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Encapsulation of Laurel Leaves Essential Oil (*Laurus Nobilis* L.) by Supercritical Fluid Extraction Of Emulsion (SFEE)

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ABSTRACT: The laurel leaves essential oil has bioactive properties. Encapsulation of a lipophilic compound in a hydrophilic encapsulating agent using supercritical fluids is a green alternative to limit the degradation or loss of the active principle. The objectives of this work are evaluated the stability of the emulsions, by of the hydrodynamic size of the emulsion droplets, and the effects laurel essential oil concentration and droplet size in efficiency of the encapsulation. Stable miniemulsions were obtained during 24 h. The increase concentrations of the essential oil and small droplet size favored the encapsulation efficiency, varying from 48.8 to 75.5%. There were the encapsulations of laurel leaves essential oil in modified starch.

INTRODUCTION

The laurel leaves essential oil has bioactive properties responsible for the preservation of food deterioration, maintenance of red color meat, pH control in meat, antibacterial and antimicrobial action, antifungal action, microsporicidal and acaricidal action, antioxidant action, besides food applications [1–6].

Encapsulation of a lipophilic compound, such as laurel leaves essential oil, in a hydrophilic encapsulating agent has the main advantage of make it disposable, facilitating its application by the food, pharmaceutical and chemical industries. So stability is an important criterion for preserving the properties of *flavor* materials, the encapsulation comes with an alternative of limiting the degradation or loss of the aroma during the processing steps that proceed its formulation or consumption[7].

Processes using supercritical fluids, such the Supercritical Fluid Extraction of Emulsions (SFEE), emerge as an alternative to the encapsulation of natural substances due to the use of environmentally safe solvents, especially CO_2, which is widely used and has low cost [8–10]. The SFEE process can be understood in two steps: the formation of the emulsion and the removal of the organic solvent producing the encapsulation, forming a suspension.

Ultrasound formation of emulsion occurs due to cavitation. During this process, bubbles can collapse near the interface of the two liquids and the shock results in an efficient mixing of the two layers. Subsequently, very fine and highly stable emulsions can be obtained, with a relatively low ultrasonic energy input [11].

Therefore, the objectives of this work are to evaluate the influence of the laurel leaves essential oil concentration (C_{oe}) and the sonication time (t_s) in the reduction of the hydrodynamic size of the emulsion droplets (Dg) formed in an ultrasonic probe, and to evaluate the effects of the variation of these parameters (C_{oe} and Dg) on the encapsulation efficiency of the SFEE process.

MATERIALS AND METHODS

Materials: modified starch Hi-Cap 100 used as coating material. Tweet 80 United States Pharmacopeia (U.S.P.). Dichloromethane (DCM) (P.A. 99.9%). The laurel essential oil was extracted by hydrodistillation.

Emulsions formulation: the experimental planning is described in Table 1, in duplicate.

Table 1: Experimental planning for emulsions formulation.

Runs	C_{oe}^1 (mg/mL)	t_s^2 (min)	$C_{oe}^1/C_{(Hi\text{-}Cap)}^3$
1	6	2	1:5
2	6	4	1:5
3	6	6	1:5
4	9	2	1:3
5	9	4	1:3
6	9	6	1:3
7	12	2	1:2.5
8	12	4	1:2.5
9	12	6	1:2.5

[1]C_{oe} = concentration of the laurel essential oil in the emulsion (mg/mL); [2]t_s = sonication time; [3]$C_{(Hi\text{-}Cap)}$ = concentration the encapsulation agent in the emulsion (mg/mL).

Emulsion stability was evaluated by determination of hydrodynamic size of the emulsion droplets (Dg) (disperse phase of the emulsions) using the dynamic light scattering (DLS) technique and the polydispersity index (PdI) in the Zetasizer Nano S equipment (Malvern Instruments Ltda) during 24 h storage.

Supercritical Fluid Extraction of Emulsions (SFEE): the temperature (T) 40 °C and pressure (P) 100 bar selected for the SFEE assays were considering the use of supercritical CO_2 as an antisolvent, the preservation of the laurel essential oil the emulsion dispersed phase, the vapor-liquid equilibrium at high pressure (ELVS) of dichloromethane/CO_2

system (CORAZZA et al., 2003). The flow rates of the emulsion (Q_E) and CO_2 (Q_{CO2}) were kept constant, Q_E = 1 ml/min and Q_{CO2} = 1 kg/h.

Suspensions drying: the suspensions were placed in a glass petri dish and frozen in an ultra freezer at -50 °C (Nuaire, USA) during 24 h.

Encapsulation efficiency (EE): the particles obtained were diluted in dimethyl sulfoxide (20 mg/mL) and homogenized in ultrasound cleaner (Ultronique, Q3.0/37A, Brazil) during 10 min. Subsequently, the absorbance was performed in a UV/VIS spectrophotometer (Perkin Elmer Lambda 10) at 270 nm, according to methodology described by Angadi et al. (2002).

RESULTS

Emulsions stability

The hydrodynamic size of the emulsion droplets (Dg) ranged from 239.5-356.9 nm after 24 h storage, indicating the formation of oil-in-water miniemulsions (O/W).

In figure 1, it can be observed influences on the Dg caused by C_{oe} and t_s studied. The results suggest that these variables have interdependent influence for the reduction of Dg and do not have linear comportment between them or on the response variable.

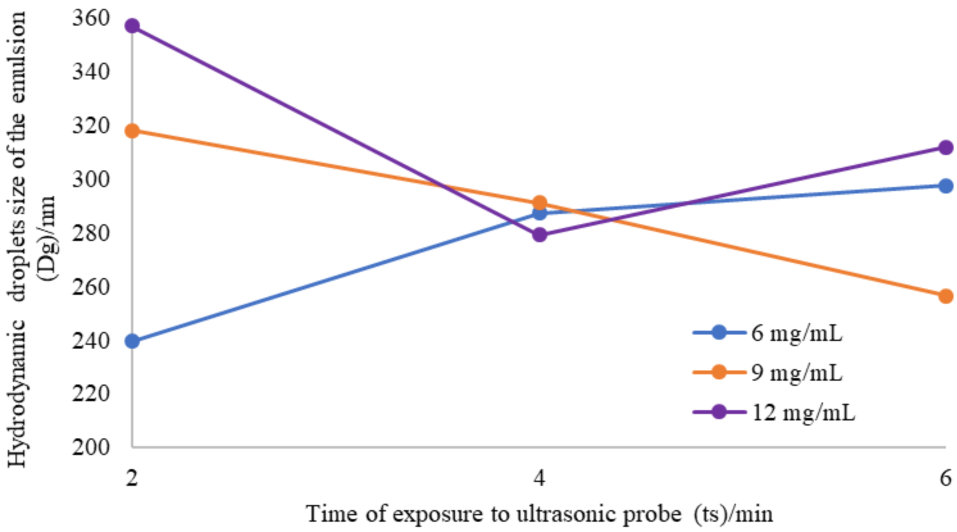

Figure 1: Hydrodynamic size of the emulsion droplets.

When keeping the sonication time (t_s) constant and varying the concentration of the laurel essential oil in the emulsion (C_{oe}), Figure 1, it was observed that the comportment of Dg varied depending on the t_s applied, not presenting linear comportment.

At 2 min, the increase of C_{oe}, expanded the Dg, exactly runs 4 and 7. Possibly the cavitation efficiency was not sufficient during the sonication time (2 min), because in the major relation fraction $C_{oe}/C_{(Hi\text{-}Cap)}$ present, consequently affecting reduction Dg and influencing the homogenization of the emulsions.

Already keeping t_s constant in 4 min, the increase of C_{oe} favored the reduction of Dg, although there is no alteration in the scale dimension of the emulsion classification, was a necessary time of the cavitation process efficiency in the formation of the emulsions studied.

However, in the 6 min t_s, the increase in C_{oe} obtained varied results in Dg. The small and major C_{oe} studied (6 and 12 mg/mL) had expansion of Dg. While the intermediate C_{oe} (9 mg/mL - 1:3 of $C_{oe}/C_{(Hi\text{-}Cap)}$ presented the decrease in Dg, comporting linearly with the increase of t_s.

Results similar to those obtained by this work were found in the production of stabilized quercetin aqueous suspensions by SFEE, concluded that ultrasound formation of emulsion, that increased quercetin concentration the particle size decreases, and the optimum emulsification time was 4 min, with larger emulsion droplet sizes when either the emulsification time was too short for a complete homogenization of the system, or too high leading to an increased droplet size probably due to coalescence and temperature effects [14].

When keeping C_{oe} constant and varying the t_s, in general, decrease of the Dg occurred with the increase of t_s for the major C_{oe} (9 and 12 mg/mL) studied, however the interval of variation was not sufficient to interfere in the order of magnitude of the Dg and, consequently, alter the classification of the emulsions. The control of the sonication time is necessary to minimize the loss of compounds caused by negative effects of cavitation of the ultrasonic probe, in particular the temperature, especially when the active principle studied by this work be a volatile compound.

The results of this work corroborate with those found by Silva et al. (2016) [15], who concluded that the intensification of the ultrasonication process, for more than 3 min, had no positive effects in the reduction of the droplets size of the annatto seed oil emulsion, demonstrating the existence of a physico-chemical limit imposed by the characteristics of the emulsifiers employed.

Evaluating the results obtained, it was concluded that the reduction of Dg is dependent on the interaction of C_{oe} and t_s, being the relation 1:3 and 1: 2.5 the $C_{oe}/C_{(Hi\text{-}Cap)}$, in ts the 4 and 6 min, indicated to minimize the Dg of laurel leaves essential oil in modified starch.

Efficiency of the encapsulation (EE) of the particles (powder)

The parameters of the SFEE process were determined with the objective of remove organic solvent (DCM) of the emulsion by supercritical CO_2, which in the process comport as an antisolvent, and minimize the removal of the active compound (1,8-cineole) which is interested in encapsulating, favoring, consequently, the efficiency of the encapsulation.

EE of the particles (powder) varied from 48.8% to 75.5%, concentration of 1,8-cineole from 3 to 9 mg/mL, respectively. Residual dichloromethane was evaluated in all particles (powder) being found a concentration of dichloromethane below 1 ppm.

Evaluating the influence of C_{oe} in EE, keeping the t_s constant, it was observed that the increase of C_{oe} favored EE, there was no significant difference between the major concentrations ($p < 0.05$), according to Figure 2, in 4 and 6 min. Probably the increase of EE occurred due to the supercritical CO_2 saturation concentration of limit by the organic solvent (DCM). Keeping constant all the parameters of the SFEE process, only the concentration of the active compound in the emulsion was varied, when the supercritical CO_2 saturated with the organic solvent (DCM), not removing other compounds, such as or 1,8-cineole, increase of EE.

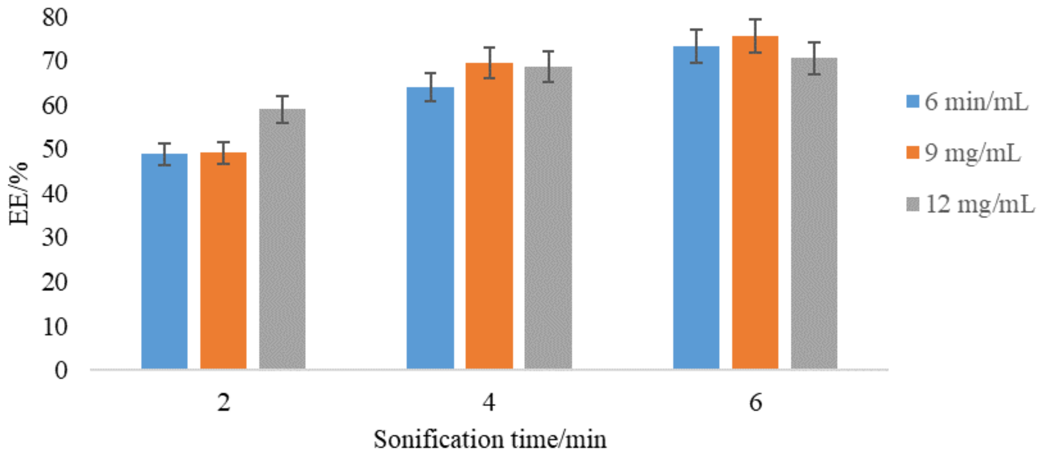

Figure 2: Influence of C_{oe} on efficiency of the encapsulation.

The Dg also influenced EE, demonstrated in Figure 3, keeping Coe constant, it was observed that in smaller Dg the major EE, the major concentrations studied, inversely proportional comported. This is related solubility to droplet, which occurred with decrease of Dg, facilitating the interactions between the active compound (1,8-cineole) and the encapsulation agent (Hi-Cap), polar compound, promoting the encapsulation and protecting the essential oil from the removal action by the supercritical CO_2.

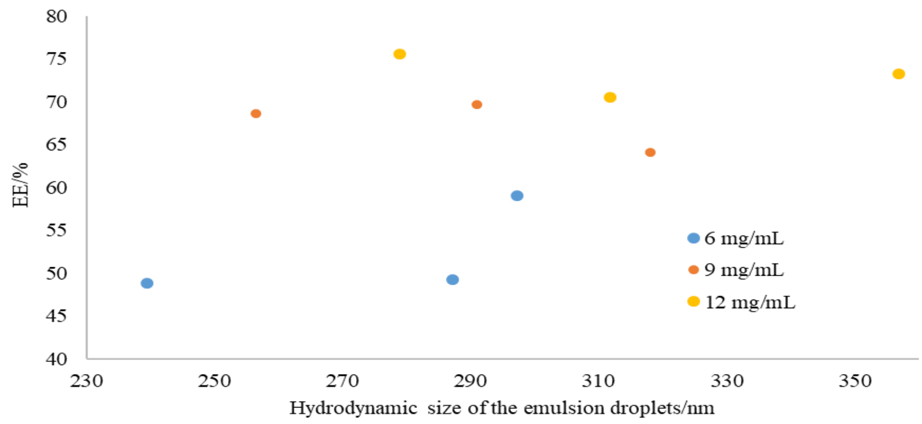

Figure 3: Influence of Dg on efficiency of the encapsulation.

The results of this work corroborate the studies on the influence of droplet size on suspensions of carotenoids encapsulated in Hi-Cap, by the SFEE process, which presented a increase EE for the suspensions with smaller particle sizes [10].

Therefore, considering the results obtained by this work, we conclude that the increase of the concentration of the active component (1,8-cineole) and the decrease in the Dg favored the efficiency of the encapsulation in the SFEE process.

CONCLUSION

Stable miniemulsions of laurel essential oil in modified starch were obtained by means of an ultrasonic probe. The efficiency encapsulation of the SFEE process is dependent the active compound of the concentration and hydrodynamic size of the emulsion droplets. Encapsulation of laurel essential oil in modified starch were formed by SFEE process.

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