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Characterization of fish oil in water emulsion produced by layer by layer deposition of soy β-conglycinin and high methoxyl pectin

Ning Xiang, Yuan Lyu, Ganesan Narsimhan*

Department of Agricultural and Biological Engineering, 225 South University Street, Purdue University, West Lafayette, IN 47907, USA

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ABSTRACT

In this study we employed a layer-by-layer (LbL) deposition technique to produce fish oil in water emulsions encapsulated with a positively charged inner soy β-conglycinin (7S) layer by high shear mixing followed by deposition of a negatively charged outer high methoxyl pectin (HMP) layer using either high shear mixing or homogenization (500 or 3000 psi) and compared them with emulsions produced by 7S-HMP complex. The size, shape, morphology and wall structure of emulsion droplets were characterized by dynamic light scattering and microscopy. Emulsion produced by high shear mixing only were unstable with a droplet size of 7.48 μm. Stable emulsions were produced by high shear mixing followed by homogenization at 500 psi with significantly lower droplet size (3 μm) and uniform two layer shell thickness (80–170 nm) around the drops. The two layer shell thickness of emulsion drops produced by homogenization at 3000 psi was non uniform although it exhibited good long term storage stability. More diffuse distribution of 7S was observed in the adsorbed layer of emulsion droplets that were produced by 7S-HMP complex compared to LbL with a higher layer thickness and a lower ζ potential for the former thereby indicating a difference in morphology of adsorbed layers in the two cases. The results of this investigation seem to indicate that the mechanism of emulsion formation in the second homogenization step is closer to layer by layer deposition than stabilization by 7S-HMP complex and demonstrated a new method of LbL to utilize 7S as an emulsifier.

1. Introduction

The utilization of emulsions and emulsifiers is significantly important in food industry. Conventional emulsion is produced by mixing or homogenizing two immiscible liquids phases, for example, oil and water, in the presence of one or more emulsifiers (Guzey & McClements, 2006). However, the conventional method has limited stability under different environmental stresses, such as pH, temperature, ionic strength, *etc.* (Guzey & McClements, 2006). One strategy to improve the emulsion stability is to employ layer-by-layer (LbL) electrostatic deposition technique, which creates multiple layers of emulsifier and polyelectrolytes around oil droplets (Gu, Decker, & McClements, 2005; Ogawa, Decker, & McClements, 2003; Surh, Gu, Decker, & McClements, 2005). LbL deposition technique was first introduced by Decher et al. (Decher & Hong, 1991a; Decher & Hong, 1991b; Decher, Hong, & Schmitt, 1992) in early 1990s. The principle of LbL deposition is sequential adsorption of oppositely charged materials on a template to form polyelectrolyte shells (Guzey & McClements, 2006; Humblet-Hua, Scheltens, Van Der Linden, & Sagis, 2011; McClements, 2005). LbL technique is a simple, inexpensive and easily controllable method to prepare polyelectrolyte capsules (Peyratout & Dähne, 2004), it can be used to encapsulate a variety of macromolecular species such as proteins, nanoparticles, dye molecules bioactive lipids, antioxidants and antimicrobials (Decher, 1997; Guzey & McClements, 2006; Shahidi & Han, 1993; Yang, Trau, Renneberg, Yu, & Caruso, 2001), and it has been applied to various fields such as food, pharmaceuticals and cosmetics (Humblet-Hua et al., 2011). In this study, soy β-conglycinin (7S) and high methoxyl pectin (HMP) were used as outer layers to stabilize fish oil in aqueous phase emulsion.

7S was used as the primary stabilizer in the LbL process. 7S is one of the most abundant storage proteins in soy, which constitute

Abbreviations: 7S, soy β-conglycinin; CLSM, confocal laser scanning microscopy; CPD, critical point dying; ETD, Everhart–Thornley Detector; HMP, high methoxyl pectin; LbL, layer-by-layer deposition; SEM, scanning electron microscopy; ThT, Thioflavin T; TLD, Through the Lens Detector; WPI, whey protein isolates.

* Corresponding author.
E-mail addresses: xiangn@purdue.edu (N. Xiang), lv10@purdue.edu (Y. Lyu), narsimha@purdue.edu (G. Narsimhan).

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30–46% of the total water-extractable proteins in soy (Saio, Kamiya, & Watanabe, 1969). 7S has a large molecular mass of 180–210 kDa (Koshiyama, 1968) and compact globular structure at physiological pH which leads to a slow rate of diffusion onto an adsorption surface, as well as to a slow rate of increase in surface pressure, without significant interfacial denaturation (Santiago et al., 2008). The emulsifying activity of 7S, thereby, is expected to be poor (Phoon, San Martin-Gonzalez, & Narsimhan, 2014). However, there are two methods of improving adsorption and interfacial properties of 7S: reducing of protein size or denaturing globular structure so that more hydrophobic residues can be exposed. β-conglycinin consist of three major subunits: α, α′ and β, the isoelectric points (pI) for these three subunits are 4.90, 5.18 and 5.66–6.00 respectively (Thanh & Shibasaki, 1977). In this study, 7S was first dissolved in pH 7 solution then the pH was decreased to 3. At pH 3, 7S is expected to be denatured so that the surface activity of 7S is increased. The second layer of our choice is HMP. Pectin is widely utilized in food production as a soluble dietary fiber (Humblet-Hua et al., 2011), and it is reported to have health benefit (Sriamornsak, 2003). HMP has a pK_a value of about 3 because of its ionized carboxylic groups along the backbone. HMP is negatively charged in mildly acidic solution (Morris, Gidley, Murray, Powell, & Rees, 1980). The 7S and HMP solutions used were at pH 3, at which pH 7S absorbed to the surface of oil droplets with positive charges, while HMP carries opposite charges.

Microscopy methods were used in this study in order to investigate the size, shape, morphology and wall structure of the 7S and HMP covered emulsion droplets, confocal laser scanning microscopy (CLSM) can be used to determine the distribution of encapsulating materials on the emulsion droplets, by using different fluorophores or dyes to attach to the encapsulating materials (Humblet-Hua et al., 2011; Humblet-Hua, van der Linden, & Sagis, 2012; Sagis et al., 2008). Scanning electron microscopy (SEM) gives a direct and closeup image of the emulsion droplet and can be used to observe the encapsulating materials on the surface of droplets (Caruso & Möhwald, 1999a, 1999b; Reihs, Müller, & Lunkwitz, 2003; Sagis et al., 2008). Cryo-SEM provides a method to investigate the inner phase of microcapsules, and also can be used to determine the thickness of the shell wall by freeze-fracturing the droplets (Humblet-Hua et al., 2012).

Use of high pressure homogenization in this technique is important in many food applications in order to reduce the drop size and therefore increase the shelf life and improve the product texture. To the best of our knowledge, this is the first study in which high pressure homogenization was employed in the second step of layer-by-layer (LbL) deposition technique for deposition of negatively charged polysaccharide onto emulsion droplet that was already coated with positively charged protein with high shear mixing in order to make oil in water emulsion. High pressure homogenization was not applied in the first step for easier separation of cream layer before the subsequent homogenization step. Previous studies of LbL have employed only high shear mixing for both the steps (Humblet-Hua et al., 2011; Humblet-Hua et al., 2012; Sagis et al., 2008) and homogenization for the first step in LbL deposition. Lee et al. (Lee, Lefèvre, Subirade, & Paquin, 2009) reported using homogenization (2000 psi) as the first step to prepare the primary emulsion, before the addition of secondary layer; 40,000 g centrifugation for 60 min was employed to separate the cream layer. Guzey and McClements (Güzey & McClements, 2006) also reported using high pressure homogenization as the first step. However, in order to provide stable emulsion, the flocs that were formed by excess protein were disrupted by rehomogenization for two passes (2000 psi and 200 psi). Preventing bridging flocculation is essential in the production of LbL emulsion. Using homogenization as the first step to produce desired small emulsion droplets requires a more difficult separation step to isolate the cream layer. In order to circumvent this, we investigated the employment of homogenization as the second step to produce stable LbL emulsion. In this study, we employed the above technique to produce fish oil in water emulsions encapsulated with a positively charged inner soy β-conglycinin (7S) layer by high shear mixing followed by deposition of a negatively charged outer high methoxyl pectin (HMP) layer using homogenization (500 or 3000 psi). The size, shape, morphology and wall structure of 7S and HMP encapsulated emulsion droplets were characterized.

2. Materials and methods

2.1. Materials

Defatted soy flour (Hodgson Mill, USA), fish Oil (CAS No. 8002-50-4, Sigma-Aldrich, USA), HMP (Modernist Pantry, USA), Thioflavin T (ThT) (CAS No. 2390-54-7, Sigma-Aldrich, USA). All other chemicals were of analytical grade and purchased from Sigma-Aldrich.

2.2. Methods

2.2.1. Purification of soy 7S

The method of purification of soy 7S was adapted from (Howard, Lehnhardt, & Orthoefer, 1983) and (Phoon et al., 2014). The protein content in defatted soy flour was 46.7%. The soy flour (5%, w/v) was dissolved in 10 mM sodium phosphate buffer at pH 8 overnight. The solution was centrifuged at 500 × *g* for 15 min to remove undissolved materials. 0.03 M of sodium chloride and 0.77 mM sodium bisulfite was added to the supernatant in order to precipitate 11S (Howard et al., 1983). The pH of supernatant was then adjusted to 6 using ≤5 N hydrochloric acid and ≤5 N sodium hydroxide. Then, the mixture was centrifuged at 500 × *g* for 15 min, the supernatant was adjusted to pH 5.5, then centrifuged at 500 × *g* for 15 min, pH of the supernatant was adjusted to 4.5, centrifuged again at 500 × *g* for 15 min. Next, the precipitate was redissolved in 10 mM sodium phosphate buffer at pH 7. Redissolved protein was subjected to dialysis overnight in distilled water for three times, using a Spectra/Por® 6 dialysis membranes (MWCO 50000, Spectrum®, USA). All the steps described above were performed at room temperature. After dialysis, the protein was freeze-dried and stored at −20 °C.

2.2.2. Emulsion preparation

The emulsion was prepared with 0.5% (w/v) 7S dissolved in 10 mM phosphate buffer, pH 7. Then, 2% (v/v) fish oil was added to the above 7S solution. Coarse emulsion was made by mixing with a rotor–stator mixer (CAS No: 225326, Virtishear, Virtis, Gardiner, N.Y., U.S.) at a speed setting of 30 for 10 min. To remove the non-adsorbed 7S, the sample was centrifuged at 1000 × *g* for 15 min. The concentrated cream was centrifuged again at 1000 × *g* for 1 min to remove excess solution. Then the 0.5% (v/v) positively charged droplets (cream) were dispersed in a pH 3 citric buffer (10 mM) or a HMP solution that was made by adding 0.05% (w/v) pectin into pH 3 citric buffer (10 mM), followed by mixing with a rotor–stator mixer at a speed setting of 30 for 10 min or homogenization (GEA Panda Plus 2000, USA) at 500 and 3000 psi under 3 passes, respectively.

2.2.3. Light scattering measurements

The average diameter of the homogenized emulsion sample was determined by light scattering. The drop size distribution in the diluted sample (1:10) was measured by ZetaSizer Nano ZS90 (Malvern Instruments Ltd., UK) at 25 °C with optical arrangement at