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Physical properties and stability evaluation of fish oil-in-water emulsions stabilized using thiol-modified β-lactoglobulin fibrils-chitosan complex

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ABSTRACT

Fish oil-in-water emulsions containing fish oil, thiol-modified β-lactoglobulin (β-LG) fibrils, chitosan and maltodextrin were fabricated using a high-energy method. The results showed that chitosan coating induced charge reversal; denoting successful biopolymers complexation. A significantly ($p < 0.05$) larger droplet size and lower polydispersity index value, attributed to the thicker chitosan coating at the oil-water interface, were observed. At high chitosan concentrations, the cationic nature of chitosan strengthened the electrostatic repulsion between the droplets, thus conferring high oxidative stability and low turbidity loss rate to the emulsions. The apparent viscosity of emulsions stabilized using thiol-modified β-LG fibrils-chitosan complex was higher than those stabilized using β-LG fibrils alone, resulting in the former's higher creaming stability. Under thermal treatments (63 °C and 100 °C), emulsions stabilized using thiol-modified β-LG fibrils-chitosan complex possessed higher heat stability as indicated by the consistent droplet sizes observed. Chitosan provided a thicker protective layer that protected the oil droplets against high temperature. Bridging flocculation occurred at low chitosan concentration (0.1%, w/w), as revealed through microscopic observations which indicated the presence of large flocs. All in all, this work provided us with a better understanding of the application of protein fibrils-polysaccharide complex to produce stable emulsion.

1. Introduction

For decades, extensive research has been conducted on fish oil due to the diverse health advantages related to *n*-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Numerous studies have shown that the dietary consumption of fish oil enhances cardiovascular activity, anti-inflammatory response and nervous system functions (Shahidi, 2015; Ward & Singh, 2005). Despite its promising beneficial health effects, PUFA is highly susceptible to oxidative rancidity due to its high level of unsaturated carbon-carbon double bonds (Kagami et al., 2003). Therefore, it is essential to employ an efficient encapsulation system, such as an oil-in-water emulsion, to protect fish oil against oxidative deterioration and the consequent off-flavor. The stabilizers used in an emulsion system can provide a protective barrier between the fish oil and oxygen (or prooxidants). Proteins (such as chickpea, lentil and pea proteins) have been found to be effective in preventing the oxidation of emulsions and powdered products (Ho, Schroën, San Martín-González, & Berton-Carabin, 2017; Karaca, Nickerson, & Low, 2013). Besides, biopolymer coacervates, such as xanthan-locust bean gums (Khouryieh, Puli, Williams, & Aramouni, 2015) or low-methoxyl (LM) pectin and high-methoxyl (HM) pectin, have been reported to effectively reduce the formation of lipid hydroperoxides (Chen, McClements, & Decker, 2010).

Principally, an oil-in-water emulsion is a thermodynamically unstable colloidal dispersion system made up of two immiscible liquids; with the oil phase dispersed within an aqueous phase. To date, an emulsion system is considered as an important component in food processing. In emulsions containing *n*-3 PUFA, biopolymers are often used as a coating agent to provide better emulsion stability. Chitosan is

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an example of a natural biopolymer synthesized from crustacean shells through the alkaline deacetylation of chitin (Mohammed, Williams, & Tverezovskaya, 2013). Chitosan is composed of a linear structure of β-linked glucosamine and *N*-acetylglucosamine units. It exists as a cation under acidic conditions due to the protonation of its amino units. However, chitosan has limited applications in emulsion systems due to its high molecular weight (~1000 kDa). This results in its low solubility in acid-free or neutral media (Maeda & Kimura, 2004). Chitosan essentially acts as a non-surface active stabilizer in emulsion systems. Hence, the combination of chitosan with an effective emulsifier, such as β-lactoglobulin (β-LG), can bring out the advantageous properties of chitosan in achieving a stable emulsion. Previous studies have shown that the coacervate of β-LG and chitosan, formed at pH 6.0–6.5, resisted precipitation and remained stable as a colloidal suspension (Lee & Hong, 2009). Furthermore, it has been reported that the emulsion droplets coated with β-LG-chitosan complex exhibited higher stability towards flocculation as compared to those coated with β-LG alone. This observation was ascribed to the electrostatic and steric repulsions between the droplets, as conferred by the β-LG-chitosan complex (Hong & McClements, 2007).

As mentioned earlier, β-LG is an effective emulsifier that is widely used in emulsion systems. In practice, β-LG can be further processed to form fibrils under high temperatures (> 80 °C), acidic conditions (pH 2) and low ionic strengths. These β-LG fibrils have diverse functionalities and exhibit more promising results than globular β-LG as emulsifying and foaming agents. Unfortunately, the pH sensitivity of both β-LG fibrils and chitosan has limited their applications in food systems. Generally, protein is widely known to be vulnerable to changes in pH and ionic strength (Zhang et al., 2015). Flocculation of protein-stabilized droplets occurs at pH values close to the isoelectric point of the protein due to weakened electrostatic repulsion between the droplets. In addition, β-LG fibrils are highly susceptible to aggregation caused by the β-sheet destruction at high pH conditions (Jones et al., 2011). In contrast, chitosan is unstable at low pH, as evidenced by its rapid degradation at pH 1. Thus, it has been a major challenge to carry out the complexation of β-LG fibrils with chitosan (Shu & Zhu, 2002).

Therefore, in this study, we employed thiol-modified β-LG fibrils for the complexation with chitosan. The formation of thiol-modified β-LG fibrils, which are more hydrophobic in nature, is driven by the esterification process. The thiol-modified β-LG fibrils employed in this study carried a negative charge, while the chitosan used had a positive charge that is attributed to the presence of protonated amino groups. It was hypothesized that the complexation between the thiol-modified β-LG fibrils and chitosan is driven by the electrostatic attraction of these opposite charges. In the present study, we performed the complexation of thiol-modified β-LG fibrils with different concentrations of chitosan. The produced thiol-modified β-LG fibrils-chitosan complexes were then used as a stabilizer to produce fish oil-in-water emulsions via high pressure homogenization. The objective of this work was to examine the effects of the thiol-modified β-LG fibrils-chitosan complex on the physical properties and stability of the resulting emulsions.

2. Materials and methods

2.1. Materials

Whey protein isolate, BiPRO (~70% β-lactoglobulin), was obtained from Davisco Foods International Inc., USA. Chitosan (100–300 kDa) and 1-propanethiol (99%) were purchased from Sigma-Aldrich (USA); and omega-3 fish oil (18/12 TG) was purchased from Jedwards International, Ins. (USA). Analytical grade chemicals and deionized water were used throughout this study.

2.2. Preparation of thiol-modified β-lactoglobulin fibrils

Whey protein isolate (WPI) was dissolved in deionized water for 2 h under magnetic stirring to create a WPI stock solution (2.5%, w/w). The WPI solution was adjusted to pH 4.6 using 6 M HCl and centrifuged at 6000 × *g* (Thermo Fisher Scientific, Waltham, USA) for 30 min, followed by filtration through a 0.2 μm regenerated cellulose filter paper to remove insoluble materials. The filtered supernatant was readjusted to pH 2 using 6 M HCl and the supernatant was heated at 80 °C for 20 h under magnetic stirring (300 rpm) to form the β-LG fibrils. The fibril solution was immediately cooled to room temperature using an ice bath. Then, the fibril solution was adjusted to pH 8 or 9 using 6 M NaOH prior to the addition of thiol reagent. The addition of 1-propanethiol to the fibril solution was performed under magnetic stirring (350 rpm) for 24 h at a constant pH (pH 8 or 9) based on the 1-propanethiol:total carboxyl residue molar ratios of 0.5:1, 1:1, 2:1, 3:1 and 4:1. The molar ratio was calculated based on the average molecular weight of a carboxyl residue (45 g/mol) by considering 27 carboxyl residues per β-LG monomer.

2.3. Emulsion preparation

The chitosan solution was prepared by dispersing different concentrations of chitosan (0.1, 0.2, 0.3, 0.4 and 0.5%; w/w) in 0.06 M acetic acid solution overnight at room temperature under magnetic stirring to ensure complete dissolution. Then, the complexation of thiol-modified β-LG with chitosan was performed. The complex was made up of a fixed amount of thiol-modified β-LG fibrils (1%, w/w) and 0–0.5% (w/w) chitosan. To produce the fish oil-in-water emulsion, a coarse emulsion was firstly prepared by adding 10% (w/w) fish oil into an aqueous phase containing 1% (w/w) thiol-modified β-LG fibrils, 0–0.5% (w/w) chitosan and 15% (w/w) maltodextrin, and subjecting the mixture to high-shear homogenization at 7500 rpm for 5 min (Silverson L4R, Buckinghamshire, UK). Then, the coarse emulsion was immediately homogenized using a high-pressure homogenizer (Panda 2 K, Niro Soavi, Deutschland, Lubeck, Germany) at 750 bar for three cycles.

2.4. Droplet size and polydispersity index (PDI)

The droplet size and PDI of the emulsion samples were measured using a Zetasizer Nano ZS (Worcestershire, U.K.) instrument at 25 °C. The samples were diluted with pH-adjusted deionized water prior to the measurements.

2.5. Zeta potential measurement

Zeta potential (mV) measurement was performed by diluting samples with pH-adjusted deionized water (0.05%, v/v) and loading them into disposable folded capillary cells (DTS1070). Then, the zeta potential of the samples was determined by using a Zetasizer Nano ZS (Worcestershire, U.K.) instrument at 25 °C.

2.6. Rheological measurement

The rheological behavior of the samples was determined by means of a dynamic controlled stress rheometer (HAAKE Rheo Stress 600, Massahusetts, USA), equipped with a cone-and-plate geometry (cone diameter = 60 mm, cone angle = 1°, gap = 0.05 mm). The samples (1.5 mL) were measured at a pre-set shear rate of 0–100 s^{-1}.

2.7. Microscopic observation

The microstructure of the samples was observed using a conventional optical microscope (Nikon ECLIPSE 50*i*) equipped with a digital camera (Nikon, COOLPLIX 4500). In brief, a drop of sample was deposited onto a microscope slide and covered with a lid. It was then left to equilibrate for 1 min at room temperature prior to photomicrographic capture at 100 × magnification.