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Protection of echium oil by microencapsulation with phenolic compounds

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ABSTRACT

The consumption of omega-3 enables the reduction of cardiovascular disease risk; however they are unstable. The aim of this work was to encapsulate echium oil (*Echium plantagineum* L.), a rich source of omega-3 fatty acids, with phenolic compounds (sinapic acid and rutin) by double emulsion followed by complex coacervation or by complex coacervation with sinapic acid in the capsule wall. Analyses of morphology, particle size, circularity, water activity, moisture, Fourier transform infrared spectroscopy, thermogravimetry, process yield, accelerated oxidation and identification and quantification of fatty acids present in the encapsulated oil were performed. Samples presented values of encapsulation process yield of phenolics and oil in the range of 39–80% and 73–99%, respectively. Moreover, all samples protected the oil against oxidation, obtaining induction time (accelerated oxidation) of 5 h for pure oil and values in the range from 10 to 18 h for samples. Thus, better protection to the oil was possible with sinapic acid applied in the capsule wall, which enhances its protection against lipid oxidation.

1. Introduction

There are several sources of omega-3 fatty acids (ω-3 FA) that can be added to food products. These sources can be from animal origin (marine oils extracted from cold water fish) or vegetable origin, such as echium oil (Whelan, 2009). *Echium plantagineum* L. has a Mediterranean and Macaronesian origin (Berti, Johnson, Dash, Fischer, & Wilckens, 2007) and contains 33% of α-linolenic acid and from 9 to 16% stearidonic acid, an intermediate in the biosynthesis of EPA and DHA, (Clough, 1993; Payne, Lad, Foster, Khola, & Gray, 2014), which are important omega-3 FA. Besides, the echium oil is being used in replacement of fish derived oils, since it presents unique ratio of omega-3 to omega-6 FA of 1.8:1, suitable for nutraceutical applications (Berti et al., 2007).

Omega-3 FA have been assessed for reducing the risk of cardiovascular disease (Poole et al., 2013); however they are very susceptible to oxidation, which hinders their application in food. Lipid oxidation could be delayed by different factors such as packaging and modified atmosphere. Moreover, other two strategies that could minimize these limitations are: (1) adding a compound with antioxidant function and (2) microencapsulation.

Phenolic compounds, such as sinapic acid and rutin, have been of great interest in the food industry, as they act as antioxidant agents (Lee et al., 2013). Sinapic acid, the main phenolic acid of canola, has been studied regarding its neuroprotective effect against Alzheimer's disease (Lee et al., 2012), cardiac hypertrophy and dyslipidemia (Roy & Prince, 2013). In relation to rutin, phenolic compound found in plants and food sources such as onions, grape, bean, apple and tomato, it has several pharmacological activities, including anti-allergenic, anti-inflammatory and vasoactive properties (Jantrawut, Assifaoui, & Chambin, 2013; Kim, Kwon, & Jang, 2011). Espinosa, Inchingolo, Alencar, Rodriguez-Estrada, and Castro (2015) studied the effect of eleven compounds on oxidative stability of emulsions prepared with echium oil and it was observed that sinapic acid and rutin were the most efficient to delay lipid oxidation, so they were distinguished from the compounds analyzed, which make them potential alternative to be applied as antioxidant in products, specially with echium oil.

The encapsulation is a process which retains a bioactive (solid, liquid or gas) inside another (wall material) in order to protect the material against adverse environmental conditions, thereby increasing the shelf-life and promoting the controlled release of the active compound in the microcapsule (Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011; Shahidi & Han, 1993).

In this context, the aim of this work was to protect echium oil. In order to achieve this aim, the echium oil was encapsulated in the presence of the phenolic compounds rutin and sinapic acid.

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2. Material & methods

2.1. Materials

As core were used echium oil (NEWmega™ Echium Oil, Ref.15200, from De Wit Specialty Oils (De Waal, Tescel, The Netherlands), sinapic acid and rutin from Sigma Chemical Co. (St. Louis, MO, USA). As wall material were used gelatin and Arabic gum from Gelita South America (Mococa/SP, Brazil) and Nexira (São Paulo/SP, Brazil), respectively. Polyglycerol ricinoleic acid (PGPR 90) (Danisco, Denmark) was used as an emulsifier.

2.2. Methods

2.2.1. Microencapsulation

For the production of microcapsules, a primary water-in-oil emulsion (W/O) consisting of sinapic acid or rutin solution in the internal aqueous phase (0.50 mg of phenolic compound/mL of water) and echium seed oil in the external oil phase was prepared at a ratio of 2:1 (v:v oil phase:aqueous phase). Polyglycerol ricinoleic acid (PGPR 90) was used as the emulsifier in the concentration of 0.5% (*w*/w) and added in the oil phase.

The primary emulsions (W/O) were emulsified in the gelatin solution (7.5%-*w*/w) with pH 6.0 to obtain a double emulsion water-in-oil-in-water (W/O/W) with different concentrations of phenolic compounds as showed in Table 1. The homogenization steps were performed at 12,000 rpm for 4 min (primary emulsion — W/O) and 10,000 rpm for 3 min (double emulsion — W/O/W) with an Ultra-Turrax apparatus (Ika, Germany). The Arabic gum solution (7.5%-*w*/w) was added to these emulsions by magnetic stirring at 40 °C for 3 min.

Besides the application of sinapic acid in the internal phase of the primary emulsion, a sample was also obtained where it was added directly to the primary emulsion, before the addition of Arabic gum solution (SIN-GEL). In this case, the double emulsion was not prepared, but only the primary emulsion composed of echium oil and gelatin solution. Moreover, the control sample composed of only echium oil and the wall materials (gelatin and Arabic gum) was also prepared.

Samples with different concentrations of the primary emulsion (50, 75 and 100% — SIN50, SIN75 and SIN100 and RUT50, RUT75 and RUT100, respectively) in relation to the total mass of the polymer (7.5%-*w*/w) were prepared, in addition to samples in which sinapic acid was used in the capsule wall and the oil was encapsulated without a phenolic compound (SIN-GEL and Control, respectively), resulting in eight samples (Table 1). The ratio of polymers (gelatin and Arabic gum) was fixed at 1:1.

Table 1
Concentrations and proportions used for each sample in the process of encapsulation.

Sample	Concentration of phenolic (μg/g of capsule)	Proportion of phenolic in relation to the oil (ppm)	Proportion of core in relation to total amount of polymer (%)	Concentration of oil (g/g of capsule)
SIN50	65.1	200	50	0.24
SIN75	87.0	200	75	0.32
SIN100	104.8	200	100	0.39
SIN-GEL	88.9	200	50	0.33
RUT50	65.1	100	50	0.24
RUT75	87.0	100	75	0.32
RUT100	104.8	100	100	0.39
Control	–	–	50	0.33

SIN50 and RUT50: sample with a 1:1:0.5 proportion of gelatin, Arabic gum and primary emulsion containing sinapic acid and rutin, respectively. SIN75 and RUT75: sample with a 1:1:0.75 proportion of gelatin, Arabic gum and primary emulsion containing sinapic acid and rutin, respectively. SIN100 and RUT100: sample with a 1:1:1 proportion of gelatin, Arabic gum and primary emulsion containing sinapic acid and rutin, respectively. SIN-GEL: sample with a 1:1:0.5 proportion of gelatin, Arabic gum and echium oil with sinapic acid as a cross-linker. Control: sample with a proportion of 1:1:0.5 of gelatin, Arabic gum and echium oil.

To promote complex coacervation, the pH was adjusted to 4.0 at 40 °C under magnetic stirring and the temperature was gradually lowered to 10 °C in an ice bath. The material was stored for 24 h at 7 °C to promote decantation. The coacervates were frozen (−18 °C) and freeze-dried (Terroni/São Carlos — SP, Brazil) for 24 h at a pressure of 1 to 0.1 kPa pressure, at a temperature of −20 °C and a final temperature of 30 °C. All the procedure of capsules fabrication is presented in a schematic representation in Fig. 1.

2.2.2. Characterization of coacervate samples

2.2.2.1. Yield of encapsulation process of phenolic compounds. The yield was determined by the mass difference of the phenolic compound contained in the microcapsules and the total quantity used in the encapsulation process. For the rupture of the capsule, 0.2 g of sample was diluted in 5 mL of methanol, 5 mL of $ZnSO_4$ solution (5%-*w*/w) and 0.8 g of KCl, subjected to ultrasound for 10 min and centrifuged at 4000 rpm at 25 °C for 10 min. The supernatant was filtered and analyzed by High Performance Liquid Chromatography (HPLC) (Shimadzu, Kyoto, Japan). A C18 column was used with water:methanol as the mobile phase at a ratio of 45:55 (water at pH 3.5), flow of 1 mL/min. The detection wavelength was 325 and 356 nm and the injection volume was 10 and 20 μL for sinapic acid and rutin, respectively. The yield of the encapsulation process was calculated from Eq. (1).

$$Yield = \frac{(Phenolic\ added - phenolic\ in\ the\ capsule) \times 100}{Phenolic\ added}. \quad (1)$$

2.2.2.2. Yield of encapsulation process of echium oil. The yield was determined by the difference in the mass of the oil contained in the microcapsules and the total quantity used in the encapsulation process. For the rupture of the capsule, the same method mentioned in Section 2.2.2.1 was used. After the rupture of the capsule, the procedure continued with the Bligh Dyer method with some modifications for oil extraction (Bligh & Dyer, 1959). In the broken capsules, it was added 5 mL of chloroform, 10 mL of methanol and 4 mL of distilled water. The mixture was lightly stirred for 1 min and then 5 mL of chloroform and 5 mL of sodium sulfate solution (1.5% *w*/w) were added to it, stirring gently in the vortex for 1 min more. The samples were allowed to stand for 30 min and a known volume of the chloroform phase was withdrawn and kept in oven at 60 °C to evaporate the solvent. The quantification of the oil was made from the remaining mass after evaporation of the chloroform. Thus, knowing the mass of oil added to the microcapsule and the volume of evaporated chloroform, it was possible to obtain the yield of encapsulated oil.

2.2.2.3. Morphological characterization of the microcapsules by optical microscopy, scanning electron microscopy (SEM) and confocal microscopy. Wet microcapsules were analyzed by optical microscopy using Bio3 equipment (Bel Photonics, Italy) and by confocal microscopy, using an Axio Observer Z.1 and an LSM 780-NLO Zeiss microscope, with a 40× objective. A laser with a wavelength of 561 nm was used for excitation along with a 569–691 nm emission filter for the fluorophore Nile Red. The pinhole was set to 1 Airy unit in each channel and the image size was 1024 × 1024, with an optical zoom of 1.7×. Lyophilized microcapsules were analyzed by SEM using a TM 3000 table-top microscope (Hitachi, Tokyo, Japan), and TM 3000 software.

2.2.2.4. Particle size analysis. To obtain the particle size, 200 microcapsules of each sample were individually measured using ImageJ software. A BIO3 microscope (Bel Photonics, Italy) was used to obtain the images.

2.2.2.5. Circularity. To determine the circularity the ratios between the smallest and the largest diameter of the capsules were used. These measurements were obtained from 100 capsules for each sample using