SUPERCRITICAL FLUID EXTRACTION OF MALAGUETA PEPPER (*Capsicum frutescens* L.) ASSISTED BY ULTRASOUND – EFFECTS ON VEGETABLE STRUCTURE

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ABSTRACT

In a supercritical fluid extraction (SFE) unit, one generally can change the temperature, pressure, extraction bed size, solvent flow rate, among others, in order to maximize the global yield. The morphology of the solid substrate particle can also influence the extraction efficiency, since the solvent must cross diffusive paths inside the solid particle, in order to extract specific compounds Moreover, the SFE process capacity may be improved by using combined extraction techniques, such as the use of different co-solvents and ultrasonic waves. The ultrasonic technology is based on the high frequency ultrasonic waves, which are capable of causing cavitations and disrupting the cell walls of vegetable materials. This favors the penetration of solvent and mass transfer, increasing the extraction yield and velocity. The objective of this study was to obtain extracts of malagueta pepper (*Capsicum frutescens* L.) using SFE assisted by ultrasound and conventional extraction method, and understand the effects of extraction on the structure of the vegetable matrix. The raw material used was malagueta pepper dried at 50 °C (5% w. b.) and triturated. The SFE conditions were 40 ± 3 °C and 15 ± 0.5 MPa. The CO_2 mass flow rate was fixed at 0.5 ± 0.1 kg/h. To study the influence of extraction on vegetable structure, SFE extractions were performed without ultrasound and with ultrasound at powers of 280 and 360 W. Conventional extractions (soxhlet) were carried out using *n*-hexane as solvent. 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 results showed that SFE assisted by ultrasound increased global yield when compared to the SFE without ultrasound. The highest yield was obtained in the conventional method (soxhlet). Images obtained by FESEM showed that the action of ultrasound waves did not cause cracks on the cell wall surface, but the images reveal morphological changes caused by disturbances on the vegetal matrix due to application of the ultrasound.

INTRODUCTION

Peppers are used as food plants, which produce the spicy/heating sensation due to its chemical components, which are able to stimulate the taste buds of the mouth. The responsible compounds for this sensation are capsaicinoids, among which capsaicin is the most representative. Many beneficial proprieties have been associated to consumption of capsaicin, for instance, antioxidant, antimicrobial, anti-inflammatory and antitumor activities, and contributions to the control of diabetes and pain relief [1].Moreover, in the last decade, the study of pepper varieties gained the attention of many research groups and industries interested in getting high quality products. Taking into account the benefits of capsaicin and the concern of industry, there is great interest in developing new technologies to obtain concentrated extracts

Supercritical fluid extraction (SFE) was developed as an alternative to traditional methods for the extraction and fractionation of active compounds. Carbon dioxide (CO_2) is the most commonly used supercritical fluid in these processes, due of its advantages, which are: low cost, nontoxicity, non-flammability, inertness and good extraction capacity [2, 3]. 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. The SFE process capacity may be improved by using combined extraction techniques, such as the use of different co-solvents and ultrasonic waves [4].

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. 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 [5].

The objective this work was to obtain extracts of malagueta pepper (*Capsicum frutescens* L.) by SFE assisted by ultrasound and conventional extraction methods, as well as to understand the effects of extraction on vegetable matrix structure through scanning electron microscopy.

MATERIALS AND METHODS

The work was conducted in the Laboratory of High Pressure in Food Engineering (LAPEA/DEA-FEA/UNICAMP). The raw material was malagueta pepper, which is a variety of chili pepper acquired at "Central de Abastecimento de Campinas/SP (CEASA)", a local market in Campinas, southeastern Brazil.

The fruits with good physical integrity were selected, washed with running water and conditioned under refrigeration (≈ 4°C) for further utilization. The raw material was subjected to a drying process in a laboratory oven, following the methodology used by Aguiar et al. [6], 70 °C for 12 hours. After drying, the samples were ground in a knife mill (Marconi, model MA 340, Piracicaba) with the objective to homogenize and decrease the resistance to mass transfer during the later stages of extraction. The pepper solid particles were characterized by size classification in a vertical vibratory sieve shaker (Bertel Metallurgic Ind. Ltda., SP, Brazil) Tyler series, Wheeling, USA) system (Bertel, model 1868, Caieiras, SP) with sequential openings of 12, 16, 24, 32, 48 and 80 mesh, and the mean particle diameter was calculated according to ASAE Standards [7] with value of 0.94 ± 0.03 mm.

The SFE experiments were performed to evaluate the influence of ultrasonic power on global yield. The fixed SFE conditions were pressure and temperature of 15 ± 0.5 MPa and 40 ± 3°C respectively, obtained by Aguiar et al. [6], who optimized SFE-CO_2 of malagueta pepper in terms of capsaicinoid recovery. In global yield experiments the ratio between solvent and feed mass (S/F) was maintained constant, at about 600 ± 2 (kg of solvent /kg raw material). All SFE experiments were carried out in 5 hours, according to results obtained by Aguiar et al. [6].

The soxhlet method was selected as conventional extraction technique, using hexane as solvent. In each extraction about 5.0 g of dried sample were packed in filter paper and inserted in the soxhlet extractor. Solvent (0.15 L) was added and the system was heated until boiling. The reflux was kept for 6 h, then the solvent was evaporated under vacuum (at 25 °C), and the recovered extract was weighed. The Soxhlet extractions were performed in triplicates.

The SFE experiments were performed in a unit consisting of a 0.295 L extraction column; a pneumatic pump (PP 111-VE MBR, Maximator, Nordhausen, Germany); two thermostatic baths to control CO_2 temperature at the pump inlet and SFE temperature; a flow totalizer and manometers to measure pressure. The ultrasonic system (US) (Unique Group, model DES500) is composed of a transducer unit with frequency of 20 kHZ and a variable output power controller. The ultrasound probe was installed inside of the SFE column. Figure 1 illustrates SFE-US unit used in experiments.

In the global yield experiments the influence of variables process on SFE of malagueta pepper assisted by ultrasound was studied, varying the power in two levels: 280 (12 W/cm²) and 360 W (15.5 W/cm²) with ultrasound application time of 60 minutes; and compared to conventional extraction method (soxhlet) and SFE without ultrasound.

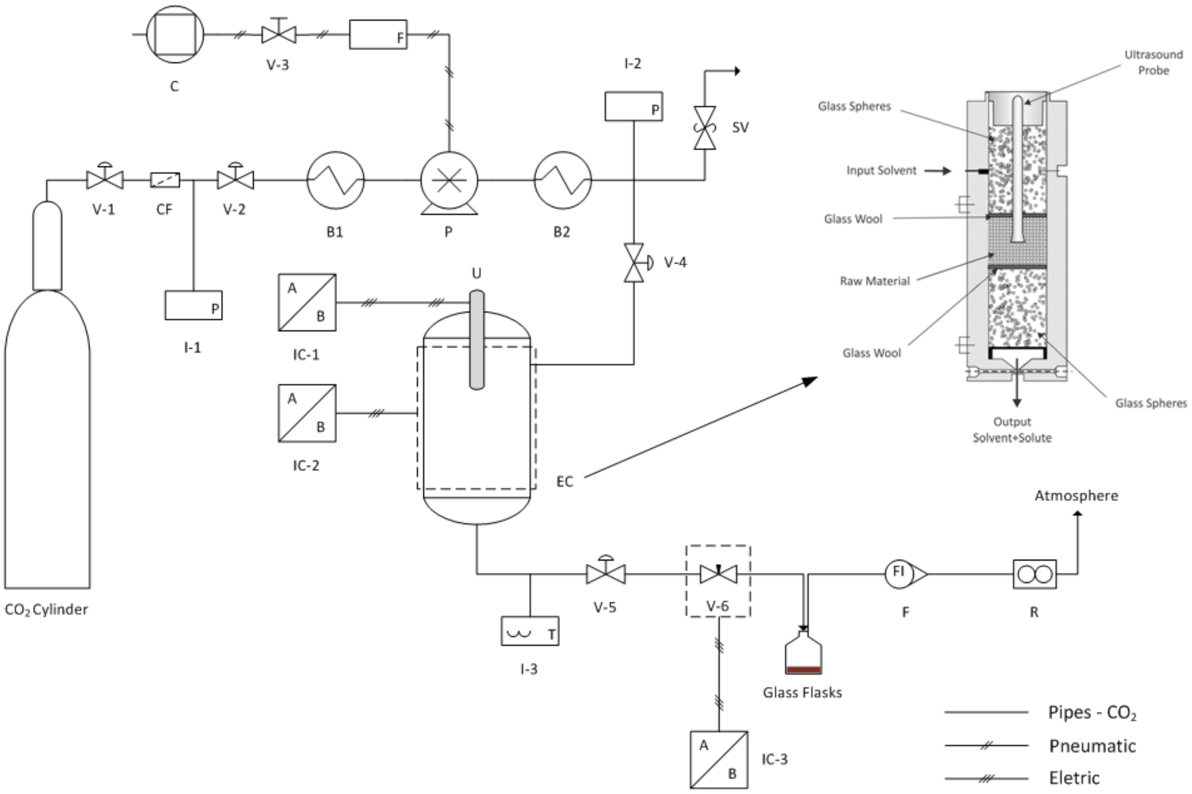


Figure 1. Diagram of the SFE unit with carbon dioxide assisted by ultrasound; V-1, V-2, V-3, V-4 e V-5 – Control valves; V-7 – Micrometer valve; SV – Safety valve; C- Compressor; F- Compressed air filter; CF – CO_2 Filter; B1 –Cooling bath; P - Pump; B2 – Heating bath; I-1 e I-2 – Pressure indicators; I-3 – Temperature indicator; IC-1, IC-2 e IC-3 – Indicators and controllers of ultrasound power, temperature of extraction column and temperature of micrometer valve, respectively; U – Ultrasound probe; F – Rotameter; T – Flow meter; EC – Extraction column and settings of the extraction bed of 295mL for SFE assisted by ultrasound.

The pericarp sample microstructures were analyzed before and after soxhlet and SFE, using a scanning electron microscope equipped with a field emission gun (FESEM - FEI Quanta 650). The pericarp particles were separated from peduncle and seeds with aid of tweezers and an optical microscope. Prior to analysis, the samples were coated with gold 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 guarantee the reproducibility of the results.

The results were statistically evaluated by of variance (ANOVA), applied using the software Statistica for Windows 6.0 (Statsoft Inc., USA) in order to detect significant differences in global yield (Y) obtained by sohxlet, SFE (15 MPa e 40 °C), and SFE+US from malagueta pepper (*Capsicum frutescens* L.). The significant differences at level of 5 % ($p < 0.05$) were analyzed by the Tukey test.

RESULTS

Table 1 shows the global yields (Y) obtained by soxhlet, SFE (15 MPa e 40 °C), and SFE+US from malagueta pepper. It can be verified that global yields obtained by soxhlet were different from those obtained by SFE with and without ultrasound in all operational conditions. This behavior is explained by the temperature conditions, solvent recycle and solvent/solute interactions of soxhlet method, which contributes to higher solute solubility. Therefore, the solvent easily reaches active sites inside the solid matrix, promoting the solubilization of solute in solvent [8, 9]. Duarte et al. [10] obtained a global yield of malagueta pepper oleoresin of 10.1% (kg of extract/kg of raw material), using the conventional method with hexane as solvent, value near to the obtained by this study (9.7±0.1), as seen in Table 1.

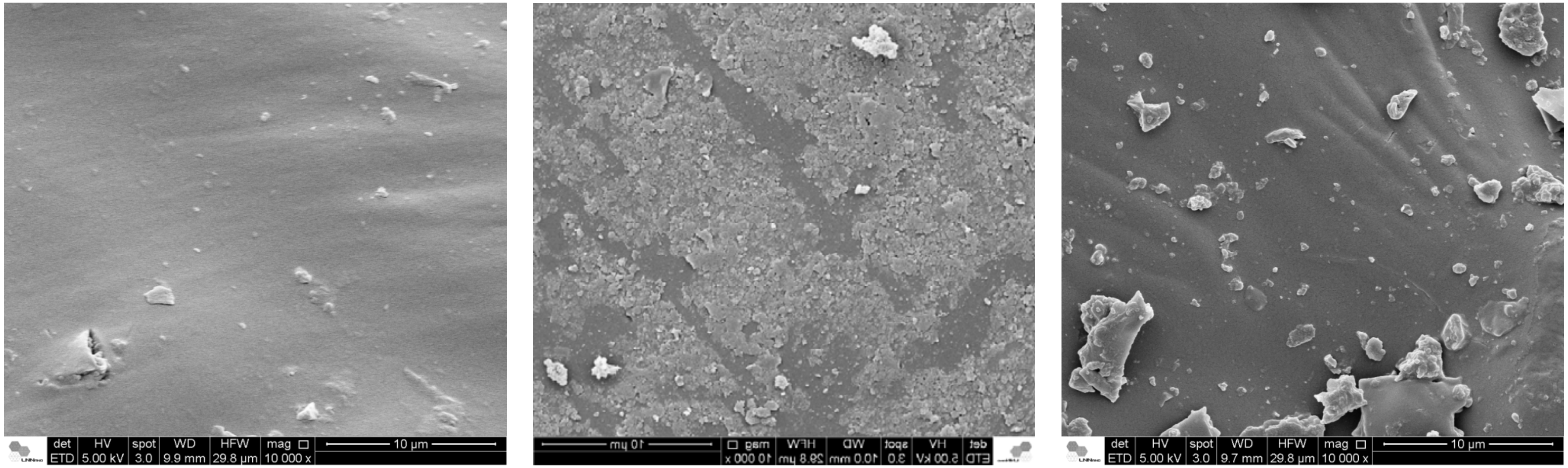
Table 1: Global yields obtained by Soxhlet, SFE (15 MPa and 40 °C), and SFE+US from malagueta pepper (*Capsicum frutescens* L.).

Method	Solvent		Y (%)
Soxhlet	Hexane		9.7 ± 0.1^{a}
SFE	Power (W)	Time[1]	
SFE	-	-	5.7 ± 0.1^{c}
SFE+US	360	60	7.3 ± 0.2^{b}
SFE+US	280	60	6.0 ± 0.5^{c}

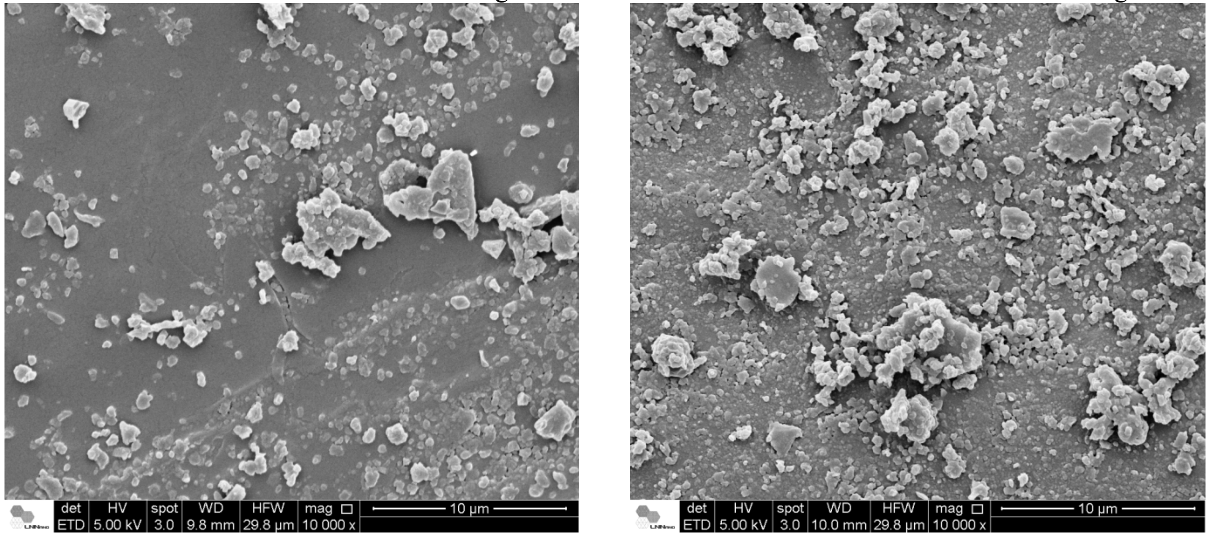
Results are expressed as mean ± standard deviation. SFE – Supercritical fluid extraction; US – Ultrasound; Y – Global yield (g of extract/g of raw material); Irradiation time expressed in minutes; Different letters in the same column indicate that the means differ significantly by Tukey test ($p \leq 0.05$).

SFE without ultrasound at 40 °C and 15 MPa achieved a global yield of 5.7 ± 0.1% (extract mass/raw material mass). Considering that the conventional method (soxhlet) with hexane extracted all the oleoresin from the raw material, the SFE extraction achieved a recovery rate of approximately 60%. Moreover, when applying ultrasonic waves the recovery rate increases to 75%, indicating an improvement in the extraction rate. In terms of global yield, when ultrasonic waves were applied, the increase was of approximately 30% (7.3±0.2%). This value is similar to other results obtained in works using SFE+US previously published in the literature [4, 11, 12].

The application of ultrasonic waves influenced the extraction kinetics. The global yield in the first hour of SFE without ultrasound was approximately 3.6%, whereas for ultrasound-assisted SFE was about 5.1%. In other words, in this period the increase in global yield was 40%. In the end of process the increase of global yield, compared to SFE without ultrasound, was 35%, as shown in Figure 3.



Vegetal matrix Vegetal matrix after Soxhlet Vegetal matrix after SFE



Vegetal matrix after SFE+US (280 W) Vegetal matrix after SFE+US (360 W)

Figure 2. FESEM pictures obtained for the raw material, extracted with soxhlet - hexane as solvent, supercritical CO_2 and supercritical CO_2 assisted by ultrasound at 280 W and 360 W. Scale bar – 100 µm.

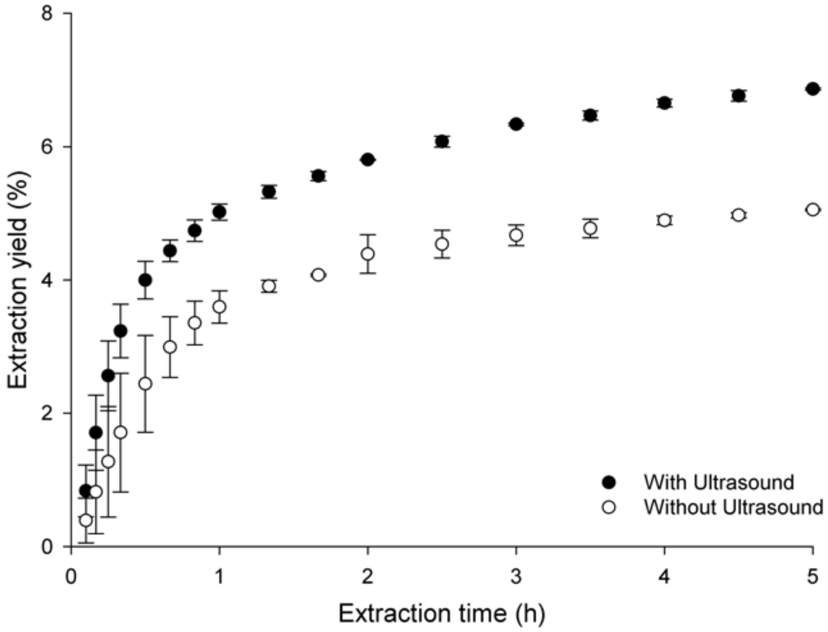


Figure 3. The supercritical carbon dioxide extraction kinetics of oleoresin from malagueta (*Capsicum frutescens* L.) pepper, at 15MPa, 40°C and solvent flow rate of 1.673 x 10^{-4} kg/s , without and with (at 360W for 60 minutes) ultrasound to mean particle diameter (0.94 ± 0.03 mm).

According to Balanchandran et al. [12] the increase in global yield was cant occurred due to the high rate extraction in the process assisted by ultrasound, which is attributed to cellular structures rupture and an increased solvent accessibility to the internal structure of particles. Also according to the authors, although in ambient conditions the cavitations are the best explanation for the increases of global yield extraction, such phenomena should exclude because the absence of phase boundaries would appear to prohibit bubble formation above the critical point. The global extraction yield increase can occur only through the turbulence associated with acoustic streaming or simply by mechanical vibration.

In order to investigate the effects of cavitations and other changes caused by ultrasonic waves on the matrix, scanning electron microscopy (FESEM) analysis was performed. Figure 2 shows the images obtained by FESEM for malagueta pepper samples before extraction, after soxhlet, SFE and after SFE assisted by ultrasound (280 and 360 W). It is clear from the images presented in Figure 2 that the samples that underwent SFE present a greater amount of particulate material deposed on the surface in comparison to the raw material. The same behavior was observed in soxhlet experiments. In the case of ultrasound assisted process, particle deposition is even more pronounced in both conditions (280 and 360 W). This effect is assigned to a disturbance caused by the supercritical fluid on the cell wall, leading to the displacement of micro particles from the internal part of the vegetable matrix to its surface. Ultrasound effects in vegetal matrix were studded by Ying, Han e Li [13], Chen et al. [14], Chittapalo and Noomhorm [15] and Balachandran et al. [12]. These works observed that ultrasonic waves were responsible for some kind of perturbation in vegetable matrix and for increases of global yield.

Another effect observed is that the raw material presented a more fragile behavior against the electron beam than the extracted samples. The non-extracted matrix becomes visually degraded under magnifications around 50.000x, which is attributed to the higher amounts of oil/resin in this material, when compared to the extracted samples. Evidences of decreased oil content in surface vegetables samples, observed by SEM, were reported by Zhang et al. [16].

The authors reported lower oil content in surface samples of flaxseed subjected to ultrasound-assisted extraction with organic solvents than in seeds before extraction process.

The increase in extraction yield cannot be explained simply by the abrasive effects or by turbulence created by ultrasonic waves. The experimental observations suggested that intensification of mass transport is due to physical effects on the surface of the particles. The SEM images show evidence of perturbations in the vegetable matrix. According to Balachandran et al., [12] it is possible that these disturbances are caused simply 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., [12] while it is not possible to prove that such phenomena occur, cavitations near vegetable matrix continue to be the most probable cause of the disruption.

CONCLUSION

The FESEM images reveal morphological changes caused by disturbances on the vegetal matrix due to application of the ultrasound, and also expose behaviors related to the increased global yield in oil/resin of malagueta pepper before the extractions. In general, the technique of ultrasonic waves coupled to SFE has shown to be effective in obtaining extracts of pepper and is an alternative technique to conventional extraction methods. However, further studies should be performed in aim to evaluate the economic viability of the process and perform the scaling of SFE assisted by ultrasound.

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