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Microencapsulation of fish oil-in-water emulsion using thiol-modified β-lactoglobulin fibrils-chitosan complex

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ABSTRACT

Fish oil was encapsulated via spray-drying using different β-lactoglobulin (β-LG) fibril variants, chitosan and maltodextrin. The effects of different wall materials and inlet temperatures on the physicochemical properties of fish oil microcapsules were investigated. A lower polydispersity index (0.29–0.37) coupled with larger mean droplet size (0.96–1.25 μm) and higher zeta potential (41.3–41.6 mV) were achieved in the reconstituted fish oil microcapsules formed using thiol-modified β-LG fibril/0.5% chitosan complex. Lower angle of repose (< 30°) displayed by the same sample suggested good flow properties, and this finding correlated well with its smooth surface, as observed using scanning electron microscopy. Microcapsules stabilized using the complex exhibited comparable encapsulation efficiency and slightly higher glass transition temperature than that of using unmodified β-LG fibrils, indicating the former's greater heat stability. This study provided valuable insight into the application of protein fibrils-polysaccharide complexes as an effective encapsulation agent.

1. Introduction

Fish oil is regarded as a nutritive functional food and is currently in great demand among health-conscious consumers. For years, clinical studies have demonstrated the protective role of omega-3 fatty acids of fish oil, such as eicosapentaenoic acid and docosahexaenoic acid, in human health. However, the major drawback of fish oil applications is their strong odor and high susceptibility to oxidative deterioration due to the presence of polyunsaturated fatty acids (Kagami et al., 2003). To better control the negative attributes of fish oil, encapsulation has been proposed as an effective approach to mask the undesirable fishy odor and protect the polyunsaturated fatty acids against oxidation. For this purpose, a fish oil-in-water (O/W) emulsion was first produced, and subsequently spray-dried. Encapsulation is a process whereby active ingredients are enclosed within a wall material, which provides a barrier for the inner core against environmental stresses.

Many studies have investigated the use of various wall materials, such as chitosan, maltodextrin, sugar, starch and whey proteins in the encapsulation of fish oil (Pourashouri et al., 2014). Maltodextrin was applied as one of the wall materials in the present study owing to its relatively low cost, good solubility and low viscosity at high solid concentrations. However, the application of maltodextrin alone is undesirable due its poor emulsifying properties (Kagami et al., 2003). Therefore, the use of additional emulsifier for the encapsulation of fish oil is required. Proteins are versatile ingredients, which have been applied in wastewater treatment (Sarrafzadeh and Sepehri, 2018) and in various types of emulsion due to its promising emulsifying properties. Whey protein is widely used as emulsifier. However, the major limitation of applying whey protein as an encapsulation agent is their poor heat stability, which may lead to droplet aggregation and subsequent disruption of the emulsion stability (Sliwinski et al., 2003). The fibrillar form of beta-lactoglobulin (β-LG) fabricated under acidic condition (pH 2) and high temperature (> 80 °C) has the same limitation as well (Liu and Zhong, 2013). Generally, protein-based encapsulation agent has limited application in the food industry due to their heat instability and susceptibility to deterioration by organic solvent (Gan et al., 2008).

To address this limitation, chitosan employed in our work as a high molecular-weight polymer was found to have higher heat stability. Specifically, chitosan is a cationic polymer composed of β-linked glucosamine and N-acetylglucosamine unit that acts as a stabilizer in

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emulsion systems. Since chitosan is a poor emulsifier, its complexation with the fibrillar form of β-LG can be beneficial as this complexation process combines the advantageous properties of both oppositely-charged biopolymers for better encapsulation efficiency. Previous works have studied similar complexation processes by applying a chitosan-lecithin complex (Klinkesorn et al., 2005) and a chitosan-maltodextrin/whey protein isolate complex (Klaypradit and Huang, 2008) in spray-dried tuna oil microcapsules. This conferred a thick coating and consequently produced an oxidatively- and physically-stable product. As reported, β-LG has an isoelectric point of 5.1 while the pKa of chitosan is in the range of 6.2–7.0. Hence, the formation of β-LG fibrils (at pH ≤ 4) and dissolution of chitosan under acidic condition (at pH ≤ 6.5) remained positively charged, and this property alone resulted in undesirable outcomes due to the electrostatic repulsion between them. In our previous work, we investigated the formation of thiol-modified β-LG fibrils at pH 9 that possessed a negative charge. The esterification process used to form the thiol-modified β-LG fibrils was found to produce fibrillar structure with greater emulsifying properties (possessing a net negative charge with reported zeta potential values ranging from −20 to −35 mV) and a high tolerance against pH changes (Chang et al., 2017). This would allow the complexation of both biopolymers by electrostatic associative interactions. Hence, the objective of our present study was to investigate the application of thiol-modified β-LG fibril-chitosan complexes as wall material for the production of fish oil-in-water emulsions that were subsequently spray-dried to form stable fish oil microcapsules.

2. Materials and methods

2.1. Materials

BiPRO whey protein isolate (WPI) containing approximately 70% β-lactoglobulin, ~15% α-lactalbumin, ~10% glycomacropeptide, ~3% bovine serum albumin and traces of immunoglobulins and lactoferrin (≤2%) was obtained from Davisco Foods International Inc., USA; 1-propanethiol (99%), chitosan (100–300 kDa) and maltodextrin (dextrose equivalent 10) were procured from Sigma Aldrich, Co, USA; omega-3 fish oil (18/12 TG) was procured from Jedwards International, Ins., USA. Analytical-grade chemicals and deionized water were employed in the present study.

2.2. Preparation of thiol-modifed β-lactoglobulin fibrils

A WPI solution was prepared at a concentration of 2.5% w/w under magnetic stirring for 2 h. Prior to casein precipitation, the pH of the WPI solution was adjusted to 4.6 using 6 M HCl. Then, the solution was centrifuged at 6000 g (Thermo Fisher Scientific, Waltham, USA) for 30 min to remove the precipitate. Thereafter, the supernatants were subjected to vacuum filtration using a 0.2-μm regenerated cellulose filter paper and was readjusted to pH 2 using 6 M HCl. For fibrillation, the protein solution was heated at 80 °C for 20 h under magnetic stirring (300 rpm). The fibril solution was then cooled in an ice-water bath for 30 min to halt the fibrillation process. With respect to thiol-modification, the fibril solution was adjusted to pH 9 using 6 M NaOH. Then, thiol reagent was added and the solution was stirred for 24 h at 350 rpm (Chang et al., 2017). Thiol-modification was performed based on the molar ratio of 1-propanethiol: total carboxyl residue considering 27 carboxyl residues in each β-LG monomer (45 g/mol).

2.3. Encapsulation by spray drying

A coarse emulsion was prepared by dispersing 10% (w/w) fish oil into the continuous phase containing 1% (w/w) β-LG variant (β-LG fibrils and thiol-modified β-LG fibrils), 0.5% (w/w) chitosan and 15% (w/w) maltodextrin using a high-shear homogenizer (Silverson L4R, Buckinghamshire, UK) at 7500 rpm for 5 min. Then, the coarse emulsion was further homogenized at 750 bar for three passes using a high-pressure homogenizer (Panda 2 K, NiroSoavi Deutschland, Lubeck, Germany). The resulting emulsion was then subjected to spray drying using a laboratory-scale spray dryer (BŰCHI Mini Spray Dryer B-290) to produce fish oil microcapsules. The inlet air temperature was set at 160, 170 and 180 °C, with a fixed flow rate of 15 mL/min.

2.4. Water activity

The water activity (a_w) of the fish oil microcapsules was measured using a water activity analyzer (AquaLab, Series 3 TE, Washington, USA) at room temperature. Duplicate measurements were performed.

2.5. Moisture content

Fish oil microcapsules (2 g) were dried in an oven at 105 °C for 24 h. The moisture content was determined gravimetrically after a constant weight was obtained.

2.6. Reconstitution properties

The reconstitution properties of the spray-dried fish oil microcapsules were assessed by dissolving 0.5 g microcapsules in 150 mL of deionized water at room temperature (25 °C) under a constant magnetic stirring (400 rpm). The refractive index of fish oil and dispersant (distilled water) was 1.467 and 1.333, respectively. The mean droplet size (intensity-weighted mean diameter), polydispersity index (PDI) and zeta potential of the resulting reconstituted dispersions were determined using a Zetasizer Nano ZS (Worcestershire, U.K.) at 25 °C.

2.7. Extraction of free oil, total oil and encapsulation efficiency

Free oil was determined by mixing 2 g of fish oil microcapsules with 15 mL of hexane and shaking for 2 min at room temperature. The solvent was then filtered and the collected solid residue was rinsed three times with 20 mL of hexane. The combined filtrate solution was evaporated using a rotary evaporator. The extracted oil was dried at 105 °C until a constant weight was obtained. Total oil was determined by dissolving 0.5 g of fish oil microcapsules in 15 mL of deionized water (60 °C). Then, the solution was added with 25 mL of hexane/isopropanol (3:1, v/v) and vortexed for 15 min. The solution was subsequently centrifuged at 8000 g for 15 min. The supernatant was re-extracted using the same solvent mixture followed by filtration. The combined filtrate solution was evaporated using a rotary evaporator at 70 °C and dried at 105 °C until a constant weight was obtained. The encapsulation efficiency was determined based on the following equation:

$$EE(\%) = \frac{\text{Total oil} - \text{Free oil}}{\text{Total oil}} x100 \quad (1)$$

2.8. Glass transition temperature

Thermograms were obtained using a differential scanning calorimeter (DSC-7, PerkinElmer) under nitrogen flow (20 mL/min) with the temperature ramped from 25 °C to 125 °C at a rate of 5 °C/min. Sample weight was taken as 5 mg. The glass transition temperature, Tg, was determined as the temperature at which the change in the heat flow curve (midpoint value).

2.9. Flow properties

Fish oil microcapsules (2 g) were poured through a funnel at a fixed distance from the ground to form a conical heap. The span angle of the heap was defined as the angle of repose (AOR). The AOR was calculated according to the measured base diameter, *d*, and the height of the heap,