



Enhancing stability of echium seed oil and beta-sitosterol by their coencapsulation by complex coacervation using different combinations of wall materials and crosslinkers

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ABSTRACT

Intake of omega-3 fatty acids and phytosterols aids in the reduction of cholesterol and serum triglycerides. However, both fatty acids and phytosterols are susceptible to oxidation. This work coencapsulated echium oil (source of stearidonic and alpha-linolenic fatty acids) and beta-sitosterol (phytosterol) by complex coacervation using different combinations of wall materials, and sinapic acid (SA) and transglutaminase as crosslinkers. High encapsulation yields were obtained (29–93% for SA; 68–100% for the mixture of oil and phytosterols) and retention of 49–99% and 16% for encapsulated and free SA, at 30 days-storage. Treatment with gelatin-arabic gum and 0.075 g SA/g gelatin showed the best results: 0.07 mg MDA/g capsule, and retention of 96, 90 and 74% for alpha-linolenic, stearidonic acid and beta-sitosterol at 30 days of storage, respectively. Thus, coencapsulation of echium oil and phytosterol using SA as the crosslinker was possible, obtaining effective vehicles for protection and application of these compounds in foods.

1. Introduction

Omega-3 and omega-6 fatty acids have been extensively studied due to their beneficial effects on health (Kralovec, Zhang, Zhang, & Barrow, 2012). Stearidonic acid (SDA) and alpha-linolenic acid (ALA) are important omega-3 fatty acids. SDA, a long chain fatty acid produced by the desaturation of ALA, is found in small amounts in plants, fish and algae, and it plays a valuable role in human nutrition because it is an intermediate in the biosynthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Berti et al., 2007; Coupland & Hebard, 2002; Lemke et al., 2013). The echium seed oil is of great interest to researchers, as it contains 9–16% SDA and 33% ALA, in addition to 14% linoleic acid and 10% gamma-linolenic acid (omega-6 fatty acids), and has been an alternative to the use of fish oil, due to its unique ratio of omega-3 to omega-6 fatty acids (Berti et al., 2007). Similarly, phytosterols, compounds obtained from refined oils of plants, seeds, whole grains and legumes, also have many health benefits (Sanclement et al., 2012).

According to Espinosa, Inchingolo, Alencar, Rodriguez-Estrada, and Castro (2015), the consumption of an omega-3 fatty acid and

phytosterol mixture leads to a reduction in triglycerides and cholesterol serum levels. However, these compounds are very susceptible to oxidation and are insoluble in water. Consequently, their application in food products is particularly challenging. An alternative to minimize such problems would be the microencapsulation of these compounds.

Microencapsulation is intended to protect compounds against adverse environmental conditions, to mask unpleasant odors and flavors, and to avoid the evaporation of volatile compounds through the coating or dispersibility of compounds (core) by one or more materials (wall materials), facilitating their application in food products (Santos, Bozza, Thomazini, & Favaro-Trindade, 2015). The complex coacervation technique consists of the electrostatic interaction between two polymers with opposite charges. According to Comunian and Favaro-Trindade (2016), it is an ideal process for the encapsulation of hydrophobic compounds, considering the first step in the process is the preparation of an oil-in-water (O/W) emulsion. The polymers used as wall materials were gelatin, arabic and cashew gum. Gelatin is one of the most used hydrocolloids in food industry, has low cost and emulsifier property (Comunian & Favaro-Trindade, 2016); arabic gum is found in Arab countries, and it is used as stabilizer, emulsifier, thickener and

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Table 1

Composition of each treatment and yield values of sinapic acid, oil and phytosterol mixture.

| Treatments | Polysaccharide in combination with gelatin | Concentration of sinapic acid (g/g of gelatin) | Concentration of transglutaminase (U/ g of gelatin) | Sinapic acid encapsulation yield (%) | Encapsulation yield of the oil + phytosterols (%) |
|------------|--|--|---|--------------------------------------|---|
| GA00 | Arabic gum | 0.000 | – | – | 98.8 ± 7.6 ^a |
| GA025 | Arabic gum | 0.025 | – | 29.2 ± 1.7 ^d | 94.8 ± 5.0 ^a |
| GA05 | Arabic gum | 0.050 | – | 84.1 ± 9.0 ^{ab} | 98.5 ± 12.4 ^a |
| GA075 | Arabic gum | 0.075 | – | 87.5 ± 2.7 ^a | 97.8 ± 16.1 ^a |
| GC00 | Cashew gum | 0.000 | – | – | 100.8 ± 5.3 ^a |
| GC025 | Cashew gum | 0.025 | – | 51.2 ± 3.0 ^c | 100.7 ± 1.0 ^a |
| GC05 | Cashew gum | 0.050 | – | 67.5 ± 0.9 ^{bc} | 103.0 ± 2.7 ^a |
| GC075 | Cashew gum | 0.075 | – | 93.1 ± 8.9 ^a | 102.9 ± 3.9 ^a |
| GAT15 | Arabic gum | – | 15.00 | – | 68.9 ± 1.5 ^b |
| GCT15 | Cashew gum | – | 15.00 | – | 95.0 ± 0.6 ^a |

Equal letters in the same column do not differ statistically at 5% level by the Tukey test.

GA00, GA015, GA05 and GA075: Treatment with a ratio of 1:1:0.5 gelatin, arabic gum and echium oil containing sinapic acid as crosslinker at concentrations of 0, 0.025, 0.05 and 0.075 g/g of gelatin and 5% (w/w) gelatin solution.

GC00, GC025, GC05 and GC075: Treatment with a ratio of 1:1:0.5 gelatin, cashew gum and echium oil containing sinapic acid as crosslinker at concentrations of 0, 0.025, 0.05 and 0.075 g/g of gelatin and 5% (w/w) gelatin solution.

GAT15: Treatment with a ratio of 1:1:0.5 gelatin, arabic gum and echium oil containing transglutaminase as crosslinker at concentration of 15 U/g of gelatin and 5% (w/w) gelatin solution.

GCT15: Treatment with a ratio of 1:1:0.5 gelatin, cashew gum and echium oil containing transglutaminase as crosslinker at concentration of 15 U/g of gelatin and 5% (w/w) gelatin solution.

flavor fixative (Ribeiro & Seravalli, 2004). Cashew gum is an exudate from *Anacardium occidentale* tree, with similar properties to arabic gum and presents great availability in the Northeast region of Brazil. In addition, cashew gum has a higher protein content than arabic gum, showing better emulsifying property (Andrade et al., 2013).

The crosslinking process has been widely used together with the complex coacervation technique, to enhance the capsule strength. Previous work from our research group showed that in addition to being an antioxidant, the phenolic compound sinapic acid (SA) is an effective crosslinker and can enhance the stability of microcapsules obtained by coacervation (Comunian, Boillon, et al., 2016; Comunian, Gomez-Estaca, et al., 2016b). Besides having several beneficial effects on health, such as a neuroprotective effect against Alzheimer's disease, and ability to ameliorate cardiac hypertrophy and dyslipidemia (Lee et al., 2012; Roy & Prince, 2013), SA represents a suitable alternative to traditional crosslinkers, such as transglutaminase, which is expensive and does not provide health benefits, and glutaraldehyde, which is toxic. In a recent study from our group, echium oil was coencapsulated with SA and/or rutin, by complex coacervation (Comunian, Boillon, et al., 2016). However, for the inclusion of these compounds in the microcapsule, a water-in-oil-in-water (W/O/W) double emulsion was produced with the addition of SA or rutin in the internal aqueous phase, as they are water-soluble compounds. The results showed that protection of the echium oil was promoted, but it was not better than when SA was applied as a crosslinker (Comunian, Boillon, et al., 2016; Comunian, Gomez-Estaca, et al., 2016). Therefore, we conducted further work on echium oil encapsulation by complex coacervation, with the specific purpose of studying the application of SA as a crosslinker. In this instance, the best parameters for the inclusion of this compound in the microcapsule were investigated (Comunian, Gomez-Estaca, et al., 2016). However, the inclusion of phytosterol and the oxidative stability of the oil and of the encapsulated compounds were not studied, and are important factors to guarantee the viability of the microcapsule and their application in food products. In addition, the encapsulation of phytosterols and the coencapsulation of this type of mixture (two lipophilic bioactive compounds with different physicochemical properties) have been little explored in the literature.

In this context, the current work aimed to coencapsulate echium oil—rich in SDA and ALA—and phytosterol, by the complex coacervation technique, using SA as a crosslinking agent (and as an antioxidant). Also, to evaluate the stability of these compounds under predetermined conditions, with the intention to protect and apply these compounds in foods.

2. Material and methods

2.1. Materials

Echium seed (*Echium plantagineum* L.) oil (NEWmega™ Echium Oil, Ref.15200; De Wit Speciality Oils, Texel, Netherlands) was used as the omega-3 source. SA, gelatin, arabic gum, and cashew gum were obtained from Sigma Chemical Co. (St. Louis, MO, USA), Gelnex (Santa Catarina, Brazil), Nexira (São Paulo, Brazil) and EMBRAPA Tropical Agroindustry (Fortaleza/Ceará), respectively. The mixture of phytosterols, composed of beta-sitosterol (70–80%), beta-sitostanol (0–15%), campesterol (0–15%), stigmasterol (0–2%) and campesterol (0–5%) was obtained from DuPont-Danisco (Barueri, Brazil).

2.2. Methods

2.2.1. Microencapsulation

For the preparation of microcapsules, the method adopted by Comunian, Gomez-Estaca, et al. (2016), with some modifications, was used. The ratios of 1:1 gelatin:arabic gum and 1:2.5 gelatin:cashew gum were fixed, with 50% core (echium oil + phytosterol mixture) relative to the total polymer mass (gelatin, arabic gum and cashew gum solution 5% w/w). Treatments with various SA concentrations (0, 0.025, 0.050 and 0.075 g sinapic acid/g gelatin), as a crosslinking agent, were prepared, which were compared to transglutaminase, totaling 10 treatments, as shown in Table 1. The SA concentrations were used according to Comunian, Chaves, et al. (2017).

The oil and the phytosterol mixture (0.132 g phytosterols/g oil) were emulsified with gelatin solution (5% w/w), to obtain a simple O/W emulsion at 10,000 rpm/3 min by using an Ultra-Turrax (IKA, Germany). The solution of arabic or cashew gum (5% w/w) was added to these emulsions under magnetic stirring at 40 °C.

The pH was adjusted to 4.0 with a hydrochloric acid solution (1 mol/L) at 40 °C under constant magnetic stirring, to promote complex coacervation and, thereafter, the temperature was gradually reduced to 10 °C in an ice bath. The coacervate material was stored at 7 °C for 24 h, to promote decantation. The supernatant (water) was removed, using a dispenser. The coacervates were then frozen (−18 °C) and freeze-dried (Terroni; São Carlos, Brazil).

2.2.2. Crosslinking process

2.2.2.1. Crosslinking with SA. Based on a previous method (Comunian, Gomez-Estaca, et al., 2016), the SA crosslinking agent was

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