ENCAPSULATION OF PEPPER OLEORESIN BY THE SUPERCRITICAL FLUID EXTRACTION OF EMULSION TECHNIQUE

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ABSTRACT

The oleoresin of capsicum peppers is rich in capsaicinoids, which are compounds that present pharmacological applications. However, its strong pungency limits their topical and oral uses. Thus, mechanisms that promote controlled release of the oleoresin, such as encapsulation techniques, can be a way to make their therapeutic use viable. The aim of this work was to evaluate the encapsulation of oleoresin by supercritical fluid extraction of emulsions (SFEE) using modified starch Hi-Cap 100® as coating material and surfactant, ethyl acetate as solvent and CO_2 as antisolvent. Firstly, emulsions were prepared using an ultrasonic probe, varying the parameters: starch concentration (6, 9, and 12 g/L), emulsion oil/water ratio (1/5, 1/4, and 1/3) and ultrasonic power (240, 480, and 720 W). The concentration of oleoresin in ethyl acetate was 20 mg/mL, and the sonication time was 5 min. The emulsions that presented higher stability were subjected to the SFEE assays. A lab-scale unit that consists of a CO_2 supply system, emulsion and CO_2 injection unit (coaxial nozzle with an internal diameter equal to 127 μm) and a high pressure column was used. The encapsulation parameters were: temperature and CO_2 flow rate fixed at 40 ˚C and 22.5 g/min respectively, pressures from 9 to 11 MPa, and emulsion flow rates from 0.5 and 1.0 mL/min. The resulting suspension was freeze-dried and the morphology of the solid material was analyzed by scanning electron microscopy. The ultrasound emulsification process was able to produce stable emulsions with mean droplet diameter (after 10 minutes) ranging from 126.4 to 286.3 nm. During the SFEE process a loss of oleoresin was observed due to dissolution in CO_2, reaching up to 40% of the initial oleoresin content, depending on the experimental conditions. The SEM images showed small spherical particles (ca. 5 to 20 μm) dispersed in an amorphous mass of starch. During the freeze-drying process, the starch dissolved in water precipitates, forming large aggregates with different morphologies. Nevertheless, small spherical particles were observed in all the conditions evaluated, which may represent the original particles after their growth during the freeze-drying process.

INTRODUCTION

Capsicum peppers are known as good sources of several nutrients, such as vitamin C, phenolics, flavonoids and carotenoids [1-3]. Hot cultivars are rich in capsaicinoids, which are the compounds responsible for the spicy flavor imparted by many peppers. Capsaicinoids have also strong pharmacological effects on health, which may be used in pain relief, cancer prevention, and weight reduction, besides providing gastrointestinal and cardiovascular benefits. The encapsulation of capsaicinoids in polymer matrices can be an alternative to the use of these compounds as pharmaceuticals and food ingredients, since the spiciness is a limiting factor for their use [4].

The process of particle formulation called Supercritical Fluid Extraction of Emulsions (SFEE) combines conventional emulsion techniques with the unique properties of supercritical fluids for the production of micro and nanoparticles [5]. Techniques based on the precipitation of emulsions are widely used for microencapsulation of active compounds. The greatest disadvantage associated with conventional emulsion precipitation methods is the difficulty to remove the residual solvent from the polymer matrix. The presence of solvent residues in the polymer causes a reduction of the activity of the encapsulated compound on the target system and increases toxicity, which can create obstacles to the development of products [6].

In the SFEE process, the extraction conditions are selected to promote the maximum extraction of the organic phase of the emulsion with the smallest loss of solute and encapsulating material by dissolution in supercritical CO_2 [7]. The advantage of SFEE over other precipitation techniques involving supercritical fluids is the correlation between the distribution of the diameter of the emulsion droplets and the final diameter distribution of the suspension particles. Therefore, it is possible to control the particle size in the suspension by varying parameters that directly influence the final size of the emulsion droplets during their formation [8].

Thus, the aim of this study was to evaluate the effect of variables of the emulsification process by ultrasound in the emulsion droplets diameter and the formation of suspensions of modified starch Hi-Cap 100® as coating material and surfactant, and oleoresin of *C. frutescens* as core material by SFEE.

MATERIAL AND METHODS

Chemicals

The coating material and surfactant used for the emulsion formation was Hi-Cap 100® (National Starch Food Innovation, Hamburg, Germany) modified starch. Ethyl acetate 99.5% (Dinâmica, SP, Brazil) was used as solvent in the SFEE process. For the extraction of oleoresin and the SFEE experiments, CO_2 with 99.9% purity (White Martins, Campinas, Brazil) was used. All the other solvents and chemicals were of analytical grade.

Oleoresin extraction

The oleoresin from *C. frutescens* used in the SFEE experiments was obtained according to Aguiar et al. [9], using supercritical CO_2 extraction at the condition of 15 MPa and 40 °C.

Emulsion formation

Twelve experiments of emulsion formation were performed, varying the parameters: Hi-Cap 100® concentration, emulsion oil/water ratio (O/W), and ultrasound power. The concentration of oleoresin in the oil phase was fixed at 20 mg/mL, the non-polar solvent was ethyl acetate, and ultrasound application time was 5 min. The parameters and their levels used to prepare the emulsions are shown in Table 1.

Table 1 Experimental conditions employed in the preparation of emulsions

Assay	[Hi-Cap 100®] (g/L)	Power (W)	O/W (v/v)	Hi-Cap 100®/Oleoresin	Creaming index (%)	Mean diameter (µm)
1	6	240	1/5	1.2	100	208.3±2.7
2	6	240	1/3	0.6	92	-
3	6	720	1/5	1.2	100	286.3±7.7
4	6	720	1/3	0.6	90	-
5	12	240	1/5	2.4	100	126.4±2.7
6	12	240	1/3	1.2	96	-
7	12	720	1/5	2.4	100	140.9±2.4
8	12	720	1/3	1.2	92	-
9	9	480	1/4	1.35	100	256.7±2.7
10	9	480	1/4	1.35	100	275.4±5.4
11	9	480	1/4	1.35	100	268.9±2.0

For each experiment, about 200 ml of emulsion were formed as described below: a) a solution of Hi-Cap 100® was prepared by dispersing the surfactant in deionized water (Milli-Q) with the aid of a magnetic stirrer, b) oleoresin was dissolved in ethyl acetate and the resulting solution was gradually added to the dispersion and stirred for 1 min, c) the raw dispersed emulsion (immersed in an ice bath in order to minimize the temperature increase) was subjected to ultrasonic probe and processed in ultrasonic power set for 5 minutes. 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.

To evaluate the stability, fresh prepared emulsions were transferred into cylindrical tubes (total volume of 25 mL), capped and stored at 25 °C for 90 min. After storage, emulsions had their stability evaluated in terms of creaming, by measuring the total height of the emulsion (H_e) and the height of the clear serum layer (H_s) that might have been formed due to emulsion creaming. Creaming index (CI) was determined using Equation 1.

$$CI = \left(\frac{H_s}{H_e}\right) \times 100 \quad (1)$$

Mean diameter of the emulsions was determined by light scattering (PCS). The instrument used was the Zeta Potential Analyser, (Brookhaven Instruments Corporation, USA), with a solid-state laser with a power of 15 mW and a wavelength of 675 nm. The effect of storage time on emulsion stability was investigated by measuring the droplet sizes at different times (5 to 20 min) with an interval of 1min. The emulsions which presented lower droplet size and higher stability were subjected to the SFEE experiments.

Supercritical Fluid Extraction of Emulsions (SFEE)

The experimental apparatus shown schematically in Figure 1 consists of a CO_2 supply system, an emulsion injection unit, and a high pressure stainless steel column. Briefly, CO_2, previously cooled in a thermostatic bath (MA184, Marconi, Campinas, Brazil) at -5 °C, was pressurized using a pneumatic pump (PP 111-VE MBR, Maximator, Nordhausen, Germany) and subsequently heated to operating temperature in a heating bath (MA184, Marconi, Campinas, Brazil). Then, CO_2 was injected into the high pressure column (712 mL of internal volume) with flow rate controlled by a heated micrometer valve coupled to a rotameter used as gas flow meter. The internal temperature of the column was maintained by using the heating bath. After temperature and pressure were stabilized, the emulsion was introduced using a HPLC pump (PU-2080, Jasco, Tokyo, Japan) through a coaxial nozzle with internal diameter of 127 µm. At column inlet previously saturated with supercritical CO_2, rapid diffusion occurs at the interface of the oil phase of the emulsion droplet and supercritical CO_2. After the injection of the emulsion, the system was kept under the same operation conditions to remove the residual organic solvent from the suspension. Afterwards, the column was slowly depressurized, the suspension was collected into glass bottles, and part of the collected volume was subjected to freeze-dried (L101-LioTop/LIOBRÁS, SP, Brazil) to obtain the dried suspension.

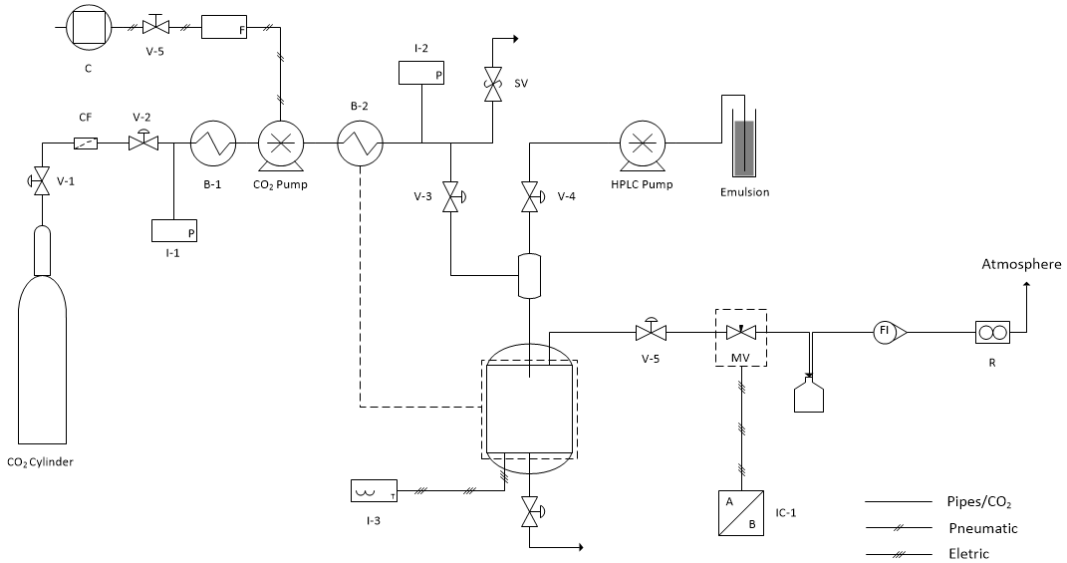

Figure 1 Schematic representation of the SFEE unit. V-1, V-2, V-3, V-4 , and V-5 – Control valves; MV – Micrometer valve; SV – Safety valve; C- Compressor; F- Compressed air filter; CF – CO_2 Filter; B1 –Cooling bath; B2 – Heating bath; I-1 e I-2 – Pressure indicators; I-3 – Temperature indicator; IC-1 – Indicators and controllers of temperature of micrometer valve, R – Gas totalizer; FL – Flow meter.

According to the literature, operating parameters such as pressure, temperature, processing time, and CO_2 and emulsion flow rates have little or no influence on the particle size of dispersions formed in SFEE [5, 8, 10]. The parameters that directly influence the size of particles on the suspension are the emulsion droplet size, concentration of solute in the feed solution, and the oil/water ratio of the emulsion (O/W) [11]. Thus, the operating pressure and temperature are selected to minimize the extraction of oil from the dispersed phase of the emulsion, and prevent further losses in the polymer phase and extract in supercritical CO_2. Information about the vapor-liquid equilibrium at high pressure (VLE) of the binary systems ethyl acetate/CO_2 may help to define the conditions under which the mixture is homogeneous, and maximize the

extraction of solvent from the emulsion [10]. A mixture of ethyl acetate/CO_2 presents critical pressure (at 38 ˚C) of 8.5 MPa, with molar fraction of CO_2 of 0.9 [12, 13]. At these operating conditions, water is only slightly soluble in supercritical CO_2 [14], while ethyl acetate is completely soluble. Consequently, the temperature conditions were set at 40 ˚C and pressure varied from 9 to 11 MPa. CO_2 flow rate (Q_{CO2}) was fixed at 22.5 g/min. The experimental conditions employed in SFEE are shown in Table 2.

Table 2 Experimental conditions employed in the SFEE experiments.

Emulsion	Pressure (MPa)	$Q_{emulsion}$ (mL/min)	Q_{CO2} (g/min)	% CO_2	Oleoresin loss (%)	Mean diameter (nm)
Exp 5	9	0.5	22.5	97.83	4.15	157.4±4.0
Exp 5	9	1	22.5	95.74	1.05	136.9±3.8
Exp 5	11	0.5	22.5	97.83	38.5	165.1±6.2
Exp 5	11	1	22.5	95.74	30.25	167.4±10.4
Exp 9	9	0.5	22.5	97.83	6.84	334.3±17.3
Exp 9	9	1	22.5	95.74	3.00	205.1±13.6
Exp 9	11	0.5	22.5	97.83	32.32	240.8±3.9

Particle morphology

The morphology of the material resulting from the freeze-drying process was analyzed using a scanning electron microscope equipped with a field emission gun (FESEM - FEI Quanta 650). 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, Campinas-SP, Brazil). Analyses of the sample surfaces were performed under vacuum, using a 5 kV acceleration voltage and a large number of images were obtained on different areas of the samples to assure the reproducibility of the results.

The mean droplet size of the emulsions was determined by the light scattering (PCS) technique, using a Zeta Potential Analyser, (Brookhaven Instruments Corporation, USA), with a solid-state laser (power of 15 mW and wavelength of 675 nm).

RESULTS AND DISCUSSION

The emulsions prepared with aqueous Hi-Cap 100® and capsicum pepper oleoresin solubilized in ethyl acetate showed high values of creaming index (CI, Table 1), which demonstrate the high stability of the system. According to the analysis of variance (ANOVA), none of the variables (Hi-Cap 100® concentration, oil/water ratio and ultrasound power) showed a significant effect on the creaming index. However, it was found that the emulsions prepared with the highest oil/water ratio (O/W = 1/3) had lower creaming indexes.

Initially, kinetic experiments were performed to determine the appropriate time for injecting the emulsion into the SFEE system, as a function of mean diameter of emulsion droplets. Figure 2 shows the values of the droplet size for each emulsion as function of time (5 to 20 min), as determined by light scattering. It can be seen that the mean diameter of the emulsion droplets increases in the first 5 minutes, becoming relatively stable after 10 minutes. Based on these results, it was determined that the time for injecting the emulsion into the SFEE system would be 10 min from the end of the

emulsification process. The mean diameter of the emulsion droplets was measured for those emulsions which had higher stability (CI = 100%).

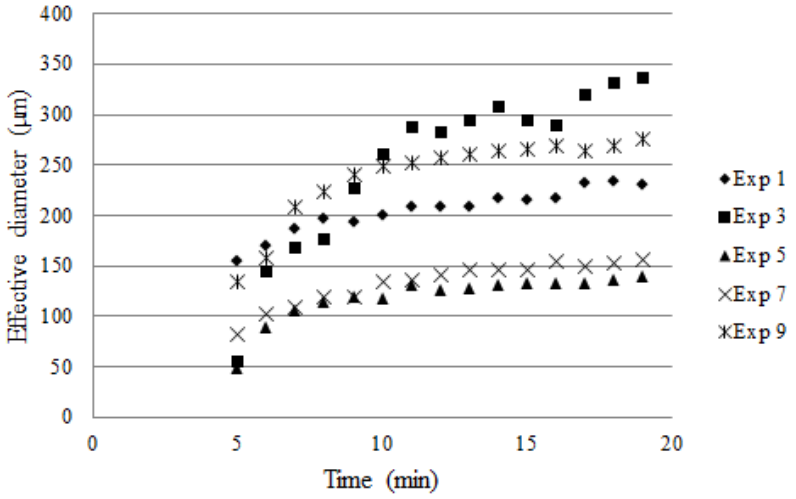

Figure 2 Mean diameter of emulsions as function of time.

The emulsions had mean droplet diameter (at 10 minutes) ranging from 126.4 to 286.3 nm for experiments 5 and 3, respectively. Smaller droplets were observed for the highest concentration of Hi-Cap 100® in aqueous solution (12 g/L), and consequently at the greatest Hi-Cap 100®/oleoresin ratio. Figure 3 shows the diameter distribution of the emulsion droplets, where the formation of emulsions with monomodal size distribution can be observed.

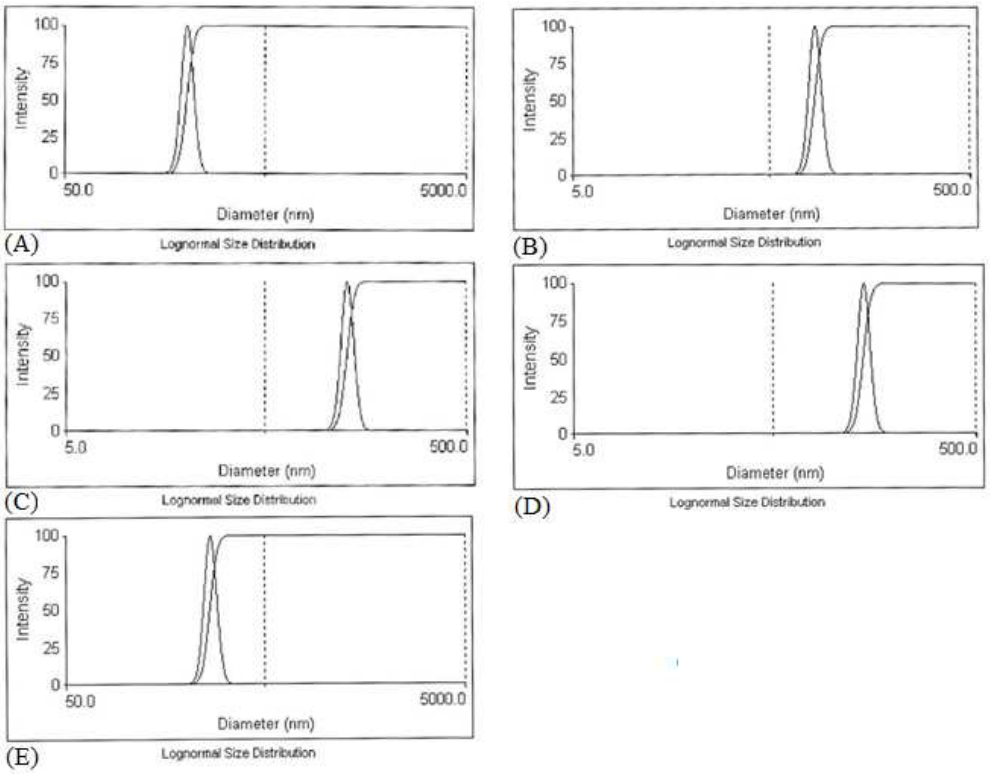

Figure 3 Distribution of droplet sizes of emulsions: (A) Exp.1, (B) Exp. 3, (C) Exp. 5, (D) Exp. 7 and (E) Exp. 9.

To perform the SFEE experiments, the emulsions resulting from experiments 5 (since it has the lowest droplet size and ultrasound power) and 9 (due to the higher concentration of oleoresin in the emulsion) were selected.

During the SFEE experiments, a loss of oleoresin was visually observed in the collection flask due to its dissolution in the supercritical CO_2. These losses were quantified and are presented in Table 2, where it can be seen that the higher the process pressure, the greater the loss of oleoresin. The experiments performed at 9 MPa resulted in a loss of oleoresin ranging from 1.05 to 6.84%, while at 11 MPa the losses varied from 38.5 to 30.25% of the initial mass of oleoresin injected into the system. Once the oleoresin used in the experiments was extracted with CO_2 at 15 MPa and 40 °C, it was expected that CO_2 could solubilize part of the extract present in the emulsions at pressures above 10 MPa.

Regarding the average diameter of the particles in the suspensions, it was found that SFEE did not alter significantly the size of emulsion droplets initially fed. This observation agrees with previous reports from Mezzomo et al. [15], while processing emulsions of carotenoids from pink shrimp residue solubilized in ethyl acetate with aqueous solution Hi-Cap 100® by SFEE technique.

In the micrographs of freeze-dried suspensions (Figure 4, left), one can note the presence of a large amorphous mass of polymer, possibly formed during the freeze-drying process by starch agglomeration. Micrometric particles (5 to 20 um) distributed over this mass can be noticed, as indicated by the arrows on Figure 4 (left). An amplification on the particle surface is shown on Figure 4 (right), revealing their spherical geometry and continuous surface. A similar result was obtained by Mattea et al. [8] during the encapsulation of betacarotene solubilized in ethyl acetate in aqueous solution of Hi-Cap 100® by freeze-drying of suspensions. These authors identify large needle type particles formed during the freeze drying step together with small spherical particles formed during the antisolvent precipitation process.

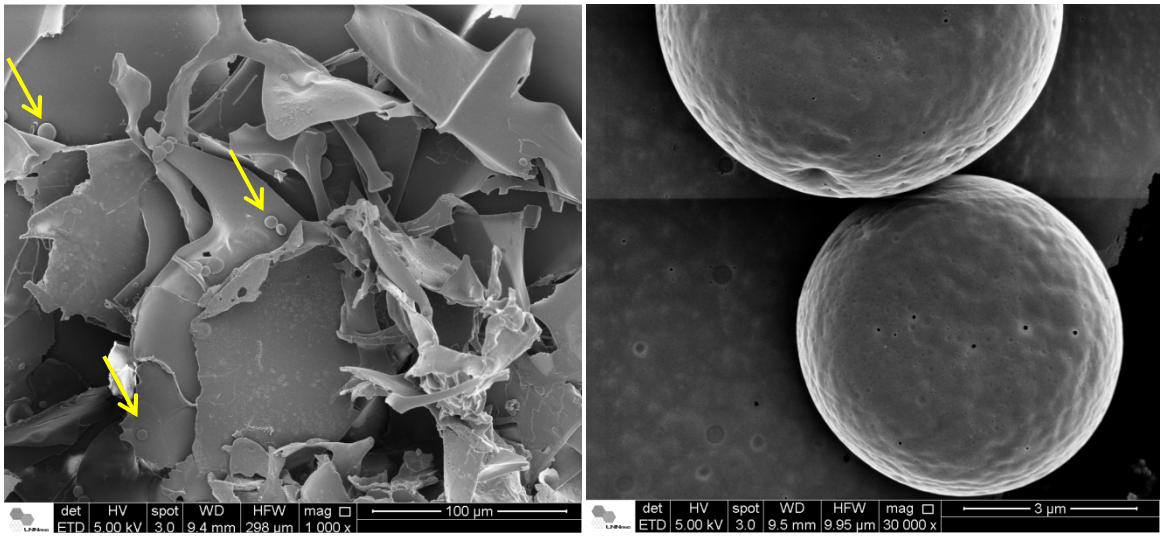

Figure 4 FESEM micrographs of the extract loaded microparticles - SFEE conditions: 11 MPa, 40 °C and 0.5 mL/min emulsion flow rate: (A) Amorphous plates formed by starch agglomeration (bar scale 100 µm) 1000x magnitude and (B) 30000x magnitude.

CONCLUSIONS

The process of ultrasound emulsification used in this work was able to produce stable emulsions, whose oil phase comprises the oleoresin of capsicum peppers solubilized in

ethyl acetate in the presence of the Hi-Cap 100® modified starch, dispersed in aqueous solution. It was observed that increasing the oil/water ratio slightly decreases the emulsion stability, and an increase in the concentration of Hi-Cap 100® in the aqueous phase causes a considerable decrease in the size of droplets dispersed in the system. During the processing of the emulsion by SFEE, there is a considerable loss by dissolution of oleoresin in supercritical CO_2. The process did not influence the resulting size of the droplets dispersed in the suspensions. The FESEM micrographies showed small spherical particles dispersed in an amorphous mass of starch, which may represent the original particles after their growth due to the freeze-drying process.

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