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Effect of different polysaccharides and crosslinkers on echium oil microcapsules

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ABSTRACT

Microencapsulation by complex coacervation using gelatin and arabic gum (AG) as wall materials and transglutaminase for crosslinking is commonly used. However, AG is only produced in a few countries and transglutaminase is expensive. This work aimed to evaluate the encapsulation of echium oil by complex coacervation using gelatin and cashew gum (CG) as wall materials and sinapic acid (S) as crosslinker. Treatments were analyzed in relation to morphology, particle size, circularity, accelerated oxidation and submitted to different stress conditions. Rounded microcapsules were obtained for treatments with AG (45.45 μm) and microcapsules of undefined format were obtained for treatments with CG (22.06 μm). The S incorporation for 12 h improved the oil stability by three fold compared to oil encapsulated without crosslinkers. Treatments with CG and S were resistant to different stress conditions similar to treatments with AG and transglutaminase, making this an alternative for delivery/application of compounds in food products.

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1. Introduction

Echium oil is a plant-based oil which contains 9–16% stearidonic acid, 14% linoleic acid, 10% gamma-linolenic acid and 33% alpha-linolenic acid. It is being considered as an alternative to fish oils, as it presents a unique ratio of omega-3 to omega-6 fatty acids that is not found in any other plant (Berti et al., 2007). However, this oil is very unstable, and is also a hydrophobic material which hampers its application in beverages, for instance. Thus, one alternative to minimize the problems related to echium oil is the microencapsulation process.

The microencapsulation technique can be defined as a process in which one or more materials are surrounded by a membrane, in order to protect against environmental conditions, and also to promote the controlled release of the materials in specific locations and conditions. The complex coacervation encapsulation technique consists of the electrostatic interaction between oppositely charged macromolecules. Microcapsules are obtained by this interaction, where the formation of a shell around the encapsulated material is possible under specific conditions of pH and temperature (Xiao, Liu, Zhu, Zhou, & Niu, 2014). Many wall material combinations are used in the complex coacervation technique, including casein and pectin (Baracat et al., 2012), gelatin and arabic gum (Comunian et al., 2013; Santos, Bozza, Thomazini, & Favaro-Trindade, 2015), alginate and pea protein isolate (Klemmer, Waldner, Stone, Low, & Nickerson, 2012), pectin and soy protein isolate (Mendanha et al., 2009) and gelatin and chitosan (Prata and Grosso, 2015). The combination of gelatin and arabic gum is the most common and effective one.

Gelatin, a hydrocolloid majorly obtained from the bones and skins of mammals and fish, has amphoteric character and cationic properties at pH below its isoelectric point (IEP) and anionic characteristics at pH values above its IEP, which makes it a great polymer to be used as wall material in the complex coacervation technique (Xiao et al., 2014). On the other hand, arabic gum has some limitations. It is produced in just a few countries (such as Senegal and Sudan), and variations in its quality and composition make its obtainment and standardization difficult. The use of new polysaccharides in the complex coacervation process is important

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to increase the range of choices and, consequently, to decrease the cost of the final product, in order to obtain innovative and excellent products. A new option is the cashew gum, a macromolecule similar to arabic gum due to its thermal and rheological behavior (Mothé and Rao, 2000). It is an exudate from the *Anacardium occidentale* tree and is composed of galactose, arabinose, glucose and rhamnose (Abreu, Oliveira, Paula, & Paula, 2012). Additionally, some studies have shown health benefits such as antitumor and antihypertensive activities (Carestiato, Aguila, & Mothé, 2009). The cashew gum has a negative charge, which makes it a feasible and effective alternative as a wall material in the complex coacervation technique.

Microcapsules obtained by complex coacervation are also known to be fragile under certain conditions. For this reason, specific compounds are used as crosslinkers in order to obtain more resistant structures. The traditional crosslinkers are formaldehyde and glutaraldehyde, which are toxic and prohibited in the food industry. Transglutaminase has also been widely used, however it is an expensive material which limits its application (Peng, Zhao, Huang, Chen, & Zhao, 2014). Furthermore, countries such as Spain do not allow the use of transglutaminase in food products.

The sinapic acid compound, extracted from fruits (lemons, oranges, tangerines, strawberries, blueberries), vegetables (onions, garlic, broccoli), cereal grains (rye, rice and oat) and herbs and spices (borage, thyme, nutmeg) (Niciforovic and Abramovic, 2014), is a phenolic compound with antioxidant functions and has been studied regarding its neuroprotective effect against Alzheimer's disease (Lee et al., 2012) and cardiac hypertrophy and dyslipidemia (Roy and Prince, 2013). Sinapic acid also has possible reactions with proteins and enzymes, which lead to covalent bonds and thereby crosslinking reactions (Rawel and Rohn, 2010). Moreover, the chemistry of hydroxycinnamic acid derivatives concerning polysaccharide-polysaccharide and lignin-polysaccharide crosslinking in grass cell walls (Ralph, Quideau, Grabber, & Hatfield, 1994) and in soluble and insoluble dietary fibers of many cereal grains (Bunzel et al., 2003) seems to be a clue towards understanding the enhanced mechanical properties of microcapsules formed through a coacervation with sinapic acid as a crosslinker. In grasses, it is well-known that ferulic acid is esterified to grass cell wall polysaccharides (arabinoxylans) at the C-5 position of α-L-arabinofuranoside moieties (Hatfield, Ralph, & Grabber, 1999). Dimerization of such polysaccharide-ferulate esters provides a pathway for crosslinking polysaccharide chains (Ralph et al., 1994). Similarly, another hydroxycinnamic acid derivative, sinapates, were also claimed to have a similar role to ferulates in crosslinking polysaccharides in cereal grains and presumably in grass cell walls in general (Bunzel et al., 2003). Thus, the use of sinapic acid as a crosslinker instead of the traditional crosslinkers can be an alternative for achieving more rigid and stable microcapsules.

The current research therefore aimed to compare the use of cashew gum and sinapic acid, instead of arabic gum and transglutaminase, as the polysaccharide-encapsulant and crosslinker, respectively, to encapsulate echium oil by complex coacervation and submit the capsules to different temperatures, pH levels, and salt and sucrose concentrations. To the authors' best knowledge, there are no published studies regarding the use of cashew gum as a wall material and sinapic acid as a crosslinker, making this an innovative idea.

2. Material and methods

2.1. Materials

Echium oil (NEWmegaTM Echium Oil, Ref.15200/De Wit Speciality Oils, Tescel, Netherlands) was used as the core. The encapsulants were gelatin, purchased from Gelnex (Santa Catarina, Brazil), arabic gum (composed by protein (0.99% – w/w)), rhamnose (4% – w/w), arabinose (46% – w/w), galactose (38% – w/w) and glucuronic acids (6.5% – w/w), purchased from Nexira (São Paulo/SP, Brazil), and cashew gum (composed by β-D-galactose (72–73% – w/w), α-D-glucose (11–14% – w/w), arabinose (4–6.5% – w/w), rhamnose (3.2–4% – w/w) and glucuronic acids (4.7–6.3% – w/w)) (De Paula, Heatley, & Budd, 1998) which was obtained from EMBRAPA Tropical Agribusiness (Fortaleza/Ceará, Brazil). The crosslinkers used were sinapic acid (Sigma, St. Louis, MO, USA) and transglutaminase (Ajinomoto, São Paulo, Brazil) with an activity of 100 U/g. Lycopene (LycoVit Dispersion 20%) from BASF (Ludwigshafen, Germany) was used to dye the oil.

2.2. Methods

2.2.1. Purification and characterization of cashew gum

For purification of cashew gum, it was dispersed in 96% ethanol and the precipitate was dried at 60 °C for 24 h. An aqueous solution of 6% (w/w) of the obtained powder was prepared and centrifuged at 5000 rpm for 15 min at 20 °C with the equipment Centrifuge 5430R, Eppendorf AG (Hamburg, Germany). The supernatant was filtered to obtain the purified cashew gum solution. This solution was stored in Petri dishes and maintained at 65 °C for 24 h in a kiln (Fanem, Model 315 SE, São Paulo/Brazil) for complete drying and obtaining powder material. The purified cashew gum is referred to throughout the text as cashew gum. The study was adapted from Torquato et al. (2004).

For the characterization of cashew gum, analysis of protein content (Baethgen and Alley, 1989), ash content (AOAC, 2005) and solubility (Cano-Chauca, Stringheta, Ramos, & Cal-Vidal, 2005) were carried out.

2.2.2. Microencapsulation process

2.2.2.1. Complex coacervation. The microcapsules were produced according to Nori et al. (2011), with some modifications. A concentration of 50% (w/w) of oil in relation to the polymer mass, with 8% (m/m) of lycopene in relation to the oil, was added to a 5% (w/w) gelatin solution and homogenized at 10,000 rpm for 3 min with an Ultraturrax T25 (IKA, Germany), obtaining an oil in water emulsion. Then, in order to perform the complex coacervation, a 5% (w/w) arabic or cashew gum solution was added to the oil in water emulsion under magnetic stirring at 40 °C. The pH was adjusted to 4.0 and the temperature reduced to 10 °C via an ice bath. After preliminary tests, the proportions of gelatin:arabic gum and gelatin:cashew gum were fixed at 1:1 and 1:2.5, respectively.

2.2.2.2. Crosslinking. Sinapic acid (0.05 g/g of gelatin) was added in two different ways: (1) after preparing the simple oil in water emulsion (oil in gelatin solution) and before the addition of the arabic gum solution, sinapic acid was added while using a magnetic stirrer over 1 min at 40 °C; (2) after the complex coacervation process, following the pH adjustment and temperature reduction, sinapic acid was added to the solution with the microcapsules and maintained under low magnetic stirring for 12 h at 15 °C in the BOD TE-391 incubator (Tecnal/Piracicaba, São Paulo – Brazil).

For the crosslinking with transglutaminase, a 100 mL aqueous solution was prepared containing the enzyme at a concentration of 15 U/g of gelatin and a pH of 6.0. The solution was added to the microcapsules after the coacervation and maintained under low magnetic stirring for 12 h at 15 °C in the BOD incubator.

In addition, the control treatments were prepared, making up ten treatments in total (Table 1 and Fig. 1).

2.2.2.3. Drying processes of microcapsules. The coacervates were stored for 24 h at 7 °C to allow the microcapsule precipitate to set-

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