Supercritical carbon dioxide extraction of capsaicinoids from malagueta pepper (*Capsicum frutescens* L.) assisted by ultrasound

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ABSTRACT

Extracts from malagueta pepper (*Capsicum frutescens* L.) were obtained using supercritical fluid extraction (SFE) assisted by ultrasound, with carbon dioxide as solvent at 15 MPa and 40 °C. The SFE global yield increased up to 77% when ultrasound waves were applied, and the best condition of ultrasound-assisted extraction was ultrasound power of 360 W applied during 60 min. Four capsaicinoids were identified in the extracts and quantified by high performance liquid chromatography. The use of ultrasonic waves did not influence significantly the capsaicinoid profiles and the phenolic content of the extracts. However, ultrasound has enhanced the SFE rate. A model based on the broken and intact cell concept was adequate to represent the extraction kinetics and estimate the mass transfer coefficients, which were increased with ultrasound. Images obtained by field emission scanning electron microscopy showed that the action of ultrasonic waves did not cause cracks on the cell wall surface. On the other hand, ultrasound promoted disturbances in the vegetable matrix, leading to the release of extractable material on the solid surface. The effects of ultrasound were more significant on SFE from larger solid particles.

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1. Introduction

Hot peppers (*Capsicum* sp.) are rich in capsaicinoids, which are substances responsible for the pungency of the fruit. Among capsaicinoids, capsaicin is the most representative [1,2]. Capsaicin is currently used in the development of new drugs due to its beneficial properties, such as antioxidant, antimicrobial, anti-inflammatory and antitumor activities, also contributing to the control of diabetes and pain relief [3]. Taking into account the benefits of capsaicinoids, there is great interest in developing new technologies to obtain extracts concentrated in such compounds. According to Aguiar et al. [4] malagueta pepper (*Capsicum frutescens* L.) has the highest levels of capsaicin and dihydrocapsaicin, when compared to other Brazilian red native peppers, such as dedo-de-moça (*Capsicum baccatum*) and bode-amarela (*Capsicum chinense*).

Extraction of active compounds from vegetal raw materials is a promising area in the food, chemical and pharmaceutical industries. On the other hand, the recovery of these compounds

is a complex task because, in most cases, they are oxidative or thermolabile substances. Furthermore, severe legal restrictions have been adopted to avoid the use of organic solvents in industrial extraction processes. Therefore, there is considerable interest in replacing traditional procedures for the recovery of active compounds, such as steam distillation and organic solvent extraction [5].

Supercritical fluid extraction (SFE) has been applied as an alternative to traditional methods for the extraction and fractionation of active compounds. Carbon dioxide (CO_2) is the most commonly used supercritical solvent in these processes, due of its advantages, which are: low cost, nontoxicity, non-flammability, inertness and good extraction capacity [5–7]. Indeed, the critical properties of CO_2 (P_c = 7.38 MPa, T_c = 304.2 K) are moderate when compared to other green solvents, allowing SFE to be carried out with low energy cost for pressurization, and at temperatures that do not damage the target compounds.

Generally, in a SFE unit, one can change the temperature, pressure, extraction bed size, solvent flow rate, among others, in order to maximize the extraction rate and the yield of a specific compound [8,9]. The morphology of the solid substrate particle can also influence the extraction efficiency, since the solvent must

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cross diffusive paths inside the solid particle, in order to extract specific compounds [5,10]. Moreover, the SFE process capacity may be improved by using combined extraction techniques, such as the use of different co-solvents and ultrasonic waves [11].

The ultrasound technique is based on the formation of high frequency ultrasonic waves, which are capable of causing cavitation due to the expansion and contraction cycles that the material goes through when submitted to ultrasound. These cycles disrupt the cell walls of the vegetable matrix, favoring the penetration of the solvent and the mass transfer, thus increasing the extraction rate and yield [12]. Low pressure extraction assisted by ultrasound with different solvents has been applied to different vegetable substrates, such as grape [13], pomegranate [14], rosemary [15], tomato [16] and pepper [17,18]. However, there are few published works that focus on ultrasound-assisted extraction at high pressure, such as SFE [11,19–21].

The objective of this work was to evaluate the effects of ultrasonic waves on the supercritical fluid extraction (SFE) of malagueta pepper (*C. frutescens* L.), as well as to evaluate the capsaicinoid and total phenolic content in the extracts. A mathematical model, based on the broken and intact cell concept, was fitted to the extraction curves, and the influence of particle diameter on the extraction kinetics was verified. Besides, field emission scanning electron microscopy (FESEM) was used to analyze the effects of ultrasonic waves on the structure of pepper particles.

2. Materials and methods

The work was carried out in the Laboratory of Supercritical Technology: Extraction, Fractionation and Identification of Vegetal Extracts – LASEFI-DEA/FEA-UNICAMP. The raw material was malagueta pepper, which is a Brazilian native variety of hot pepper (*C. frutescens* L.), purchased at the "Central de Abastecimento de Campinas/SP (CEASA)", a local market in Campinas, southeastern Brazil.

2.1. Sample preparation

The fruits with good physical integrity were selected, washed with running water and stored under refrigeration ($\approx$4 °C) for further procedures. The raw material was oven-dried at 70 °C for 12 h, according to the methodology used by Aguiar et al. [4]. After drying, the samples were ground in a knife mill (Marconi, model MA 340, Piracicaba), in order to homogenize the substrate and decrease the resistance to mass transfer during the later stages of extraction.

The solid pepper particles were separated according to their size in a vertical vibratory sieve shaker (Bertel Metallurgic Ind. Ltda., SP, Brazil) Tyler series (Wheeling, USA) system (Bertel, model 1868, Caieiras, SP, Brazil) with sequential openings of 12, 16, 24, 32, 48 and 80 mesh. The mean particle diameter was calculated according to ASAE Standards [22]. To check the influence of particle diameter on the kinetics of ultrasound-assisted SFE, samples were classified in three groups: 1. Larger particles, formed by particles retained in a sieve of 16 mesh (1.18–1.68 mm, mean particle diameter (dp) of 1.43 ± 0.35 mm); 2. Smaller particles, formed by the material retained in a sieve of 48 mesh (0.177–0.342 mm, dp = 0.23 ± 0.16 mm); and 3. Particles of mean diameter, formed by the material retained in all sieves. The density of the particles was measured by helium pycnometer (Quantachrome Instruments, Ultrapyc 1200e, Boynton Beach, USA), whereas bulk density was measured by weighing a known volume of the solid material. Both solid and bulk densities data needed to apply the broken and intact cell model to the SFE kinetic curves.

2.2. Soxhlet extraction

The Soxhlet method was selected as a conventional extraction technique, in order to determine the total capsaicinoid content of the samples and to compare them with the results of SFE. Soxhlet extraction was performed using four solvents, with different polarities [23]: hexane, dichloromethane, ethyl ether and ethyl acetate. Each extraction was carried out using 0.15 L of solvent and 5.0 g of dried sample packed inside filter paper. The reflux of boiling solvent was kept for 6 h. Then, the liquid extract was recovered by solvent evaporation under vacuum (at 25 °C), weighed and stored under freezing (−18 °C) for further analyses. The capsaicinoid and the total phenolic content in the extracts were determined according to the methodologies presented in Sections 2.4.1 and 2.4.2, respectively. The Soxhlet extractions were performed in triplicates.

2.3. Supercritical fluid extraction (SFE) experiments

The conditions of the SFE experiments were fixed at pressure of 15 ± 0.5 MPa and temperature of 40 ± 3 °C, which were optimized by Aguiar et al. [4] for supercritical CO_2 extraction from malagueta pepper. Experiments were carried out using constant substrate mass, and aiming to obtain enough extract to perform the subsequent chemical analyses. In global yield experiments, the ratio between solvent and feed (S/F) was kept constant at 600 ± 2 kg CO_2/kg feed. This value of S/F is high when compared those used by Duarte et al. [24], Daood et al. [25], and Perva-Uzunalic et al. [8], which were 170, 30 and 120 kg of solvent per kg of pepper, respectively. The high applied value of S/F guarantees the solute exhaustion in the vegetal matrix and the consequent achievement of SFE global yield at the performed condition. Finally, the influence of particle size in the kinetics of

Table 1
Experimental conditions of SFE from malagueta pepper (*Capsicum frutescens* L.) with and without ultrasound on global yield and kinetics experiments.

Global yield (X_0)			
Method	Solvent		Polarity[1]
Soxhlet	Ethyl acetate		0.0
	Dichloromethane		3.1
	Ethyl ether		4.4
	Hexane		0.0

	Power (W)	Time (min)[2]	Energy (kJ/cm^2)
SFE (40 °C/15 MPa)	–	–	–
SFE + US	360	240	223.0
SFE + US	360	150	139.4
SFE + US	360	60	55.7
SFE + US	280	240	173.5
SFE + US	280	150	108.4
SFE + US	280	60	43.3
SFE + US	200	240	123.1
SFE + US	200	150	77.4
SFE + US	200	60	31.0

Kinetics Experiments				
	dp (mm)	Power (W)	Time (min)[2]	Energy (kJ/cm^2)
SFE	0.94 ± 0.03	–	–	–
SFE	1.43 ± 0.35	–	–	–
SFE	0.23 ± 0.16	–	–	–
SFE + US	0.94 ± 0.03	360	60	55.7
SFE + US	1.43 ± 0.35	360	480	446.1
SFE + US	0.23 ± 0.16	360	480	446.1

SFE – supercritical fluid extraction; US – ultrasound; X_0 – global yield (g of extract/g of raw material).
[1] Source: Byers [23].
[2] Irradiation time expressed in minutes; dp – particle diameter (mm).

the ultrasound-assisted SFE process was studied, according to the methodology presented in Section 2.4.1. In all SFE experiments (global yield and kinetics experiments with or without ultrasound) the solvent used was CO_2 (Gama Gases, Campinas-SP, Brazil) with 99% purity. Table 1 shows all the extraction experiments performed in this study.

The SFE experiments were carried out in a ultrasound-assisted supercritical fluid extraction (SFE + US) unit consisting of a 0.295 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 to measure pressure. The ultrasonic system (Unique Group, model DES500, Campinas, Brazil) is composed by a transducer unit with frequency of 20 kHz and a variable output power controller. The ultrasound probe was installed inside the SFE column. Fig. 1 illustrates the SFE + US unit, with special focus on the SFE + US bed.

2.3.1. Global yield (X_0) experiments

In the global yield experiments, two parameters of the SFE + US process were varied at three levels. Ultrasound power was evaluated at 180 W (8.6 W/cm^2), 280 W (12 W/cm^2) and 360 W (15.5 W/cm^2); and ultrasound application time varied between 60, 180 and 240 min. The SFE + US yield was compared to those of conventional extraction method (Soxhlet) and SFE without ultrasound.

The response variables were the global yield (X_0), which was calculated by the ratio between mass of extract (m_{ext}) and of feed (F), in a dried weight basis, according to Eq. (1), and the concentrations of capsaicin (C), dihydrocapsaicin (DHC); nordihydrocapsaicin (n-DHC); homodihydrocapsaicin (h-DHC), total capsaicinoids (Total) and total phenolic compounds. The quantification methods of capsaicinoids and phenolics are described in Sections 2.4.1 and 2.4.2, respectively.

$$X_0 = (m_{ext}/F) \times 100 \tag{1}$$

2.3.2. Kinetics experiments

The SFE kinetics experiments consisted in determining the mass of extract, or the global yield, as function of time. The collecting flasks were weighed before and after the extracts were recovered using an analytical balance (Sartorius, Model A200S, Gottingen, Germany). The flasks were then stored at 4 °C prior to analyses.

The effect of particle size on the SFE + US kinetics was verified by testing two groups of samples with different mean diameters: (1) larger particles, with mean diameter of 1.43 ± 0.35 mm; and (2) smaller particles, with mean diameter of 0.23 ± 0.16 mm. Kinetics experiments were conducted using the same conditions of the global yield experiments: temperature of 40 °C, pressure of 15 MPa and a solvent flow rate of 1.673×10^{-4} kg/s. The SFE particle bed was formed by about 20 g of dried and milled peppers, and
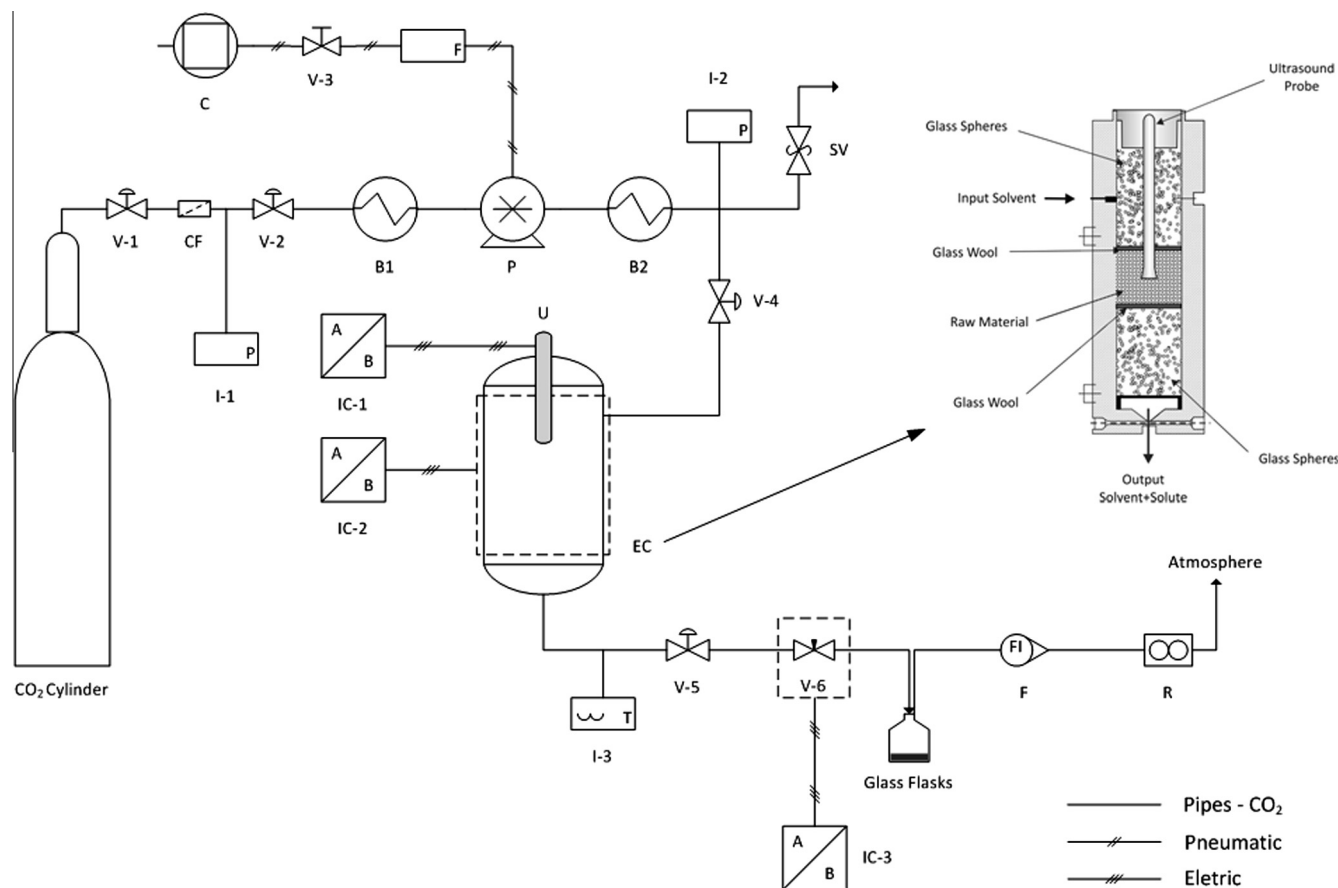

Fig. 1. Diagram of the SFE + US unit: V-1, V-2, V-3, V-4 and V-5 – control valves; V-6 – micrometer valve; SV – safety valve; C – compressor; F – compressed air filter; CF – CO_2 filter; B1 – cooling bath; P – pump; B2 – heating bath; I-1 and I-2 – pressure indicators; I-3 – temperature indicator; IC-1, IC-2 and IC-3 – indicators and controllers of 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 295 mL for SFE + US used in the kinetic experiments.

the remaining volume of the column was filled with glass spheres. Glass wool was added to separate the raw material from the glass spheres, as shown in Fig. 1.

2.4. Evaluation of the extracts

About 1 g of the extracts obtained by Soxhlet, SFE and SFE + US was dissolved in 10 mL of methanol and filtered on a millex PVDF 0.22 µm filter (Millipore). Subsequently, the samples were sonicated (135 W/25 kHz – *Unique ultracleaner* 1450) for 10 min with the purpose of homogenizing the extracts. The dried raw material was submitted to the same procedure, according to the methodology described by Barbero et al. [18]. An aliquot of 1.5 mL of the methanol/extract solution was stored for chromatographic analysis and the remaining content was used for analysis of total phenolics. The chemical analyses are described as follow.

2.4.1. Analysis of capsaicinoids

The HPLC-PDA analysis was carried out on a Dionex chromatographic system (Sunnyvale, CA, USA). Capsaicinoids were separated using a LiChrospher column RP-18e (250 mm × 4 mm, 5 µm i.d., Merck). The wavelength employed for detection was 280 nm.

The chromatographic method used a gradient of two solvents: acidified water (0.1% acetic acid, solvent A) and acidified methanol (0.1% acetic acid, solvent B), working at a flow rate of 1 mL/min. The following gradient method applied was: 0 min, 0% B; 2 min, 55% B; 6 min, 55% B; 7 min, 60% B; 12 min, 60% B; 14 min, 65% B; 15 min, 65% B; 20 min, 70% B; 25 min, 70% B; 27 min, 100% B. The temperature of the column was held constant at 25 °C.

The HPLC method was used to prepare calibration curves for capsaicin and dihydrocapsaicin, which are the two commercially available capsaicinoid standards. Regression equations and the correlation coefficient (r^2) (0.9997 for capsaicin and 0.9999 for dihydrocapsaicin), limits of detection (9.76 mg L^{-1} for capsaicin and 4.10 mg L^{-1} for dihydrocapsaicin) and quantification (32.55 mg L^{-1} for capsaicin and 13.67 mg L^{-1} for dihydrocapsaicin) were calculated.

The four major capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin and homodihydrocapsaicin) present in the analyzed extracts from malagueta pepper were quantified using this method. Capsaicin and dihydrocapsaicin were quantified based on the calibration curves obtained from the standard solutions. Since there are no commercial standards for nordihydrocapsaicin and homodihydrocapsaicin, these compounds were quantified from the calibration curve of dihydrocapsaicin (for nordihydrocapsaicin and for homodihydrocapsaicin), given the structural similarities between these molecules and taking into account their molecular weights. All analyses were run in triplicate.

2.4.2. Total phenolics

The total phenolic content was determined by spectrophotometry using the Folin–Ciocalteau method, based on the methodology proposed by Singleton, Orthofer and Lamuela-Raventos [26] with modifications. Briefly, 2.5 mL of the diluted Folin–Ciocalteau reactive (1:10 v/v) was added to 0.5 mL of solution of extract in methanol. After 5 min, 2.0 mL of a sodium carbonate solution (7.5%) was added. The absorbance was measured at 760 nm after keeping the mixture in the dark for 2 h. Gallic acid was used as standard, and the results were expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.

2.5. Mathematical modeling

The mathematical model of Sovová [27] was applied through the individual adjustment of each experimental curve. This model assumes that part of the extractable material is readily available to the solvent, due to the breaking of cells caused by milling. The remaining extract keeps inside intact cells, where the solvent needs to enter through diffusion. Therefore, three model parameters must be adjusted: the intact solid ratio (X_k), the fluid phase mass transfer coefficient (k_{ya}), which is convective, and the solid phase mass transfer coefficient (k_{xa}), which is rather diffusive. To apply the model, the routine of Powell [28] was used. 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. Within this range, the routine searches the parameter values that minimize the objective function (f), which was defined as the sum of squared errors.

Some process data are needed to apply model, such as global yield (X_0), extraction bed dimensions (H – height and d – diameter), solid feed (F), solvent flow rate (Q), solvent and solid densities (ρ_s and ρ_a, respectively), extract solubility (Y^*) and particle diameter (dp). The extract solubility in supercritical CO_2 (Y^*) was assumed to be 0.0032 kg of extract/kg of solvent, as obtained by Silva [29], who performed the mathematical modeling of malagueta pepper (*C. frutescens* L.) SFE with CO_2 at 40 °C, 15 MPa and various CO_2 flow rates.

2.6. Field emission scanning electron microscopy (FESEM)

The microstructures of the pepper pericarp samples were analyzed before and after the extractions using a scanning electron microscope equipped with a field emission gun (FESEM – FEI Quanta 650). The pericarp particles were previously separated from peduncles seeds using an optical microscope. Prior to analysis, the samples were coated with Au in a SCD 050 sputter coater (Oerlikon-Balzers, Balzers, Liechtenstein). Both equipments were available at the National Laboratory of Nanotechnology (LNNano) located in Campinas-SP/Brazil. Analyses of the sample surfaces were performed under vacuum, using a 5 kV acceleration voltage and a large number of images was obtained on different areas of the samples (at least 20 images per sample) to assure the reproducibility of the results.

2.7. Statistical analysis

The variance (ANOVA) of the results was evaluated, using the software Statistica for Windows 6.0 (Statsoft Inc., USA). This allowed the detection of significant differences in global yield (X_0) and in the concentrations of capsaicin (C), dihydrocapsaicin (DHC), nordihydrocapsaicin (n-DHC), homodihydrocapsaicin (h-DHC) and total phenolics obtained by Sohxlet, SFE, and SFE + US. The significant differences at level of 5% ($p < 0.05$) were analyzed through the Tukey's test.

Table 2
Physical and chemical characteristics of malagueta pepper (*Capsicum frutescens* L.) used in global yield and kinetic SFE experiments.

Characteristic	*Capsicum frutescens* L.
Mean particle diameter	0.94 ± 0.03 mm
Real density	1.20 ± 10 kg m^{-3}
Equilibrium moisture	5.68 ± 0.04%
Total lipids	9.7 ± 0.1%
Total capsaicinoids	3.94 ± 0.2 (mg/g raw material)
Capsaicin	2.3 ± 0.5 (mg C/g raw material)
Dihydrocapsaicin	1.5 ± 0.3 (mg DHC/g raw material)
Nordihydrocapsaicin	0.10 ± 0.02 (mg n-DHC/g raw material)
Homodihydrocapsaicin	0.04 ± 0.02 (mg h-DHC/g raw material)
Total phenolics	0.858 ± 0.005 (mg GAE/g raw material)

Table 3
Global yield, capsaicinoids and total phenolic concentrations obtained by Sohxlet, SFE (15 MPa e 40 °C), and SFE + US from malagueta pepper (*Capsicum frutescens* L.).

Method	Solvent	Polarity[1]	X0 (%)	Recovery of X0 (%)	C[3]	DHC[3]	n-DHC[3]	h-DHC[3]	Total[3]	Recovery of Total(%)	Phenolics[4]	Recovery of phenolics (%)
Soxhlet	Ethyl acetate	0.0	9.4 ± 0.20^a	94.8	2.16 ± 0.20	1.20 ± 0.09^{ab}	0.10 ± 0.010^a	0.04 ± 0.003^a	3.50 ± 0.30^{ab}	88.8	0.39 ± 0.02^a	45.5
	Dichloromethane	3.1	9.3 ± 0.37^a	95.9	2.27 ± 0.30^a	1.22 ± 0.17^a	0.10 ± 0.015^a	0.04 ± 0.006^a	3.63 ± 0.48^a	92.1	0.37 ± 0.01^{ab}	43.1
	Ethyl ether	4.4	8.6 ± 0.40^{ab}	88.71	1.76 ± 0.12^b	0.97 ± 0.07^b	0.08 ± 0.003^a	0.03 ± 0.006^a	2.84 ± 0.20^b	72.1	0.37 ± 0.01^{ab}	43.1
	Hexane	0.0	9.7 ± 0.10^a	–	1.88 ± 0.17^{ab}	1.05 ± 0.09^{ab}	0.09 ± 0.015^a	0.03 ± 0.004^a	3.05 ± 0.27^{ab}	77.4	0.30 ± 0.01^d	35.0

Method	Power (W)	Time (min)[2]	Energy (kJ/cm²)	X0 (%)	Recovery of X0 (%)	C[3]	DHC[3]	n-DHC[3]	h-DHC[3]	Total[3]	Recovery of Total(%)	Phenolics[4]	Recovery of phenolics (%)
SFE	–	–	–	5.7 ± 0.05^f	58.8	1.83 ± 0.01^{ab}	1.00 ± 0.02^{ab}	0.07 ± 0.006^a	0.02 ± 0.004^a	3.00 ± 0.03^{ab}	76.1	0.31 ± 0.01^d	36.1
SFE + US	360	240	223.0	7.2 ± 0.10^{cde}	74.2	1.87 ± 0.21^{ab}	1.05 ± 0.11^{ab}	0.08 ± 0.005^a	0.02 ± 0.003^a	3.03 ± 0.32^{ab}	76.9	0.38 ± 0.02^{ab}	44.3
SFE + US	360	150	139.4	7.1 ± 0.07^{cde}	73.2	1.95 ± 0.09^{ab}	1.07 ± 0.04^{ab}	0.09 ± 0.006^a	0.03 ± 0.008^a	3.13 ± 0.14^{ab}	79.4	0.37 ± 0.01^{ab}	43.1
SFE + US	360	60	55.7	7.3 ± 0.23^{bc}	75.3	1.93 ± 0.05^{ab}	1.01 ± 0.03^{ab}	0.07 ± 0.021^a	0.03 ± 0.003^a	3.10 ± 0.05^{ab}	78.3	0.37 ± 0.02^{ab}	43.1
SFE + US	280	240	173.5	7.2 ± 0.86^{cd}	74.2	1.82 ± 0.02^{ab}	1.01 ± 0.04^{ab}	0.09 ± 0.012^a	0.03 ± 0.006^a	3.00 ± 0.06^{ab}	76.5	0.32 ± 0.01^{cd}	37.3
SFE + US	280	150	108.4	6.4 ± 0.15^{cdef}	66.0	1.85 ± 0.15^{ab}	0.99 ± 0.04^{ab}	0.07 ± 0.004^a	0.03 ± 0.005^a	3.00 ± 0.20^{ab}	76.1	0.31 ± 0.01^d	36.1
SFE + US	280	60	43.3	6.0 ± 0.50^{def}	61.9	1.94 ± 0.10^{ab}	1.07 ± 0.06^{ab}	0.08 ± 0.006^a	0.03 ± 0.004^a	3.13 ± 0.16^{ab}	79.4	0.33 ± 0.01^{bcd}	38.5
SFE + US	200	240	123.1	6.9 ± 0.16^{cdef}	71.1	2.15 ± 0.11^a	1.18 ± 0.11^a	0.09 ± 0.016^a	0.03 ± 0.004^a	3.46 ± 0.17^a	87.8	0.36 ± 0.02^{abc}	42.0
SFE + US	200	150	77.4	6.0 ± 0.02^{cdef}	61.9	1.80 ± 0.24^{ab}	1.00 ± 0.11^{ab}	0.08 ± 0.015^a	0.03 ± 0.005^a	2.93 ± 0.36^{ab}	74.4	0.33 ± 0.03^{bcd}	38.5
SFE + US	200	60	31.0	5.9 ± 0.10^{ef}	60.8	1.78 ± 0.15^b	0.97 ± 0.07^{ab}	0.07 ± 0.007^{ab}	0.02 ± 0.007^a	2.85 ± 0.23^b	72.3	0.39 ± 0.02^a	45.5

Results are expressed as mean ± standard deviation of the analysis. SFE – supercritical fluid extraction; US – ultrasound; X0 – global yield (g of extract/g of raw material);C – capsaicin, DHC – dihydrocapsaicin; n-DHC – nordihydrocapsaicin; h-DHC – homodihydrocapsaicin; Total – total capsaicinoids (Sum C, DHC, n-DHC and h-DHC); phenolics – total phenolic compounds.
Different letters in the same column indicate that the means differ significantly by Tukey's test ($p \leqslant 0.05$).
[1] Source: Byers [23].
[2] Irradiation time expressed in minutes.
[3] Values expressed in mg of respective capsaicinoid/g of raw material.
[4] mg of GAE/g of raw material.

3. Results and discussion

The physical and chemical characteristics of the raw material used in the extraction experiments are shown on Table 2. The initial moisture content of the raw material was 61 ± 1% (w.b.), and after 20 h of drying (70 ± 2 °C), the equilibrium moisture of 5.68 ± 0.04% was achieved. This value is close to that obtained by Nogueira et al. [30], which was of 5%. The total lipids, i.e., global yield obtained by Soxhlet method with hexane was 9.7 ± 0.1%. Jordão and Bonnas [31] evaluated the malagueta pepper (*C. frutescens* L.) centesimal composition obtained at 11.1% of total lipids, reaching a value close to that obtained in this work. However, due to the large number of pepper genotypes, the possibility of cross-species and the sensitivity to physiological stress, large variations may be found in physical properties and chemical composition data [32–34].

3.1. Global yield

Table 3 shows the effect of ultrasound power and its irradiation time in the global yield (X_0), concentrations of capsaicin (C), dihydrocapsaicin (DHC), nordihydrocapsaicin (n-DHC), homodihydrocapsaicin (h-DHC), and total phenolics obtained by Sohxlet, SFE (15 MPa and 40 °C), and SFE + US from malagueta pepper (*C. frutescens* L.). It can be verified that the global yield obtained by Soxhlet was different from those obtained by SFE with and without ultrasound, in all operational conditions. This behavior can be explained by the temperature conditions, the solvent recycle and the solvent/solute interactions of the Soxhlet method, which contributes to enhance the solubility of most of the sample compounds. Furthermore, in the Soxhlet method, the solvent boiling temperature, the surface tension and the viscosity are high compared to those used in SFE. Therefore, the solvent reaches active sites inside the solid matrix more easily, leading to solubilization of the solute into the solvent [36,37]. Besides the factors influencing the conventional method, the highest global yields of Soxhlet were obtained with low polarity solvents, such as hexane, dichloromethane and ethyl acetate, approximately 9.7%, 9.3% and 9.2% (kg of extract/kg of raw material), respectively. Duarte et al. [24] obtained a global yield of malagueta (*C. frutescens* L.) pepper oleoresin of 10.1% (kg of extract/kg of raw material), using the conventional method with hexane as a solvent.

The SFE at 40 °C and 15 MPa without ultrasound achieved a global yield of 5.7%. Assuming that the Soxhlet extraction with hexane recovered all the oleoresin from the raw material, SFE achieved a recovery of approximately 58%. Moreover, when applying ultrasonic waves, the recovery increased up to 75.3%, indicating an improvement in the extraction. The maximum increase of global yield obtained with ultrasound was approximately 30%. This value is similar to other results obtained in previously published works using SFE + US. For instance, Riera et al. [19] obtained an increase of 30% in the global yield in extraction from almonds (*Prunus amygdalus*); Balachandran et al. [20] achieved an increase of 30% in SFE from ginger (*Zingiber officinale*); Gao et al. [11] obtained 61% of increase in SFE from marigold (*Tagetes erecta* L.); and Hu et al. [21] had an increase around 14% in SFE from adlay seeds (*Coix lachrymal-jobi* L. var. *Adlay*).

The results presented on Table 3 indicate that SFE without ultrasound and SFE with ultrasound at low power and irradiation time (200 W for 60 min) were not significantly different according to Tukey's test ($p \leqslant 0.05$). There is not a significant uptake in the global yield when short time and low power ultrasonic waves are applied. However, when ultrasonic waves of high power are applied (360 W), there is a positive effect in SFE global yield. The effects of irradiation time ($F_{cal} = 10.53$) and ultrasound power
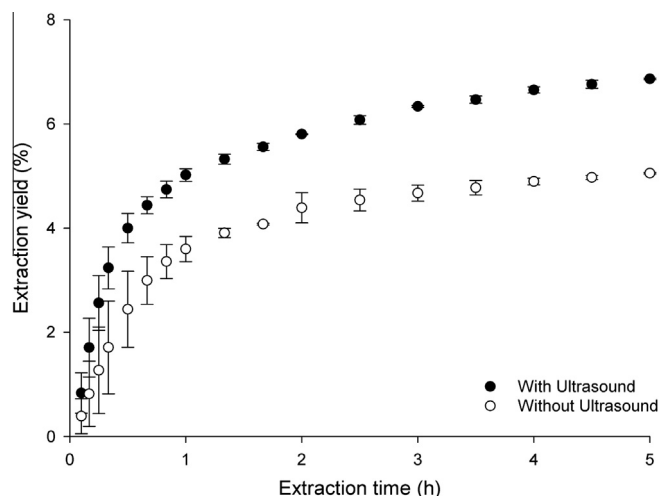
Fig. 2. Kinetics of SFE from malagueta pepper (*Capsicum frutescens* L.) at 15 MPa, 40 °C and CO_2 flow rate of 1.673×10^{-4} kg/s, without and with (at 360 W for 60 min) ultrasound with particles of mean diameter (0.94 ± 0.03 mm).

(F_{cal} = 7.08) were statistically significant, but the interaction between those factors was not significant at a level of 5% ($F_{tab(8;9;0.05)}$ = 3.23).

The best conditions of SFE + US, in terms of global yield, were found at 360 W for 60, 150 and 240 irradiation minutes, and at 200 and 280 W, with 240 min of irradiation. As shown in Table 3, the differences between the global yields of these conditions were not significant. Thus, it was decided to perform the kinetics experiments at 360 W with 60 min, since this condition has the least energy (kJ per area of extractor vessel) consumed with ultrasound.

3.2. Extraction kinetics

Fig. 2 shows the SFE curves obtained from the particles with diameter of 0.94 ± 0.03 mm, with and without ultrasound. The extraction kinetics shown in Fig. 2 illustrates the typical behavior of a SFE curve. The same behavior was observed by Daood et al. [25] studying the SFE of paprika oleoresin, and by Kwon et al. [35] studying red pepper SFE, under six and four different conditions of pressure and temperature, respectively.

It can be noted that the application of ultrasonic waves influenced the extraction kinetics. In the first hour of extraction, the global yield increased from 3.6%, without ultrasound, to 5.1% using ultrasound (SFE + US). Thus, an increase of 40% in the global yield was verified in the mentioned period. At the end of the process, the increase promoted by ultrasound on the global yield was of 35%. This behavior is consistent with results obtained in previous works [11,19–21]. According to Balanchandran et al. [20], the increase in the global yield is assigned to the high extraction rates achieved in the process assisted by ultrasound. The enhanced efficiency of the process is attributed to the cell rupture that leads to an increased accessibility of the solvent to the inner structures of the particles. Moreover, although at ambient conditions the phenomenon of cavitation is the best explanation for the increase in global yield, such effect should not be considered in SFE, because the absence of phase boundaries above the critical point would avoid bubble formation. The increase of global yields in SFE processes may be related only to the turbulence associated with acoustic streaming, or simply to mechanical vibrations.

Considering the operational costs, the solvent mass to feed mass ratio (S/F) to obtain 85% of extract by SFE + US was approximately 240 (kg solvent/ kg raw material), which is 60% lower than the ratio used in SFE without ultrasound to obtain the same yield.

A lower S/F ratio corresponds to less solvent being pressurized, i.e., less required energy for the process and lower extraction time. Consequently, it results in higher productivity without compromising the extract's chemical profile.

3.3. Analysis of capsaicinoids

Table 3 presents the content of capsaicinoids (C, DHC, n-DHC h-DHC and Total) found in the extracts obtained by Soxhlet and SFE without and with ultrasound, at different powers and irradiation times. It can be observed that ultrasound conditions did not affect the concentration of capsaicinoids in the extracts. This may be taken as a positive result, since SFE + US was able to recover higher extract yields with equal capsaicinoids concentration. Therefore, the total yield of capsaicinoids was also increased.

The only significant difference in total capsaicinoid concentration was found between the dichloromethane extract and the extracts obtained with ethyl ether and SFE + US at 200 W–60 min. Even so, extracts obtained using dichloromethane are not recommended for further uses in food or pharmaceutical products, due to the toxicity of the solvent. The other results of capsaicinoids concentrations were not statistically different according to Tukey's test ($p \leqslant 0.05$). Significant differences were not observed by the Tukey's test ($p \leqslant 0.05$) for nordihydrocapsaicin (n-DHC) and homodihydrocapsaicin (h-DHC) for all extraction methods and ultrasound conditions, because these are minor compounds compared with capsaicin (C) and dihydrocapsaicin (DHC).

The recoveries of capsaicinoids by SFE + US at 200 W–240 min, SFE without ultrasound and Soxhlet with hexane were of 87.8%, 76.1% and 77.4%, respectively. SFE + US reached capsaicinoids recoveries 10% higher than the conventional methods, and 11% higher than SFE without ultrasound. The highest recoveries were achieved by Soxhlet extractions with dichloromethane and ethyl acetate (92.1% and 88.8%, respectively). This behavior can be explained by the higher extraction temperature. Even applying high temperatures, degradation of capsaicinoids was not observed. According to Barbero, Palma and Barroso [38] capsaicin and dihydrocapsaicin, which are the major compounds of capsaicinoids, are stable at temperatures above 60 °C. Boonkird, Phisalaphong e Phisalaphong [17], studying the extraction of capsaicinoids with ethanol assisted by ultrasound, obtained the same recovery as the conventional extraction methods of this work.

The capsaicinoid profiles of the extracts obtained by the investigated methods were similar. However, to select the best extraction method, one must take into account other factors, such as process safety, purity of the extract, amount of residual solvent and economic issues. Therefore, SFE with CO_2 has advantages over extractions with toxic and pollutant organic solvents and, when applied together with ultrasonic waves, it leads to a gain in global yield without changes in the capsaicinoid profile.

In order to understand the extraction capsaicinoids along time, SFE and SFE + US kinetics were performed. Fig. 3 shows SFE kinetics of total capsaicinoids (Total), capsaicin (C), dihydrocapsaicin (DHC), nordihydrocapsaicin (n-DHC) and homohydrocapsaicin (h-DHC) from malagueta pepper at 15 MPa, 40 °C and CO_2 flow rate of 1.673×10^{-4} kg/s, without and with ultrasound (360 W for 60 min).

The application of ultrasonic waves did not change the capsaicinoids extraction kinetics. In the first 2 h of extraction, it was possible to obtain 85% of the total capsaicinoids extracted in 5 h. This behavior may be related to the location of capsaicinoids in the pepper fruit, which is mostly in the placenta surface [3]. Therefore, most capsaicinoids are easily accessible to supercritical CO_2 and can be removed by convection. The solubilities of capsaicinoids in CO_2 at 15 MPa and 40 °C are probably higher than the solubilities of other compounds found in pepper oleoresin, such as
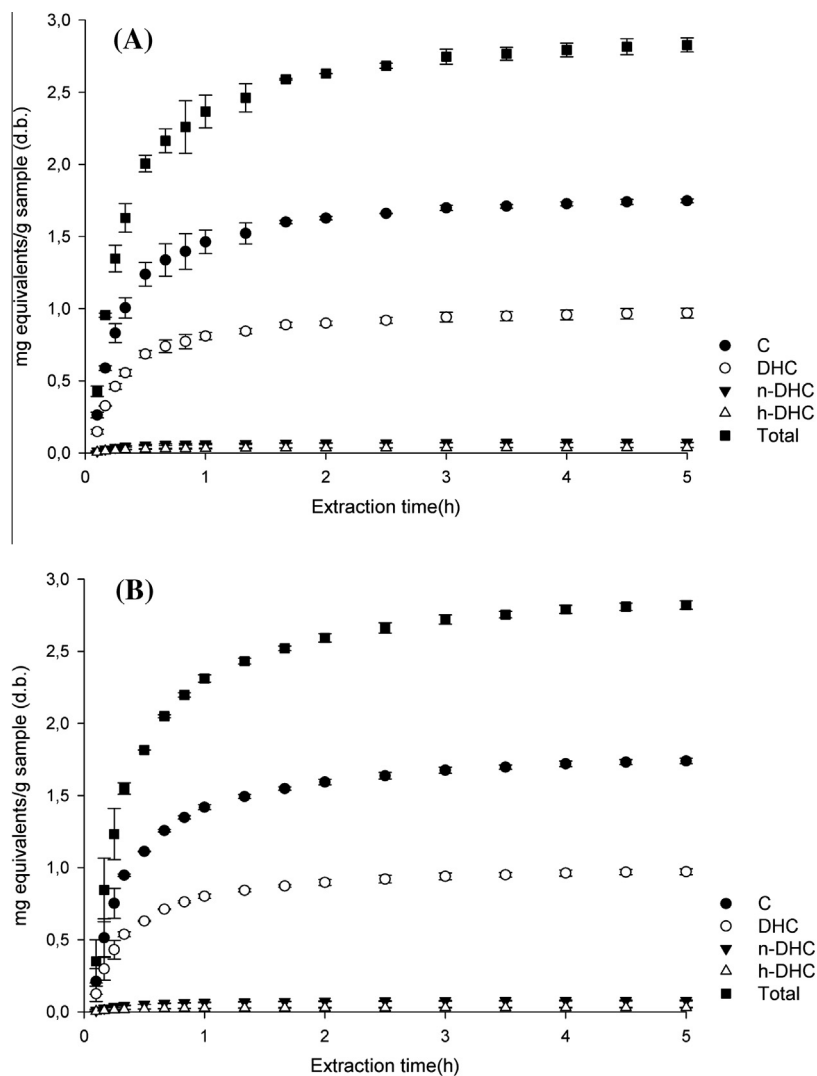
Fig. 3. SFE kinetics of capsaicin (C), dihydrocapsaicin (DHC); nordihydrocapsaicin (n-DHC); homodihydrocapsaicin (h-DHC) and total capsaicinoids (Total) from malagueta pepper at 15 MPa, 40 °C and CO_2 flow rate of 1.673×10^{-4} kg/s, without (A) and with ultrasound (B), at 360 W for 60 min.

triacylglycerols and carotenoids. Del Valle et al. [39] observed the same behavior for the SFE kinetics of capsaicin from Jalapeño red pepper. These authors concluded that the extraction rate of total pepper oleoresin was slower than that of capsaicinoids at 12 MPa and 40 °C, due to the predominant amount of triacylglycerols present in the resin, which are much less soluble than capsaicin in those conditions.

3.4. Analysis of total phenolics

The phenolic content of the extracts, expressed in mg of GAE/g of raw material, are presented in Table 3. It can be observed that the conventional extraction method with ethyl acetate, dichloromethane, ethyl ether, SFE + US at 360 W for any irradiation time, and at 200 W for 60 and 240 min were not significantly different. All those experimental runs obtained recoveries of at least 42%, while the other experimental conditions achieved recoveries of about 36%. According to Carrera et al. [13] the energy supplied by ultrasound helps releasing the phenolic compounds from the vegetal matrix. However, it also accelerates the degradation process of such compounds. Therefore, SFE + US was able to recover the same amount of phenolics as conventional processes with organic solvents.

Biesaga [40] analyzed the influence of the extraction method on the stability of flavonoids, and verified that the lowest recoveries or highest degradation rates were observed for extraction assisted by ultrasound. According to Carrera et al. [13] the application of ultrasonic waves may enhance the formation of free radicals, while the phenolic compounds can sequestrate the compounds of reactive oxygen species, leading to oxidation reactions.

Table 4
SFE process conditions needed to apply the kinetic model [27].

Condition	Larger particle	Smaller particle
T (K)	313.15 ± 3	313.15 ± 3
P (MPa)	15 ± 0.3	15 ± 0.3
dp (m)	0.00143 ± 0.00035	0.00023 ± 0.00016
X_0 (kg solute/kg solid)	0.062 ± 0.001	0.10 ± 0.01
ρ_s (kg/m^3)	1320.0	1320.0
ρ (kg/m^3)	780.23	780.23
Q_{CO_2} (kg/s)	0.0001673	0.0001673
H_b (m)	0.02	0.02
d_b (m)	0.05	0.05
F (kg)	0.0204 ± 0.0003	0.0205 ± 0.0007

T – temperature; P – pressure; dp – particle diameter; X_0 – global yield; ρ_s – solid density; ρ – solvent density; Q_{CO_2} – solvent mass flow rate; H_b –extraction bed height; d_b – extraction bed diameter; F – mass of raw material in the extraction bed.

The best condition of SFE + US, taking into account the recovery of phenolic compounds, total capsaicinoids and the kinetic profile, was 360 W for 60 min. After determining the best condition for SFE + US and the influence of ultrasonic waves on the vegetal matrix, the study of the effect of particle sizes and ultrasonic waves on the extraction kinetics was performed. With this purpose, mathematical modeling and SEM analysis were used.

3.5. Mathematical modeling

Table 4 shows the values of the input parameters needed to apply the mathematical model of Sovová [27], obtained for SFE of malagueta pepper. As can be observed, the extraction bed dimensions were maintained constant for SFE with and without ultrasound, for larger (1.43 ± 0.35 mm) and smaller particle diameters (0.23 ± 0.16 mm).

It is well known that X_0 (kg solute/kg solid) represents the amount of material (solute) that can be extracted by a SFE process at a given condition. As observed in Table 3, the application of ultrasonic waves clearly increases X_0, so the value obtained by SFE + US at 360 W during the total extraction time (8 h) was adopted. It can be verified in Table 4 and Fig. 4 A and B that X_0 varies with particle diameter, from 6.2% to 10%, for large and small diameters, respectively. Such behavior is due to a larger contact area of the particles of smaller diameter, which leads to a higher mass transfer of solute from the solid to the solvent. Instead, in the case of particles of larger diameter, the contact area provided to the solvent is smaller, causing a decrease in the mass transfer rate. This is in agreement with results found in other works [20,41,42]. Moreover, larger particles contain higher concentration of intact seeds in the raw material, which might not be penetrated by the solvent, causing this significant difference in the value of global yield obtained from different particle sizes. These hypotheses are reinforced in Fig. 4, which illustrates the experimental SFE curves and those fitted by the model [27], for the SFE of malagueta pepper without and with ultrasound (360 W), for particles of large (A) and small (B) diameters.

The curves in Fig. 4 show the behavior of SFE kinetics. The process begins with a period of constant extraction rate (CER), which is characterized by the extraction of compounds that are readily available to the solvent. When this easily accessible solute begins to exhaust, intraparticle diffusion becomes the driving mechanism of mass transfer in SFE. Then, the extraction curves assume a typical shape of a diffusion curve, with reduced extraction rate until the global yield (X_0) is achieved.

Fig. 4A and B show that the influence of ultrasound in the constant extraction rate period (CER) is higher than that on the decreasing rate and diffusion periods, this fact can be reinforced observing the increase of k_f values with application of ultrasonic waves. This behavior can be explained considering the disturbance caused by the ultrasonic waves near the cell walls, which help releasing the intraparticle material. Other possible explanation may be the desorption phenomena of solutes absorbed on a vegetal matrix, in which some interactions between solute and solid matrix could have been affected by the energy supplied to the solid in the form of ultrasonic waves. This energy may help releasing the adsorbed solute from the solid's surface to the solvent. Probably, the same interaction phenomenon was responsible for particle deposition on the vegetal matrix surface, which is discussed in Section 3.6.

The curves presented in Fig. 4 show that the model fitted well to the experimental data. Table 5 displays the values of the adjusted parameters: mass transfer coefficients in the solid phase (k_s), fluid phase (k_f), concentration of solute inside the unbroken cells (X_k), and the objective function (f) for the SFE without and with ultrasound (360 W). The values of the objective function, which is defined as the sum of squared errors, were below 1×10^{-7}, evidencing the good fitting of the model, in accordance to Fig. 4A and B. The values of the solid phase mass transfer coefficient (k_s) were lower than those of the fluid phase mass transfer coefficient (k_f) for the evaluated curves. According to Weinhold et al. [43] the solute located internally in the particles is more difficult to be removed, and thus it takes longer to cross the interface between the fluid and the solid solute, located on the surface of the particles. Therefore, the smaller values obtained for k_s indicate that the mechanism of diffusion is slower than convection in the SFE from malagueta pepper.
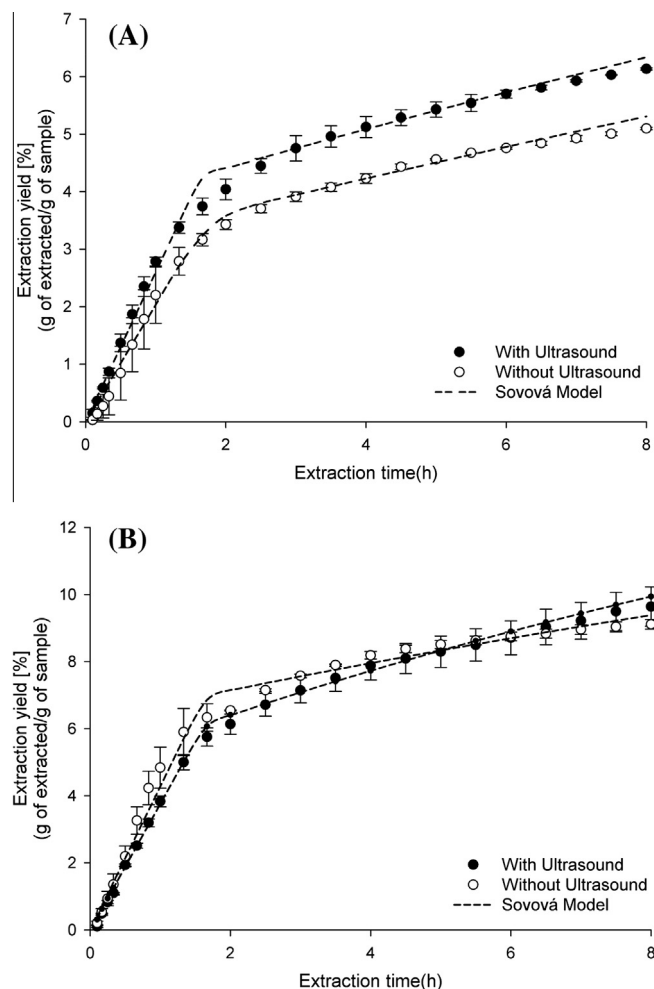

Fig. 4. Experimental and modeled SFE curves from malagueta pepper at 15 MPa, 40 °C, CO_2 flow rate of 1.673×10^{-4} kg/s, without and with ultrasound at 360 W, for larger (dp = 1.43 ± 0.35 mm (A)) and smaller (dp = 0.26 ± 0.16 mm (B)) particle diameters, with the respective amplitude of the replicates.

Table 5
Adjusted parameters, objective function (f) and constant rate period (t_{cer}) calculated with the Sovová's model [27] applied to SFE from malagueta pepper at 15 MPa and 40 °C with (360 W) and without ultrasound for larger and smaller particle diameters.

Parameters	Larger particle		Smaller particle	
	Without US	With US	Without US	With US
X_k^*	0.0276	0.0289	0.0395	0.0447
$k_f\,(s^{-1}) \times 10^4$	1.3697	1.9103	3.3636	2.7832
$k_s\,(s^{-1}) \times 10^6$	3.0575	8.9616	4.9794	6.2212
$f \times 10^8$	1.4554	0.6694	2.7608	1.8561

where: *kg of solute/kg of raw material.

Larger Particle
Raw Material

Smaller Particle
Raw Material

Raw Material after SFE

Raw Material after SFE

Raw Material after SFE+US
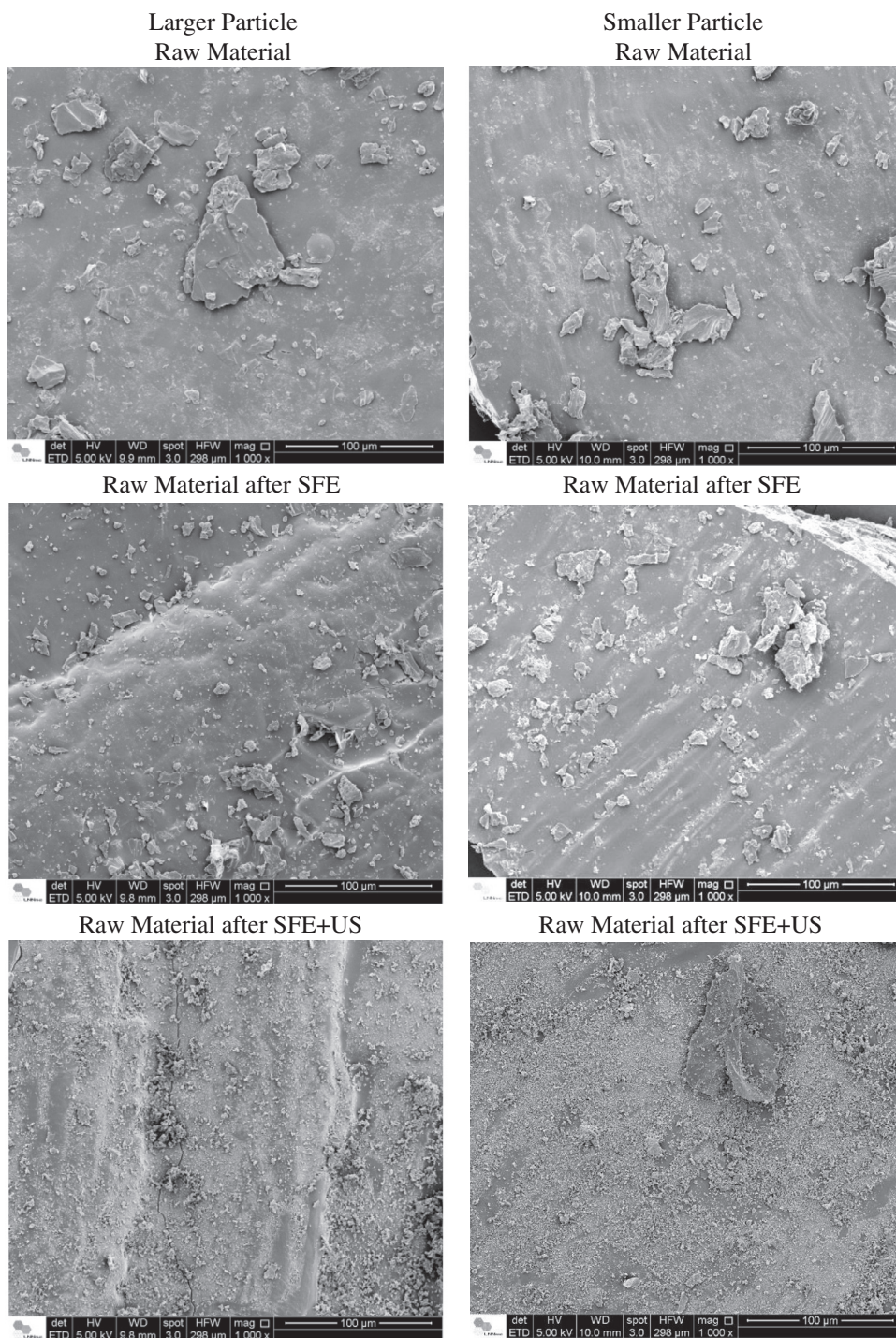
Raw Material after SFE+US

Fig. 5. FESEM images obtained from the pericarp of malagueta pepper fruits before extraction (raw material), extracted with supercritical CO_2 and extracted with supercritical CO_2 assisted by ultrasound (360 W 480 min) for two different particle diameters. Scale bar – 100 μm.

The solid phase mass transfer coefficients (k_s) in SFE are higher for smaller particles, as shown in Table 5, independently of the application of ultrasound. Since k_s is related to mass transfer inside the particles, the changes caused by particle reduction must have enhanced the intraparticle transport mechanisms. According to Fick Law's [44], in smaller particles solute and solvent face fewer barriers to diffusion from the inner part of the particles to their surface. Indeed, effective diffusivity may be increased by reducing the tortuousness of the particle pores. Therefore, smaller particle diameters result in lower internal resistance to mass transfer, which increases k_s.

Analyzing the effect of ultrasound, the values of both mass transfer coefficients increased when ultrasonic waves were applied in SFE from the larger particles. On the other hand, for smaller particles, the increase was noted only in k_s, with less intensity. The magnitude of the changes in k_f and k_s caused the increase promoted by ultrasound on global yield. While in smaller particles an increase of 7.8% was observed, in larger particles, the increase achieved 20%. For smaller particles the milling process could have been sufficient to promote the contact between solute and solvent, making the role of ultrasound superfluous. Thus, the application of ultrasound in SFE seems to be more effective for larger particles,
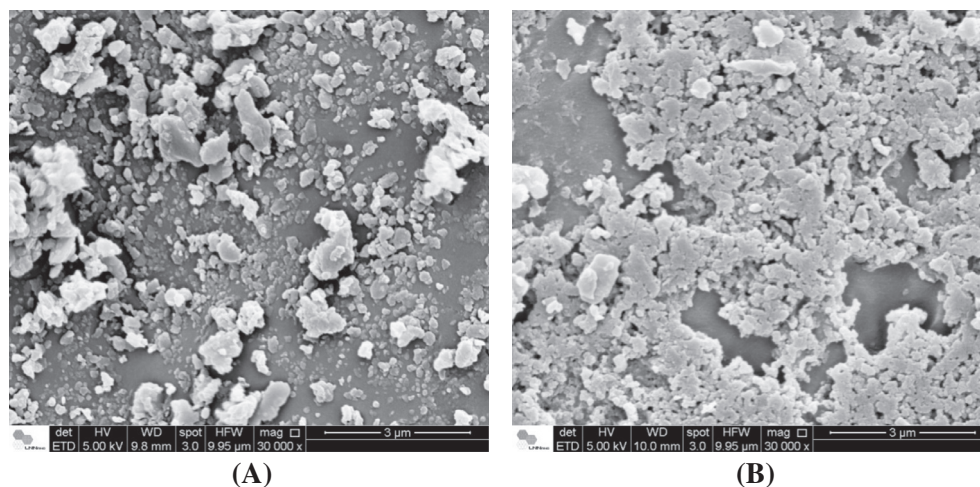
Fig. 6. FESEM images obtained for the malagueta pepper extracted with supercritical CO_2 assisted by ultrasound (360 W for 480 min) for two different particle diameters: larger (A) and smaller (B) particle. Scale bar – 3 μm.

and ultrasound might be used instead of exhaustive milling procedures on raw material.

3.6. Scanning electron microscopy (FESEM) analysis

In order to investigate the effects of ultrasonic waves on the solid matrix, scanning electron microscopy (FESEM) analysis was performed. Fig. 5 shows the images obtained by FESEM on the pericarp of malagueta pepper fruits before extraction, after SFE and after SFE + US (360 W–480 min), for both particle diameters studied in this work. It is clear from the images presented in Fig. 5 that the samples that underwent SFE present a greater amount of particulate matter deposited on the surface in comparison to the raw material. In the case of the ultrasound assisted process, particle deposition is even more pronounced in both sample sizes. The flux of the supercritical fluid and mainly the ultrasound waves disturbed the cell walls, leading to the displacement of microparticles from the internal part of the vegetable matrix to its surface.

Another remarkable issue is that the surface of the samples below the particle deposits does not present cracks or any other sign of disruption. Therefore, particulate deposits must be formed by particles removed from the internal part of the cell wall, but that reached the surface coming from the lateral cuts of the samples (formed by knife milling), not passing though the surface. Fig. 6 shows a magnified view of the deposits of particulate matter on the surface of a pepper sample extracted with SFE + US (360 W–480 min) (scale bar = 3 μm). These micrographs show the intact surface of the vegetable matrix, without cracking and covered by material from the inner regions of the cell wall. Ultrasound effects in the vegetal matrix were studied by Ying, Han e Li [45], Chen et al. [46] and Chittapalo and Noomhorm [47]. These authors observed that different methods of extraction result in different morphological effects on the vegetable matrix. Balachandran et al. [20] also verified by analyzing FESEM images that the application of ultrasonic waves disturbed the cell walls of ginger particles, facilitating the removal of the cell contents.

Another effect that was observed is that the raw material presented a more fragile behavior when reached by the electron beam than the extracted samples. The non-extracted matrix becomes visually degraded under magnifications around 50,000 times, which is assigned to the higher amounts of oleoresin in this material, when compared to the extracted samples. Evidences of decreased oil content in vegetables samples observed by scanning electron microscopy were reported by Zhang et al. [48]. The authors reported lower oil content on the surfaces of flaxseed samples subjected to ultrasound-assisted extraction with organic solvents than in seeds before the extraction process.

The increase in extraction yields cannot be simply explained by the abrasive effects or by the turbulence created by ultrasonic waves. The experimental observations suggested that the intensification of mass transport is due to physical effects on the surface of the particles. The FESEM images show evidence of perturbations in the vegetable matrix. The results of mathematical modeling for samples of greater diameter confirm that the mass transfer coefficients for both phases increase when the solid matrix is exposed to such disturbances. According to Balachandran et al. [20], these disturbances are possibly caused by the rapid changes in density associated with pressure fluctuations induced by ultrasonic waves. However, the authors also consider the possibility of a collapse as a cavitation mechanism. Also according to Balachandran et al. [20] while it is not possible to prove that such phenomena occurs, the cavitations near vegetable matrix continues to be the most probable cause of the disruption.

4. Conclusions

Supercritical extraction assisted by ultrasound (SFE + US) increased the global yield of malagueta pepper oleoresin up to 30% when compared to SFE, without changing the total capsaicinoids and phenolics profile. The best operating condition of SFE + US was 360 W for 60 min of irradiation, at temperature, pressure and CO_2 flow rate of 40 °C, 15 MPa and 1.673×10^{-4} kg/s, respectively.

The broken and intact cell model of Sovová [27] proved to be effective in predicting the kinetics of SFE assisted by ultrasound, and the values of the calculated mass transfer coefficients help understanding the possible structure changes caused by ultrasound on the samples. The highest increase was obtained on the global yield of SFE from larger particles, while the application of SFE + US from smaller particles resulted in less pronounced increases. The FESEM images revealed morphological changes caused by disturbances on the vegetal matrix due to application of the ultrasound, such as the formation of dense deposits of particulate material on the surface. Besides this, differences in the oleoresin amount on the malagueta pepper samples before and after extraction were also indicated by this technique.

In general, the ultrasonic waves have proved to be effective in obtaining extracts from malagueta pepper, and it can be an alternative technique to conventional extraction methods. However, further studies should be performed in order to evaluate the economic viability of the process and perform the scale-up of supercritical extraction assisted by ultrasound. Indeed, the influence of process pressure and temperature on SFE + US should also be explored.

Acknowledgments

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