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Evaluation of the fatty matter contained in microcapsules obtained by double emulsification and subsequent enzymatic gelation method

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ABSTRACT

The objective of this study was to evaluate encapsulated fatty matter using double emulsification and subsequent enzymatic gelation method using soy protein isolate cross-linked by transglutaminase. For this purpose, six extractions methods (acids, alkaline and enzymatic methods) were used. The extraction methods showed differences, total or partial extraction being observed when using the acid method, alkaline enzymatic method and the acid method with direct determination of the fatty acid composition. The presence of triglycerides and ethyl ester was investigated in the microcapsules by easy ambient sonic-spray ionization mass spectrometry (EASI-MS), high performance size exclusion chromatography (HPSEC) and the fatty acid composition as determined by gas chromatography (GC). The microcapsules were shown to contain 1.07 g omega-3 fatty acids / 100 g microcapsules.

1. Introduction

Epidemiological and nutritional studies suggest that the consumption of omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has several benefits for human health, such as anti-carcinogenic activity, anti-inflammatory effects, reduction in the risk of heart disease, and the prevention of osteoporosis and neurological disorders (Alzheimer's disease, Crohn's disease, etc.), also aiding in the reduction of depression (Conto, Grosso, & Gonçalves, 2013; Riediger, Othman, Suh, & Moghadasian, 2009).

According to Rubio-Rodríguez et al. (2010) and Cohen, Norman, and Heimer (1995), fish and microalgae are the traditional sources of EPA and DHA-rich oils, and the production of concentrated methyl or ethyl ester derivatives from fish oil stands out, since these are more stable to oxidation. However, the application of fish oil and its derivatives in food systems is limited by their oxidative instability during storage, presenting off-flavors (Cho, Shim, & Park, 2003). Therefore techniques have been developed aimed at protecting these compounds against oxidation, such as microencapsulation, which simultaneously facilitates incorporation of these ingredients into food formulations (Ackman, 2006).

Various wall materials and encapsulation methods have been tested for the microencapsulation of fish oil. Protein films are generally excellent oxygen and aroma barriers and are used to produce microcapsules using coacervation techniques (Conto et al., 2013). Others techniques that consist of double emulsions and subsequent reticulation with glutaraldehyde or heat gelation have also been investigated (Lee & Rosenberg, 2000). Although proteins from plant sources display emulsifying and/or film-forming properties, there are few proteins which have been studied as carrier or wall materials in the microencapsulation applications (Nesterenko, Alric, Silverstre, & Durrieu, 2013). This is because their uses for sensitive ingredients are limited by their heat instability and organic solvents sensibility. However, the use of reticulating agents such as enzyme transglutaminase (TGase) to convert the proteins into stabler forms could lead to an increment in their industrial applications (Babiker, 2000).

Many studies have been investigating the microcapsules structural characteristics, process conditions, applications and release methods (Lamprecht, Schafer, & Lehr, 2001; Polovarapu, Oliver, Ajlouni, & Augustin, 2011; Velasco, Dobarganes, & Márquez-Ruiz, 2000). This release can occur by different methods, such as fracture, diffusion, dissolution or degradation. Fractures or breaks can be achieved by pressure, shear and sonication. Diffusion occurs by concentration gradient or attractive forces between chains. Dissolution occurs by the action of heat or solvents. Finally, biodegradation occurs due to susceptibility of the wall material to certain compounds, such as enzymes (Mascarenhas, 2010). However, in this context, few works have been devoted to define the integrity of the core material after its release, which can be accompanied by specific tests, such as gas chromatography — GC and mass spectrometry — MS (Yeo, Bellas, Firestona, Languer, & Kohane, 2005). Another possible analysis is the content of lipid compounds using high performance liquid chromatography size exclusion — HPSEC (Velasco

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et al., 2000). EASI-MS has been extensively used for the analysis of oils and biofuels (Riccio et al., 2010), which makes it a new alternative for determining the integrity of the encapsulated material.

The objective of the present research was to study six extraction methods (acids, alkaline and enzymatic methods) and the composition of the lipid matter extracted of the encapsulated core material by an enzymatic gelation process, using soy protein isolate as the wall material, the enzyme transglutaminase as cross-linker agent and fish oil ethyl ester (EE) as the core material.

2. Materials and methods

2.1. Materials

Fish oil ethyl ester (EE) (62% EPA + DHA, Vital Atman, Uchoa, SP), corn oil (Qualitá, Bunge Alimentos S.A., Gaspar, SC, Brazil), Span 80 (Surfactant sorbitan mono-oleate, Croda do Brasil Ltda, São Paulo, SP, Brazil), soy protein isolate (The Solae Company, Porto Alegre, RS, Brazil, 88% protein w.b.), and active transglutaminase TG-S® microbial (TGase) (Ajinomoto, Limeira, SP, Brazil).

2.2. Methods

2.2.1. Microcapsule production

Microcapsules were produced using the enzyme transglutaminase (TGase) as the gelling agent, using the methodology adapted to Cho et al. (2003). The microcapsules were prepared using a double-emulsification process followed by enzymatic gelation like described below:

(a) A 10% solution of the protein (SPI) was first prepared in 100 mL deionized water, and 0.025% (2.7 UA/g of protein) of the enzyme TGase added at room temperature and pH 6.0 by adding 0.5 mol/L and 0.1 mol/L HCl.
(b) The first emulsion was formed by mixing the EE with the initial protein solution for 10 min in a basic T18 Ultraturrax (Ika-Werk, Brasília, DF) at 11000 rpm. The ratio of wall material to core material was 2:1 (*w*/*w*).
(c) 400 mL of corn oil, pre-heated to 50 °C was used to form the second emulsion, adding the surfactant (1%) Span 80.
(d) Immediately after forming the first emulsion, this was slowly added to the second emulsion with mild magnetic stirring. After mixing, the double-emulsion was maintained at 37 °C for 17 h in a chamber (BOD — Biochemical Oxygen Demand incubator), this time was necessary to form capsules (pretest not showed).

The microcapsules were filtered and washed with ethanol three times and then once with petroleum ether, air-dried, frozen for 12 h and then freeze-dried in a Pirani 50 freeze-dryer (Edwards, USA) for 24 h.

2.2.2. Free lipid content

The free lipid content was extracted using the methodology described by Velasco et al. (2000), with some adaptations of the scale.

To determine the free lipid content, 20 mL of petroleum ether were added to 0.8 g microcapsules and stirred for 15 min at 25 °C. The microcapsules were then filtered through anhydrous Na_2SO_4, the solvent evaporated and the samples dried with nitrogen.

The microcapsules recovered after this determination, were used to determine the extraction methods of core material, and its constitution.

2.2.3. Encapsulation yield and efficiency

The encapsulation yield was determined from the mass of freeze-dried microcapsules (d.b.) obtained divided by the total initial mass used (EE + SPI, d.b.).

The encapsulation efficiency was obtained after the acid extraction of the core, where 0.2 g of sample was added to 4.5 mL of boiling deionized water and 5.5 mL 8 M hydrochloric acid, and maintained in a boiling water bath for 30 min (until complete degradation of the wall material). The mixture was subsequently filtered and washed with 10 mL boiling deionized water. The filter papers with the hydrolyzed samples were dried in an oven and then extracted according to the methodology for the determination of the oil content (AOCS, 2009) of compounds with high protein content. The encapsulation efficiency was determined using Eq. (1) as described by Davidov-Pardo, Roccia, Salgado, León, and Pedroza-Islas (2008).

$$\%\,\text{Encapsulation efficiency} = \frac{(\text{Total lipid content} - \text{free lipid content}) \times 100}{\text{Total lipid content}} \quad (1)$$

2.2.4. Microcapsule morphology

The morphology of the microcapsules was determined using a model TM 3000 high vacuum scanning electronic microscope (SEM) (Hitachi, Tokyo, Japan). Three samples were arranged on aluminum stubs containing a double-faced copper tape, using carbon glue to secure the material. The best fields were selected, where the microcapsules were isolated.

2.2.5. Size distribution and average size of the microcapsules

The average size and size distribution of the microcapsules freeze-dried were determined using a Mastersizer 2000 (Malvern Instrument LTDA, Worcestershire, UK). Three readings were made giving a total of nine repetitions, with agitation at 3500 rpm and ultrasound to disperse the particles in water.

2.2.6. Extraction of the encapsulated fatty matter

The microcapsules obtained were subjected to six different extraction methods to extract the encapsulated material, so as to determine which method extracted more material with higher quality. The amount of encapsulated fatty matter extracted (weight) was determined using Eq. (2):

$$\%\,\text{Encapsulated Matter} = \frac{g\ \text{fatty material extracted} \times 100}{g\ \text{microcapsules}} \quad (2)$$

In parallel, the physical degradation of the microcapsule wall by each method was determined by optical microscopy (eclipse E800, Nikon, Tokyo, Japan), photographing the images using a digital camera controlled by the image-pro plus 6.0 program, using the 12×, 25× and 40× objectives.

Table 1 shows the methods difference and the code adopted for the samples, the descriptions of methods follow below:

TM1 = alkaline extraction method, using the methodology described by Velasco et al. (2000), where 0.1 g of microcapsules free of surface oil were dispersed in 1 mL deionized water at 65 °C with stirring, and 0.2 mL 25% NH_4OH then added and heated at 65 °C for a further 15 min. The solution was cooled, transferred a beaker by a separating funnel been washed with 10 mL ethanol. The encapsulated material was extracted three times in the following sequence: a) 50 mL of a 1:1 (*v*/*v*) solution of ethyl ether:petroleum ether; b) 5 mL of ethanol, 30 mL of a 1:1 (*v*/*v*) solution of ethyl ether:petroleum ether; c) 30 mL of a 1:1 (*v*/*v*) solution of ethyl ether: petroleum ether. After filtration through anhydrous Na_2SO_4, the solvent was evaporated off with nitrogen to constant weight.

TM2 = enzymatic extraction method using the methodology described by Mascarenhas (2010) with some adaptations, where 0.1 g of microcapsules free of surface oil were dispersed in 4 mL of enzyme solution containing 0.5% Protex 6 L liquid bacterial protease (Genencor, Danisco, Cotia, SP, Brazil) and 0.5% liquid bacterial