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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

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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).

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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.

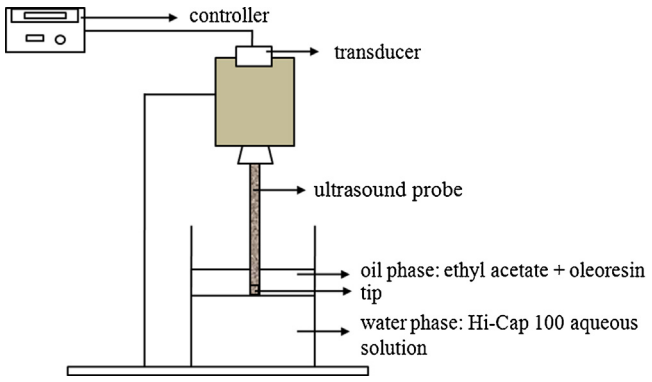

Fig. 1. Schematic representation of the ultrasound emulsification system.