J. of Supercritical Fluids 112 (2016) 37–43

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Encapsulation of pepper oleoresin by supercritical fluid extraction of emulsions

Ana Carolina de Aguiar[a,b], Luiz Paulo Sales Silva[a,c], Camila Alves de Rezende[b], Gerardo Fernandez Barbero[d], Julian Martínez[a,*]

[a] College of Food Engineering, University of Campinas, UNICAMP, DEA/FEA, Campinas 13083-862, SP, Brazil
[b] Institute of Chemistry, University of Campinas, UNICAMP, Campinas 13083-862, SP, Brazil
[c] UCP, ENSTA ParisTech, Université Paris Saclay, 828 Boulevad des Maréchaux, Palaiseau, Paris 91762, France
[d] Department of Analytical Chemistry, Faculty of Sciences, University of Cádiz, Agrifood Campus of International Excellence (CeiA3), PO Box 40, Puerto Real 11510, Cádiz, Spain

ARTICLE INFO

Article history:
Received 15 December 2015
Received in revised form 12 February 2016
Accepted 16 February 2016
Available online 18 February 2016

Keywords:
Supercritical fluids
Supercritical fluid extraction of emulsions
Ultrasound emulsification
Capsicum peppers
Particle formation

ABSTRACT

Capsaicinoids, which are the responsible for the pungency of peppers, have strong pharmacological effects. The encapsulation of capsaicinoids can be an alternative for its industrial application. The aim of this work was to evaluate the effect of various ultrasound emulsification conditions, such as surfactant concentration, oil/water ratio, and ultrasound power on the emulsion droplet size. Emulsions formed by Hi-Cap 100 and oleoresin of *Capsicum frutescens* pepper were then applied in a SFEE process. Ultrasound emulsification resulted in high emulsification efficiency and stability. The selected time for emulsion injection into the SFEE system was 10 min after its preparation, based on the coalescence kinetics. The SFEE process resulted in a considerable loss of oleoresin by dissolution in the supercritical CO_2 and promoted a droplet volume expansion, reflected by the increase in the diameter of the droplets in suspension. The formation of emulsions by ultrasound emulsification in the evaluated conditions showed promising results, but more studies are required to improve the SFEE process.

1. 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 characteristic of many peppers [3–5]. Capsaicinoids have also strong pharmacological effects, which may be used in pain relief, cancer prevention, and weight reduction, besides providing gastrointestinal and cardiovascular benefits [6,7].

The encapsulation of capsaicinoids in polymer matrices can be an alternative for the application of these compounds as pharmaceuticals and food ingredients, since the spiciness is a limiting factor for their use [8]. Many encapsulation techniques of Capsicum oleoresin and capsaicinoids are reported, most of them aiming pharmaceutical application of the resulting particles, films, emulsions or suspensions [9].

The process of particle formulation known as Supercritical Fluid Extraction of Emulsions (SFEE) combines conventional emulsion techniques with the unique properties of supercritical fluids for the production of micro and nanoparticles [10]. In the SFEE process, the extraction conditions are selected to promote the maximum extraction of the organic solvent from the emulsion with the smallest loss of solute and encapsulating material by dissolution in supercritical CO_2 [10,11]. The advantage of SFEE over other precipitation techniques involving supercritical fluids is the correlation between the distribution of the diameter in emulsion droplets and the final distribution of diameters in the particle suspension. Therefore, it is possible to control the particle size in the final suspension by varying parameters that directly influence the final size of the emulsion droplets during their formation [11]. This technique has been successfully applied in various fields such as the production of bactericidal nanocomposites of titanium dioxide in PLA [12], encapsulation of food constituents as quercetin [13] and carotenoids [11,14], production of stimuli-responsive drug delivery systems [15], among others.

Ultrasound emulsification is classified as a method of high energy and allows obtaining emulsions with droplet size in the nanometer range and low polydispersity [16]. Basically, the method

* Corresponding author. Tel.: +55 19 35214046; fax: +55 19 35214027.
E-mail address: julian@unicamp.br (J. Martínez).

http://dx.doi.org/10.1016/j.supflu.2016.02.009
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consists in the use of ultrasound waves with frequency above 20 kHz provided by a probe, resulting in mechanical vibration followed by the formation of acoustic cavitation. The collapse of the formed bubbles generates shock waves that lead to the disruption of the droplets of the crude emulsion [17]. In this process, the droplet size can be controlled by the optimization of the following parameters: oil concentration in the emulsion, viscosity of the continuous phase, emulsification time and ultrasound power [18].

To the best of our knowledge, there are no publications dealing with the use of ultrasound emulsification integrated to high pressure techniques to produce Capsicum oleoresin or capsaicinoids capsules. Therefore, the aim of this work was to evaluate the effect of variables of the ultrasound emulsification in the emulsion formed by modified starch Hi-Cap 100, which is used as coating material and surfactant, and the oleoresin of *Capsicum frutescens* pepper, used as core material in the droplets. Besides this, the emulsification efficiency was evaluated and the emulsion prepared under one of the conditions tested in this work was applied in a SFEE process.

2. Material and methods

2.1. Chemicals

The coating material and surfactant used for the emulsion formulations was Hi-Cap 100 modified starch (National Starch Food Innovation, Hamburg, Germany). Ethyl acetate 99.5% (Dinâmica, SP, Brazil) was used as solvent in the SFEE process. Carbon dioxide (CO_2) with 99.9% purity (White Martins, Campinas, Brazil) was used for the extraction of oleoresin of *C. frutescens* and as antisolvent in the SFEE experiments. For the chromatography analyses, capsaicin (C) and dihydrocapsaicin (DHC) standards were purchased from Cayman Chemical (Cayman Chemical, USA. Purity > 95%). All the other solvents and chemicals were of analytical grade.

2.2. Oleoresin extraction

The oleoresin from dried *C. frutescens* fruits used in the SFEE experiments was obtained according to Aguiar et al. [19], using supercritical CO_2 extraction (SFE) at 15 MPa, 40 °C, CO_2 flow rate of 1.98×10^{-4} kg/s and solvent to feed mass ratio (S/F) of 950 kg CO_2/kg dried pepper. The extraction yields were 41.8 g oleoresin/kg dried pepper and 4.6 g total capsaicinoids/kg dried pepper.

2.3. Ultrasound emulsification

An experimental design consisting of twelve experiments of emulsion formation by ultrasound emulsification was proposed in order to evaluate the effect of Hi-Cap 100 concentration (6 g/L, 9 g/L, 12 g/L), oil/water ratio (O/W v/v) in the emulsion (1/5, 1/4, 1/3), and ultrasound power (240 W, 480 W, 720 W) on the emulsification efficiency and the effective hydrodynamic diameter (EHD) of the emulsion droplets. The variables and their levels used to prepare the emulsions are shown in Table 1. The concentration of oleoresin in the oil phase was fixed at 20 mg/mL, based on preliminary experiments of emulsion stability. The non-polar solvent was ethyl acetate and the ultrasound application time was 5 min.

Hi-Cap 100, a modified food starch, was selected since it simultaneously plays the roles of coating material and emulsifier. For each experiment, about 200 mL of emulsion were formed as follows: (a) a solution of Hi-Cap 100 was prepared by dispersion in deionized water (Milli-Q) with the aid of a magnetic stirrer; (b) the oleoresin extracted by SFE was dissolved in ethyl acetate and the resulting solution was slowly added to the dispersion and stirred for 1 min; (c) the crude dispersed emulsion (immersed in an ice bath to minimize the temperature increase resulting from high energy input of the ultrasound emulsification) was subjected to the ultrasonic probe and processed in a ultrasonic power set for 5 min. 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, as shown in Fig. 1.

Table 1
Experimental conditions used in ultrasound emulsification.

Exp	[Hi-Cap 100] (g/L)	US Power (W)	O/W (v/v)	Hi-Cap 100/Oleoresin (g/g)
1	6	240	1/5	1.2
2	6	240	1/3	0.6
3	6	720	1/5	1.2
4	6	720	1/3	0.6
5	12	240	1/5	2.4
6	12	240	1/3	1.2
7	12	720	1/5	2.4
8	12	720	1/3	1.2
9	9	480	1/4	1.35
10	9	480	1/4	1.35
11	9	480	1/4	1.35

2.4. Evaluation of emulsion stability

The stability of the emulsions was evaluated by transferring 25 mL of the freshly prepared emulsions (immediately after preparation) to cylindrical tubes, capped and stored at 25 °C for 90 min. After 90 min the emulsions had their stability evaluated in terms of emulsification efficiency (E%), which is the ratio between the volume of the emulsified dispersed phase (V_e) (read 90 min after preparation) and the initial volume of the dispersed phase (V_0), calculated with the following equation [20]:

$$E\% \left(\frac{V_e}{V_0}\right) \times 100 \quad (1)$$

2.5. Effective hydrodynamic diameter (EHD) of emulsions and suspensions

The effective hydrodynamic diameter of the emulsions and suspensions was determined by light scattering (PCS) using a Zeta Potential Analyzer, (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 the emulsion stability was investigated by measuring the droplet sizes at different times (5 to 20 min) with intervals of 1 min. The emulsions that presented lower droplet size and higher stability were used in the SFEE experiments.

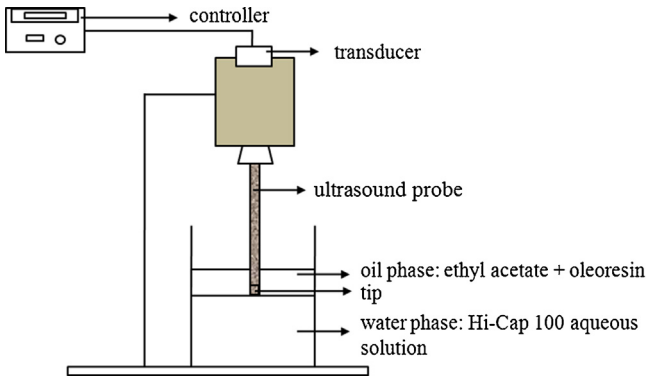

Fig. 1. Schematic representation of the ultrasound emulsification system.

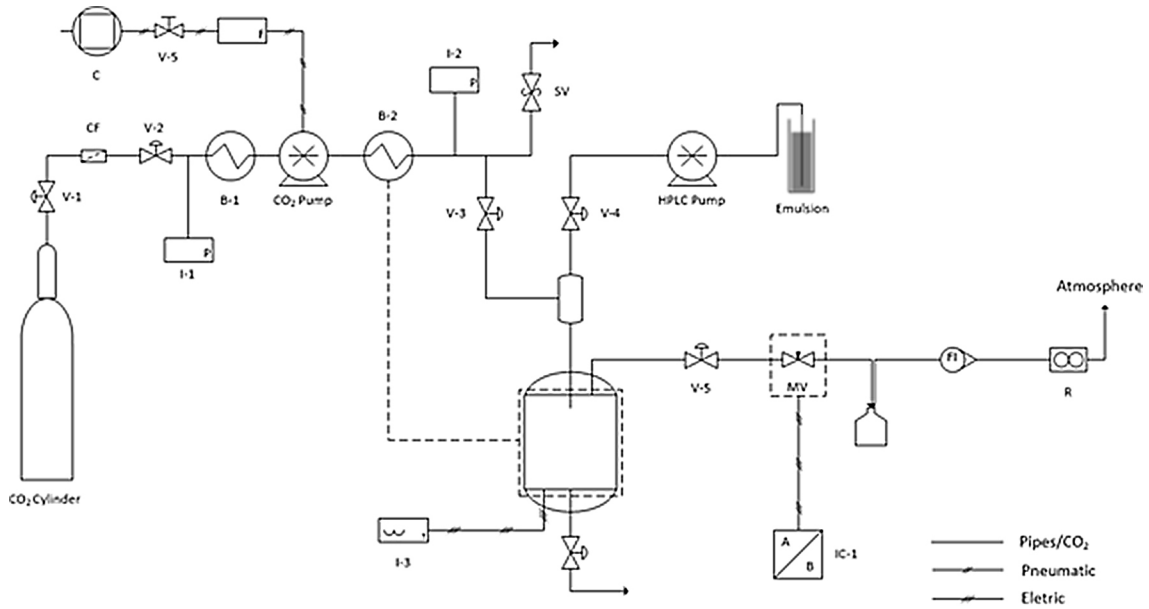

Fig. 2. Schematic representation of the SFEE unit.V-1, V-2, V-3, V-4, and V-5—Control valves; MV—Micrometer valve; SV—Safety valve; C- Compressor; F—Compressed air filter; CF–CO_2 Filter; B1—Cooling bath; B2—Heating bath; I-1 e I-2—Pressure indicators; I-3—Temperature indicator; IC-1—Indicators and controllers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

2.6. Supercritical fluid extraction of emulsions (SFEE)

The experimental SFEE apparatus shown schematically in Fig. 2 consists of a CO_2 supply system, an emulsion injection unit and a high pressure stainless steel column (712 mL of internal volume). 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 at the supercritical state was injected into the high pressure column 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 kept constant 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 the 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 for 30 min 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 material was subjected to freeze–drying (L101-LioTop/LIOBRÁS, SP, Brazil) to obtain the dried suspension.

According to the literature, operating parameters such as pressure, temperature, processing time, CO_2 and emulsion flow rate have little or no influence on the particle size of dispersions formed by SFEE [10,11,21]. The parameters that mostly influence the particle size of the suspension are the emulsion droplet size, concentration of solute in the feed solution, and the oil/water ratio of the emulsion (O/W) [22]. 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 defining the conditions where the mixture is homogeneous, and maximizing the extraction of solvent from the emulsion [21]. A mixture of ethyl acetate/CO_2 at 38 °C has critical pressure of 8.5 MPa, with molar fraction of CO_2 of 0.9 [21,23]. At these operating conditions, water is only slightly soluble in supercritical CO_2 [24], while ethyl acetate is completely soluble. Therefore, the SFEE temperature was set at 40 °C and pressure varied from 9 to 11 MPa. CO_2 flow rate $\left(Q_{CO_2}\right)$ was fixed at 22.5 g/min based on previous experiments and equipment limitation. The experimental conditions employed in the SFEE experiments are shown on Table 2. The effective hydrodynamic diameter of the suspensions was determined as described in Section 2.5.

2.7. Particle morphology

The morphology of the freeze-dried suspensions 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. A large number of images were obtained on different areas of the samples to assure the reproducibility of the results.

Table 2
Experimental conditions of the SFEE experiments.

Exp	Pressure (MPa)	[a]Q_{emu} (mL/min)	[b]Q_{CO_2} (g/min)	[c]%CO_2
1	9	0.5	22.5	97.83
2	9	1	22.5	95.74
3	11	0.5	22.5	97.83
4	11	1	22.5	95.74

[a] Q_{emu}: emulsion flow rate (mL/min).
[b] Q_{CO_2}: carbon dioxide flow rate (g/mL).
[c] %CO_2: carbon dioxide mass percentage in the supercritical CO_2 + emulsion mixture.

2.8. Total capsaicinoid content of the suspensions

The capsaicinoids were extracted from the suspensions produced by SFEE with methanol according to the method proposed by Barbero et al. [25], which consists in adding 1 g of suspension to 25 mL methanol and subject the mixture to an ultrasonic bath for 15 min. After extraction, the mixture was filtered on millex PVDF 0.20 μm (Millipore) before chromatographic analysis.

The analysis of capsaicinoids was performed using a U-HPLC-DAD-MS/MS Thermo LCQ Fleet system (Thermo Fisher, San Jose, CA, USA). The column used was a Hypersil Gold C18 (1.9 μm, 3 mm i.d. × 100 mm L) (Thermo Scientific, Waltham, MA, USA). An isocratic mobile phase (0.5 mL/min) consisting of water and acetonitrile (4:6, v/v) was used and the column temperature was set at 30 °C. The mass spectrometer was equipped with an APCI source in positive mode of ionization working with vaporizer temperature set at 300 °C, sheath gas pressure at 50 (arbitrary unit), auxiliary gas pressure at 5 (arbitrary unit), corona voltage of 6 kV and ion trap detection system operating in selected monitoring mode for ions *m/z* 80–310 and the fragments for each capsaicinoid. Data handling was performed with Xcalibur software package. The identification was based by the relative retention time to standards and comparing the mass spectrum between standards, literature data and samples. Quantification was performed in DAD (280 nm) by the external standards calibration of C and DHC through calibration curves obtained from the standard solutions. The curves (C and DHC) were prepared in real triplicates.

2.9. Retention percentage of total capsaicinoids (%CR)

The retention percentage of total capsaicinoids was defined as the ratio between the real concentration of capsaicinoids in the suspension (C_r) (determined by U-HPLC-MS—Section 2.8) and the theoretical concentration of capsaicinoids in the suspension (C_t), which was defined assuming that SFEE results in a suspension free from residual solvent and there are not capsaicinoids losses during the process, calculated according to the following equation:

$$\%CR = \frac{C_r}{C_t} \times 100 \quad (2)$$

2.10. Residual content of ethyl acetate in suspensions

The suspensions were filtered on millex PVDF 0.20 μm (Millipore) and injected in a gas chromatograph system Thermo Fisher Scientific (Thermo Fisher, San Jose, CA, USA), equipped with an autosampler AI 1310 (Thermo Scientific, Waltham, MA, EUA), a gas chromatograph Focus GC (Thermo Scientific, Waltham, MA, EUA), DB-Wax column (60 m × 0.25 mm, 0.25 μm particle size) (J&W Scientific, Agilent Technologies, Santa Clara, CA, EUA), mass detector DSQ II (Thermo Scientific, Waltham, MA, EUA), and the control and data acquisition software Xcalibur TM 2.0.7.

The analyses were performed in duplicate by direct injection of the sample. The operating conditions were 1 mL sample with 100 μL of internal standard, injection volume of 1 μL, injection in split mode with 2 min splitless and He gas flow rate at constant pressure of 65 kPa. Molecule ionization was achieved by electron impact (EI), with temperature source of 250 °C and detection of positive ions in full scan mode in the range of 28–200 *m/z*. Quantification was performed considering the relative peak area of ethyl acetate *m/z* 45 with respect to the area of the internal standard *m/z* 70. Results are expressed as ppm of ethyl acetate in the suspension.

Table 3
Emulsification efficiency (*E*%), effective hydrodynamic diameter (EHD; nm) and polydispersity of the emulsions formed by ultrasound emulsification process.

Exp	Hi-Cap 100/Oleoresin	[a]*E*%	[b]EHD (nm)	Polydispersity
1	1.2	100	208 ± 3	0.005
2	0.6	92	–	–
3	1.2	100	286 ± 8	0.005
4	0.6	90	–	–
5	2.4	100	126 ± 3	0.005
6	1.2	96	–	–
7	2.4	100	141 ± 2	0.005
8	1.2	92	–	–
9	1.35	100	257 ± 3	0.005
10	1.35	100	275 ± 5	0.005
11	1.35	100	269 ± 2	0.005

[a] *E*%: Emulsification efficiency (measured 90 min after emulsion preparation).
[b] EHD: effective hydrodynamic diameter (measured 10 min after emulsion preparation).

3. Results and discussion

3.1. Emulsions formation

The emulsions prepared with aqueous Hi-Cap 100 and Capsicum pepper oleoresin solubilized in ethyl acetate showed high emulsification efficiencies (*E*%), which demonstrate the high stability of the system until 90 min after preparation, as shown on Table 3. According to the analysis of variance (ANOVA), none of the evaluated variables (Hi-Cap 100 concentration, oil/water ratio and ultrasound power) showed a significant effect on the emulsification efficiency. However, the emulsions prepared with the highest oil/water ratio (O/W = 1/3) had lower values of emulsification efficiencies.

The effective hydrodynamic diameter of the emulsions was determined for the emulsions that showed higher stability (*E*% = 100%). These emulsions had effective hydrodynamic diameters (at 10 min) ranging from 126 to 286 nm for experiments 5 and 3, respectively (Table 3). Smaller droplets were observed for the highest concentration of Hi-Cap 100 in aqueous solution (12 g/L), and at the greatest Hi-Cap 100/oleoresin ratio. According to Capote and Castro [20], the surfactant concentration in the emulsion is one of the parameters that influence the ultrasound emulsification process. Generally, the emulsification efficiency increases with the surfactant concentration in the medium, since the coalescence of the emulsion droplets is hindered under this condition. Ionic surfactants can act as an electrostatic barrier against droplets approach, while nonionic surfactants prevent coalescence due to steric hindrance [26].

The polydispersity of the emulsions formed by the ultrasound process was low (0.005), indicating that the effective hydrodynamic diameter of the emulsion droplets shows little variation, resulting in a homogeneous mono modal distribution, as can be noted in Fig. 3.

Experiments on kinetic stability were initially performed to determine the appropriate time for injecting the emulsion into the SFEE system, as a function of the effective hydrodynamic diameter of the emulsion droplet. Fig. 4 shows the effective hydrodynamic diameters of each emulsion as a function of time (5 to 20 min), as determined by light scattering. It can be observed that the diameter of emulsion droplets increases in the first 5 min, and become relatively stable after 10 min. This behavior is typical of emulsions and dispersions with polydisperse droplets, where the small droplets tend to coalesce forming droplets of larger diameters, thus homogenizing the distribution of diameters (Ostwald ripening) diameter [26].

Based on these results, the chosen time for injecting the emulsion into the SFEE system was 10 min after the emulsification process, to ensure the relative stability of the emulsion. This

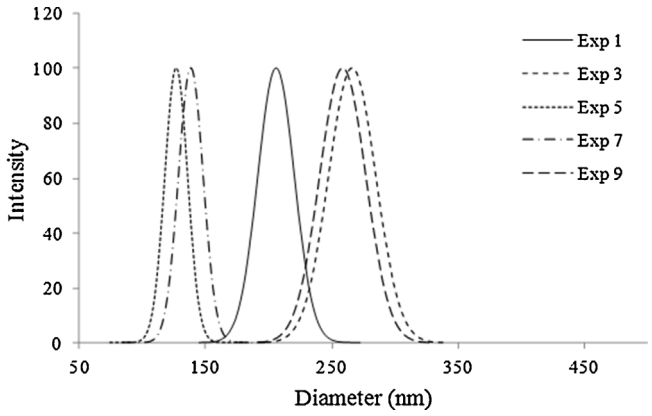

Fig. 3. Distribution of effective hydrodynamic diameter of emulsion droplets.

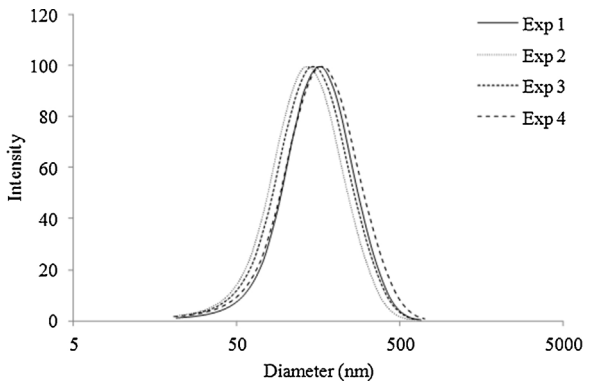

Fig. 5. Distribution of effective hydrodynamic diameter of suspension particles produced by SFEE.

condition should assure that, during the SFEE process, the emulsion will be kinetically stable. The effect of supercritical CO_2 on the stability of emulsions during the SFEE process was evaluated by Chattopadhyay, Huff and Shekunov [10], who concluded that the high pressure and the supercritical CO_2 do not modify the thermodynamic properties of the emulsion, which would lead to coalescence of the droplets and phase separation in the resulting suspension.

3.2. SFEE experiments

The emulsion resulting from experiment 5 (Hi-Cap 100 concentration = 12 g/L, ultrasound power = 240 W and O/W = 1/5 v/v) was selected to perform the SFEE experiments, since it achieved the lowest effective hydrodynamic diameter with a minimum ultrasound power, thus demanding the lowest energy consumption.

During the SFEE experiments, some 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 on Table 4, where it can be observed that the loss of oleoresin increases with the process pressure. The experiments performed at 9 MPa resulted in a loss of oleoresin ranging from 1.05% to 4.15%, while in the experiments carried out at 11 MPa, the losses varied from 30.25% to 38.5% 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 would solubilize part of the extract present in the emulsions at pressures above 10 MPa. Another relevant factor that could lead to the loss of oleoresin is the presence of ethyl acetate together with the supercritical CO_2 in the SFEE medium. In this case, the ethyl acetate may act as a cosolvent in the oleoresin extraction process during SFEE.

As observed on Table 4, the diameters of the suspension droplets lie below 300 nm. The definition proposed by Campardelli et al. [27] consider nanoparticle diameter to a maximum of 250 nm, since this dimension can represent a good comprise between the fundamental researchers that frequently set nano-objects to a maximum dimension of 100 nm and to the concept used in pharmaceutical and medical applications, where the term "nanometric" is applied to a more extended range. Therefore, the particles present in the suspensions formed by SFEE can be considered as nanoparticles.

As expected, regarding the effective hydrodynamic diameter of the particles in the suspension, the SFEE process promoted a droplet volume expansion, reflected by the slight increase in the diameter of the droplets in suspension. The initial effective hydrodynamic diameter of the emulsion fed into the SFEE system (result of the experiment 5—Table 3) increased from 126 nm to 137 nm or to 167 nm, after being subjected to SFEE, considering the experiments carried out at P = 9 MPa; Q_{emul} = 1 mL/min (Exp 2) and P = 11 MPa; Q_{emul} = 1 mL/min (Exp 4), respectively. These observations are in agreement with previous reports of Della Porta and Reverchon

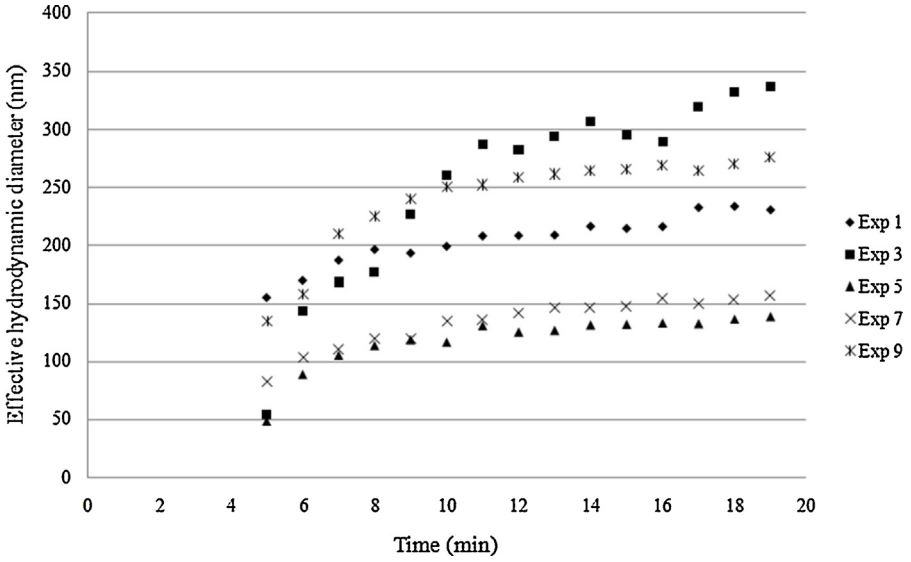

Fig. 4. Effective hydrodynamic diameter of emulsion droplets as function of time.

Table 4
Experimental conditions and results obtained from the SFEE experiments.

Exp	Pressure (MPa)	[a] Q_{emul} (mL/min)	Oleoresin loss (%)	[b] EHD (nm)	[c] C_r (mgcap/g)	Polydispersity	[d] EA (ppm)	[e] %CR
1	9	0.5	4.15	163 ± 6	0.177 ± 0.002	0.238	24,100	39.73
2	9	1	1.05	137 ± 3	0.178 ± 0.001	0.259	20,500	39.98
3	11	0.5	38.5	152 ± 13	0.096 ± 0.001	0.271	5900	21.53
4	11	1	30.25	167 ± 4	0.152 ± 0.001	0.289	11,500	34.31

[a] Q_{emul}: emulsion flow rate.
[b] EHD: effective hydrodinamic diameter.
[c] C_r: real concentration of capsaicinoids in the suspension (mg capsaicinoids/g suspension).
[d] EA: residual content of ethyl acetate (ppm).
[e] CR: retention percentage of total capsaicinoids.

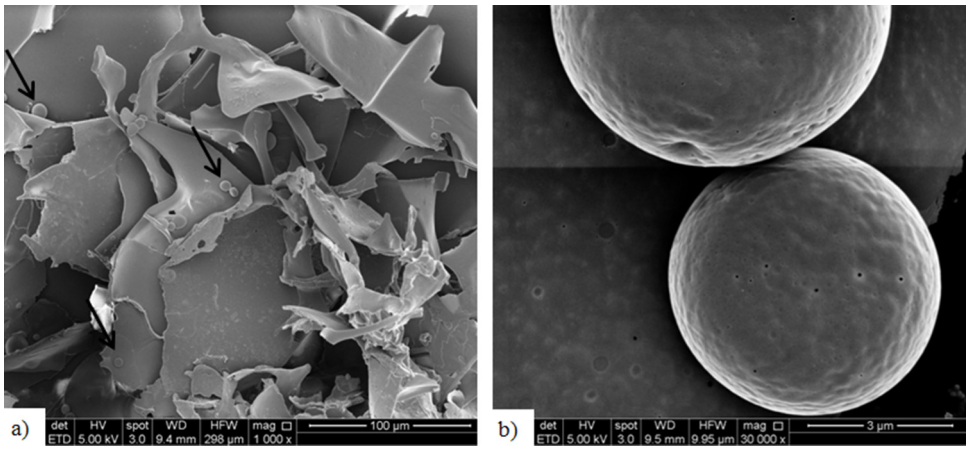

Fig. 6. FESEM images of the extract loaded microparticles. SFEE conditions of 11 MPa, 40 °C and 0.5 mL/min emulsion flow rate: (a) amorphous plates formed by starch agglomeration, with spherical micrometric particles indicated by the arrow (bar scale 100 μm) and (b) amplification in the microparticle surface (bar scale 3 μm).

[21] for the SFEE process applied to emulsions of PLGA/piroxicam and Mezzomo et al. [14], who evaluated the emulsification of pink shrimp waste solubilized in ethyl acetate with aqueous Hi-Cap 100 by SFEE, which justifies the ability to control the final particle size by modification of the initial drop size, claimed for the SFEE process [28]. As can be observed from the effective hydrodynamic diameter curves shown in Fig. 5, the emulsion prepared in the experiment 5, which initially showed a monomodal distribution, maintained this characteristic after the SFEE process. However, there was an increase in the polydispersity of the injected emulsion to the final dispersion in CO_2 (Table 4), indicating that there was a variation in the effective hydrodynamic diameter range of the particles.

Regarding the capsaicinoid retention, the suspensions obtained at lower pressure (9 MPa) showed the highest percentages, achieving a maximum of 39.98% at 9 MPa and Q_{emul} = 1.0 mL/min (Exp 2—Table 4). The low retention of capsaicinoids observed are assigned to the loss of oleoresin in the SFEE process, which lead to the loss of the capsaicinoids initially contained in the emulsions. Capsaicinoids have high solubility in supercritical CO_2, and thus they can be extracted in the early stages of the SFE process from a solid matrix [19]. This observation corroborates the fact that the high extraction rate of capsaicinoids at the initial minutes negatively influenced the retention of these compounds during SFEE.

In general, it is observed that the smaller the oleoresin loss, the greater the residual concentration of ethyl acetate in the suspension (Table 4). This indicates that, although some evaluated conditions showed low oleoresin losses during the SFEE process (experiment 2, Table 4), the supercritical CO_2 was also not able to remove adequately the solvent from the liquid phase of particles suspensions under these conditions. The high solubility of ethyl acetate in water (8.3 g/mL at 20 °C) must also be considered here, since it hardens the removal of the ethyl acetate from the liquid phase of particle suspensions by CO_2 due to mass transfer and phase equilibrium limitations. Although ethyl acetate is a Class 3 solvent, which can be considered as low toxic and of lower risk to human health (exposure limit of 5000 ppm per day) [29], its residual concentration in the obtained suspensions (5900 to 24,100 ppm) are far above those usually verified with a SFEE process. Thus, an exhaustive washing of the particles and their subsequently separation from the liquid phase of the suspension would be necessary to obtain the residual ethyl acetate content in the particles.

In the micrographs of the freeze-dried suspensions formed at 11 MPa, 40 °C and 0.5 mL/min of emulsion flow rate (Fig. 6a), one can note the presence of a large amorphous polymer mass, possibly formed during the freeze-drying process by starch agglomeration. Micrometric particles (5 to 20 μm) distributed all over the polymer mass can be observed, as indicated by the arrows in Fig. 6a. An amplification on the particle surface is shown in Fig. 6b, revealing their spherical geometry and continuous surface. A similar result was reported by Mattea et al. [11], as observed in scanning electron microscopy of freeze-dried samples obtained by encapsulation of β-carotene solubilized in dicloromethane and buffer aqueous solution of Hi-Cap 100. These authors identified large needle type particles formed during the freeze drying step, together with small spherical particles formed during the antisolvent precipitation process.

4. Conclusions

The application of ultrasound resulted in high emulsification efficiency and the produced emulsions showed high stability. The increase of the oil/water ratio slightly decreased the emulsification efficiency, and an increase in the concentration of Hi-Cap 100 in the aqueous phase promoted a considerable decrease in the effective hydrodynamic diameter of the emulsions. During the processing of

the emulsion by SFEE, there was a considerable loss of oleoresin by dissolution in the supercritical CO_2. The SFEE process promoted droplet volume expansion, reflected by the increase in the diameter of the droplets in suspension. The FESEM images of the dried suspensions 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. Additionally, an alternative to the use of toxic solvents would be the use of GRAS solvents, e.g. edible oils, in the emulsion oil phase. The adoption of these substrates would make the process environmental friendly and would reduce the exhaustive consumption of CO_2 to the removal of organic solvents at ppm levels in the suspensions formed.

Acknowledgements

The authors wish to thank CAPES (2952/2011), CNPq (Project 473342/2011-1) and FAPESP (Projects 2011/08656-7, 2012/22119-7 and 2013/02203-6) for the financial support and the LME/LNNano/CNPEM for the technical support during the electron microscopy work.

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