.11 ± 0.03^{I}	0.24 ± 0.09^{I}	0.11 ± 0.01^{I}	0.103 ± 0.005^{I}	0.15 ± 0.02^{I}	0.089 ± 0.002^{I}	0.134 ± 0.001^{I}
16:0	11.5 ± 0.5^{FG}	11.1 ± 0.6^{FG}	11.6 ± 0.2^{FG}	11.8 ± 0.1^{FG}	11.1 ± 0.2^{FG}	10.9 ± 0.2^{FG}	11.0 ± 0.2^{FG}
16:1 *n*-9	0.21 ± 0.03^{I}	0.258 ± 0.005^{I}	0.199 ± 0.006^{I}	0.197 ± 0.004^{I}	0.171 ± 0.001^{I}	0.183 ± 0.001^{I}	0.184 ± 0.001^{I}
18:0	3.7 ± 0.7^{H}	3.01 ± 0.06^{H}	4 ± 1^{H}	3.03 ± 0.03^{H}	5 ± 2^{H}	3.1 ± 0.2^{H}	3.7 ± 0.5^{H}
18:1 *n*-9	16.5 ± 0.5^{DE}	19 ± 2^{DE}	15.89 ± 0.09^{DE}	15.9 ± 0.1^{DE}	18.2 ± 0.8^{DE}	16.3 ± 0.3^{DE}	17 ± 1^{DE}
18:2 *n*-6	67 ± 2^{BC}	65 ± 2^{BC}	67 ± 1^{BC}	67.91 ± 0.03^{BC}	63 ± 1^{C}	68.2 ± 0.9^{BC}	66.9 ± 0.6^{BC}
18:3 *n*-3	0.461 ± 0.007^{I}	0.461 ± 0.008^{I}	0.460 ± 0.001^{I}	0.462 ± 0.004^{I}	0.6 ± 0.2^{I}	0.455 ± 0.006^{I}	0.47 ± 0.02^{I}
20:0	0.24 ± 0.02^{I}	0.169 ± 0.006^{I}	0.17 ± 0.01^{I}	0.21 ± 0.04^{I}	0.283 ± 0.002^{I}	0.25 ± 0.03^{I}	0.243 ± 0.004^{I}
20:1 *n*-9	0.113 ± 0.001^{I}	0.114 ± 0.008^{I}	0.102 ± 0.002^{I}	0.102 ± 0.001^{I}	0.122 ± 0.003^{I}	0.114 ± 0.003^{I}	0.115 ± 0.002^{I}
Others	0.31 ± 0.02^{I}	0.40 ± 0.08^{I}	0.32 ± 0.03^{I}	0.34 ± 0.06^{I}	0.40 ± 0.04^{I}	0.4 ± 0.2^{I}	0.31 ± 0.03^{I}
Total	100.0 ± 0.0	99.96 ± 0.04	100.0 ± 0.0	100.002 ± 0.002	99.8 ± 0.1	100.0 ± 0.0	99.9 ± 0.1
Unsaturated	84 ± 1^{A}	85.1 ± 0.7^{A}	84 ± 1^{A}	84.58 ± 0.07^{A}	83 ± 2^{A}	85.3 ± 0.6^{A}	84.7 ± 0.5^{A}
Saturated	16 ± 1^{F}	14.9 ± 0.7^{FG}	16 ± 1^{FG}	15.42 ± 0.07^{FG}	17 ± 2^{FG}	14.7 ± 0.6^{FG}	15.2 ± 0.6^{FG}
Polyunsaturated	67 ± 2^{BC}	66 ± 2^{BC}	68 ± 1^{BC}	68.37 ± 0.04^{B}	64 ± 2^{BC}	68.7 ± 0.9^{B}	67.4 ± 0.6^{BC}
Monounsaturated	16.8 ± 0.5^{DE}	19 ± 2^{D}	16.2 ± 0.1^{DE}	16.2 ± 0.1^{DE}	18.5 ± 0.8^{DE}	16.6 ± 0.3^{DE}	17 ± 1^{DE}

Values expressed as mean (%) ± standard deviation. Means followed by the same letters do not differ significantly according to Tukey's test (0.05%).

Table 5
Adjusted parameters by the mathematical model of Sovová [15] for the SFE kinetics of passion fruit seed oil at 40 °C.

Pressure	Ultrasonic power	0 W	160 W	640 W
16 MPa	$f \times 10^{-8}$	2.88	1.30	2.11
	t_{CER} (s)	4855	5374	5172
	$k_{ya} \times 10^{3}$ (s^{-1})	5.793	6.952	6.277
	$k_{xa} \times 10^{3}$ (s^{-1})	1.746	1.653	1.697
	Xk^{*}	0.051	0.040	0.035
26 MPa	$f \times 10^{-8}$	4.81	2.08	7.39
	t_{CER} (s)	566	942	904
	$k_{ya} \times 10^{2}$ (s^{-1})	6.871	4.299	4.299
	$k_{xa} \times 10^{4}$ (s^{-1})	2.028	2.290	1.726
	Xk^{*}	0.0407	0.0422	0.0405

Where: minimized function to make the adjustment (sum of squared residues); t_{CER}: CER time; k_{ya}: Fluid phase mass transfer coefficient; k_{xa}: solid phase mass transfer coefficient; *Xk*: solute ratio inside the particles; *kg solute/kg raw matter.

Table 2 presents the values of DPPH radical scavenging activity of the extracts. In order to evaluate the correlation between these the antioxidant activity and the tocopherol and tocotrienol contents, the Pearson's coefficient (r) was calculated and the obtained value was $r = +0{,}872$, with a significance higher than 99% by the F test. Thus, one can state that tocopherols and tocotrienols are the main responsible for the DPPH radical scavenging activity observed in the passion fruit seed oil

3.6. Fatty acid composition

Table 4 reports the fatty acid profile obtained for the passion fruit seed oil extracted by different methods and conditions. Based on the Tukey's test, there are not significant differences between the fatty acid compositions obtained at the different conditions. The most abundant fatty acids were linoleic (18:2 n-6), from 63 to 68%, oleic (18:1 n-9), from 15 to 19%, palmitic (C16:0), about 11% and stearic (18:0), from 3 to 5%. This composition is similar to that described by Ferreira et al. [5] for passion fruit seed oil extracted by Soxhlet and cold press. The absence of significant differences between the fatty acids composition indicates that the different conditions applied to the SFE in this work do not modify the fatty acid profile.

3.7. SFE kinetics

The experimental data needed to apply the mathematical model of Sovová [15] are: Temperature: 40 °C; Raw material apparent density: 417 kg/m^3; Raw material real density: 1280 kg/m^3; Porosity: 0.674; Solvent flow rate: 1.75×10^{-4} kg/s; Extraction bed high: 5×10^{-3} m; Extraction bed diameter: 0.05 m; Particle mean diameter: 7.4×10^{-4} m; Raw material mass: 5×10^{-3} kg; Solvent density at 16 MPa: 805 kg/m^3; Solvent density at 26 MPa: 896 kg/m^3; Solubility of the extract in CO_2 at 16 MPa: 0.0007 kg extract/kg CO_2; Solubility of the extract in CO_2 at 26 MPa: 0.0024 kg extract/kg CO_2.

Fig. 3 shows the SFE kinetics obtained at the conditions expressed on Table 5, with different ultrasonic powers. The proximity of the modeled kinetic curves to the experimental values evidences the good adjustment achieved. The parameters adjusted using the model of Sovová [15] are expressed in Table 5.

The SFE kinetics where ultrasound was applied presented greater CER times. This may be caused by the release of extractable oil to the particle's surface due to ultrasound. Thus, the ratio of easily accessible extract is higher, increasing the CER period.

The differences between the SFE kinetics with ultrasound at 160 W and 640 W could be explained by the effect of ultrasound on the system temperature. When the ultrasonic power is higher,

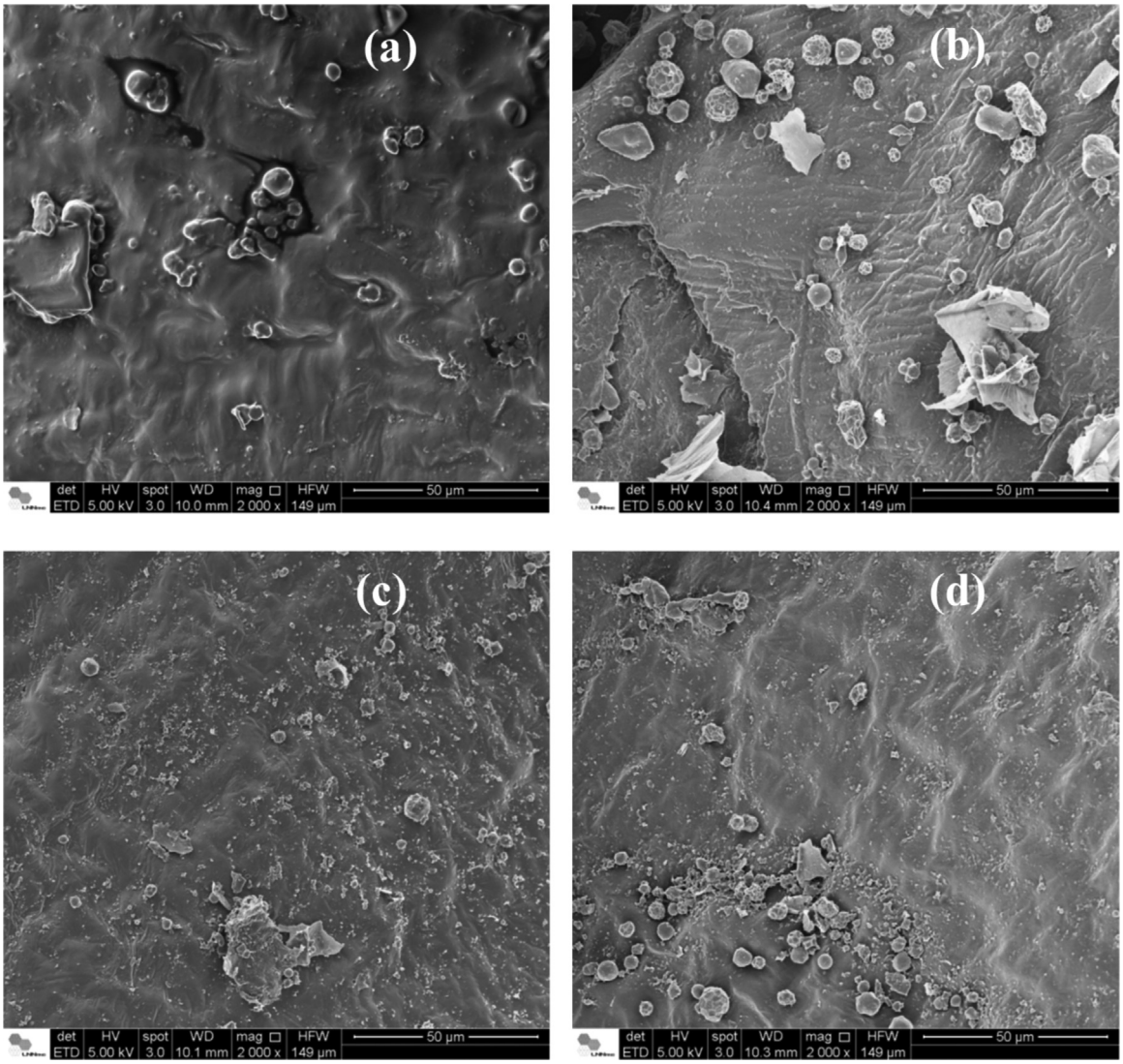

Fig. 4. FESEM images of passion fruit seed particles: (a) unextracted; (b) after Soxhlet extraction with hexane; (c) after SFE without ultrasound and (d) after SFE assisted by ultrasound (160 W).

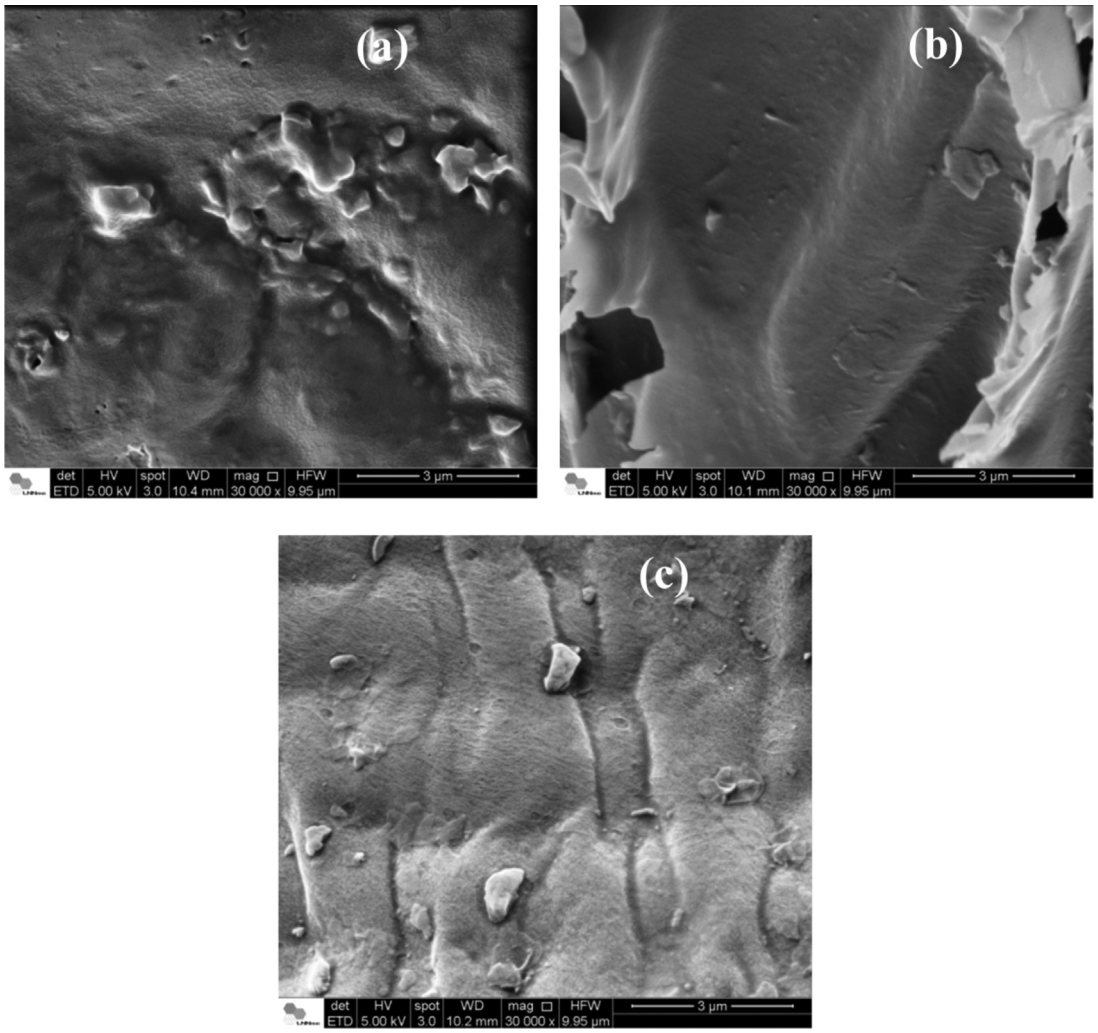

Fig. 5. FESEM images of passion fruit seed particles: (a) unextracted and after SFE assisted by ultrasound with power of (b) 160 W and (c) 640 W. Scale bar of 3 μm.

the increase of temperature is higher too, so the solvent density decreases and its solvation power in CO_2 turns lower. On the other hand, the vibrational effect of the ultrasonic waves must be considered. As already stated, the vibration caused by ultrasound could lead to the reorganization of particles, which could form preferential paths inside the extraction bed and reduce the efficiency of the mass transfer process.

Table 5 shows that at 16 MPa the fluid phase mass transfer coefficient (k_{ya}) increased when ultrasound was applied, meanwhile the solid phase mass transfer coefficient (k_{xa}) practically did not change. The fraction of oil inside the particles (X_k) was also reduced when ultrasound was used. This reinforces the hypothesis of disruptions in the cell walls that cause the release of extractable material. With a higher fraction of easily accessible oil, the extraction rate at the beginning of the kinetics remains constant for a larger time, resulting in a longer CER period. On the other hand, at 26 MPa, when ultrasound was applied, k_{ya} decreased and k_{xa} was not modified, as well as X_k. It seems that the effect of the reduction of k_{ya} was absorbed the CER time increase, which could explain the limited effects of ultrasound over the global extraction yield and the behavior of the SFE kinetics at 26 MPa. In Fig. 3(b) one can note that the effect of ultrasound on the SFE kinetics at 26 MPa is lower than at 16 MPa, which is presented in Fig. 3(a). This may be caused because of the high efficiency of the oil extraction at high pressures. Therefore, ultrasound is effective only to enhance the global yield at 160 W.

3.8. FESEM

Fig. 4 shows FESEM images of the passion fruit seeds before extraction (a), and after Soxhlet (b) and SFE without (c) and with ultrasound (d). It is possible to observe that the surfaces of the particles are generally smooth, without striations or pores, both before and after the extracting processes. On the images (b–d) a greater amount of material deposed on the samples' surfaces is observed, especially for those extracted by SFE with or without ultrasound. That could be attributed to the removal of material from inside of the seed particles and also from their boundaries that, by mechanical action of the extraction processes, are released and are deposed on the particle surface.

The samples that have undergone extraction processes have smaller sizes compared to the unextracted ones, and since the samples suffer more disruptions in SFE with ultrasound, the presence of boundaries and pieces of superficial tissues is more frequent, as shown in Fig. 4(d).

It is important to remark that the observed effect of the extraction processes is limited to the removal of the superficial tissue that covers the seed particles and of the broken material that is deposed on the surface. When higher magnification images are taken (scale bar 3 μm), it is observed that, in the greater portion of the tissue that covers the seed particles, the surfaces remain smooth and without striations or pores after the extraction, as they were in the unextracted samples. This may be observed in Fig. 5, which shows

images with great magnification of the samples of unextracted seed particles (a) and after SFE, assisted by ultrasound with power of 160 (b) and 640 W (c). Figure 5(c) also shows a cell wall of the superficial tissue, where tiny cellulose fibers appear.

4. Conclusions

Passion fruit seed oil was extracted using SFE assisted by ultrasound. The extracts presented DPPH radical scavenging activity, which was not modified by the ultrasound power, but it was affected by the different pressures used in SFE. Tocopherols and tocotrienols were identified in the extracts, and they had a good correlation with the DPPH radical scavenging activity. The fatty acid profile of the extracted oil showed linoleic, oleic, palmitic and stearic acids as the major oil components, and it was not affected by the process conditions.

The application of ultrasound helped increasing the global yield up to 29% when compared to SFE without ultrasound. The effect of ultrasound on SFE global yield and kinetics was more pronounced at 16 MPa than at 26 MPa. The mathematical model of Sovová [15] was able to describe the extraction process, showing that when ultrasound was applied at 16 MPa and 40 °C, the mass transfer coefficient of the fluid phase and the CER time increased and the extract fraction inside the particles decreased. This finding indicates the role of ultrasound in SFE, which is to release extractable material from inside the particles. The enhancement of SFE with ultrasound has shown potential to improve the global extraction yields, allowing working at moderate pressures, which could compensate the energetic cost of ultrasound application. Meanwhile, when ultrasound was applied at 26 MPa and 40 °C, the decrease of the mass transfer coefficient on the fluid phase absorbed the effect of the increased CER time, thus demonstrating no positive effect of ultrasound at this condition. There was no evidence of cavitation effects in the extraction bed. However, damages induced by friction due to the vibration generated by the ultrasonic waves were observed on the surfaces of the particles in the FESEM images.

This work has shown the great potential of SFE and ultrasound to obtain valuable products from passion fruit processing waste. More work is expected on the supercritical and ultrasound technologies applied to food by-products.

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