



Characteristics and oxidative stability of soy protein-stabilized oil-in-water emulsions: Influence of ionic strength and heat pretreatment



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ABSTRACT

Some physicochemical characteristics, microstructure and stability of native and preheated (95 °C, 15 min) soy protein isolate (SPI)-stabilized emulsions, formed at varying protein concentrations (c ; 0.5–4.0%, w/v) in the absence or presence of 300 mM NaCl, were characterized. The emulsifying ability, flocculated state of droplets, microstructure, interfacial protein concentration (Γ) of the fresh emulsions, as well as stability of these emulsions against coalescence, flocculation, creaming and even lipid oxidation upon storage up to 2 weeks were evaluated. In general, increasing c was favorable for the emulsification efficiency, but the flocculated state of oil droplets or size of the flocs in the fresh emulsions was more affected by the presence of salt, and/or the heat pretreatment. Increasing ionic strength or application of a heat pretreatment resulted in remarkable increases in extents of droplet flocculation in the fresh emulsions, as well as amount and concentration of adsorbed proteins at the interface. All the emulsions exhibited an extraordinary stability against coalescence and/or flocculation. Increasing c led to a progressive increase in stability against creaming, especially for the preheated SPI emulsions with 300 mM NaCl. All the emulsions at $c = 1\%$ or above exhibited a similarly high oxidative stability upon storage up to 9 days. Even at $c = 0.5\%$, the oxidative stability of the formed emulsions could be greatly improved by increasing ionic strength, and/or application of a heat pretreatment. The findings have important implications for the development of an important kind of protein-stabilized emulsions with industrial relevance.

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1. Introduction

Soy proteins have been widely applied in a wide range of food formulations, due to its good nutritional, and functional properties, and even health effects (FDA, 1999). Besides the excellent heat-induced gelling properties, these proteins also exhibit good emulsifying properties (Akoi, Taneyama, & Inami, 1980; Hettiarachchy & Kalapathy, 1998). To date, the emulsifying properties of soy proteins are mainly utilized as processing aids in concentrated emulsions, such as meat emulsions in the comminuted meat field, and their use as an emulsifying agent in dilute emulsion products is very limited. One of the main reasons causing this situation is that soy proteins are a mixture of multi-components (e.g., 7S and 11S globulins) with complex oligomeric structure, and their properties

not only highly dependent on the type, composition and nature of the proteins, e.g., extent of thermal denaturation and/or aggregation, but also affected by a variety of processing and environmental parameters, e.g., pH, temperature, salts and the presence of other components. For emulsions to be evaluated, the choice of emulsification or homogenization technique is also important for the emulsion properties. Thus, it is not surprising that much less information is available addressing the emulsion properties of soy protein products as compared to milk proteins, e.g., whey protein and caseins (Damodaran, 2005; Dickinson, 1994; McClements, 2004).

Using a shear homogenization as the emulsification technique (e.g., 20,000 rpm, 30 s), Wagner and coworkers mainly investigated the influence of protein denaturation and protein concentration (c) on the coalescence stability of soy proteins (whey and isolate)-stabilized emulsions, formed at oil fraction (ϕ) = 0.25–0.33 and $c = 0.1$ –1.0% (w/v), and found that 1) irrespective of the applied c , native soy protein emulsions exhibited better stability against coalescence than those stabilized by denatured proteins; 2) the

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presence of NaCl (e.g., 0.15 M) improved the coalescence stability of these emulsions, especially in the case at higher c values (e.g., $>0.5\%$) (Mitidieri & Wagner, 2002; Palazolo, Mitidieri, & Wagner, 2003). In these works, the creaming stability was not addressed, though the emulsions formed under the conditions investigated might be very unstable against creaming. In a following work addressing this issue, they observed a seemingly contrasting phenomenon that the cream phase of denatured soy protein emulsions was more stable against coalescence and flocculation than that of native protein counterparts (Palazolo, Sorgentini, & Wagner, 2005). The inconsistency might be due to the differences in interfacial nature and flocculated state of droplets in the system. They attributed the higher stability in the cream phase (of denatured proteins-stabilized emulsions) to formation of hydrated flocs with a gel-like network structure (Palazolo et al., 2005).

On the other hand, several studies have been available addressing the properties and microstructure of soy protein-stabilized emulsions, produced by a series of emulsification techniques with high energy levels (Floury, Desrumaux, & Legrand, 2002; Puppo, Sorgentini, & Añón, 2003; Roesch & Corredig, 2002, 2003). In these works, the applied c was relatively high ranging from 1 to 10%, with ϕ varying within the range 0.1–0.6. The test soy proteins included soy protein concentrate (SPC), soy protein isolate (SPI) and soy 11S globulin. Interestingly, it was found that the creaming stability of the emulsions could be greatly improved by increasing the c or ϕ , and even a preheating treatment of the proteins prior to the emulsification. The improvement of creaming stability was largely attributed to gel-like structure of the emulsions (Roesch & Corredig, 2002, 2003). In a recent work of ours, we characterized the rheological properties and microstructure of these gel-like emulsions stabilized by unheated and preheated SPI, at a relatively high c value of 6% and varying ϕ values of 0.2–0.6, and found that increasing the ϕ , or increasing the ionic strength progressively increased the stiffness of these gel-like emulsions (Tang & Liu, 2013). Thus, the gel-like network formation seems to be highly associated with bridging flocculation of oil droplets, through inter-droplet hydrophobic interactions of the proteins adsorbed at the interface (Puppo et al., 2003; Tang & Liu, 2013).

Based on the mentioned-above works, it can be generally recognized that the stability of soy protein emulsions, e.g. against coalescence and creaming, is not only highly related to the denatured state of proteins, the applied c and ionic strength in the aqueous phase and ϕ , but also depends on the energy level of emulsification technique. However, we can see that the properties and stability of soy protein emulsions at relatively low c and ϕ values, produced by a high energy level of emulsification, are little characterized. The development of these dilute soy protein emulsions has important implications for many food formulations, e.g. soy protein beverages with emulsified ingredients.

Therefore, the present work was to systematically investigate the influence of c in the aqueous phase (0.5–4.0%, w/v), the presence of 300 mM NaCl and/or a heat pretreatment (at 95 °C for 15 min) on the stability against coalescence, flocculation and creaming of SPI-stabilized emulsions. The emulsions were formed by microfluidization at a specific ϕ value (0.2). The emulsifying ability (or efficiency), flocculated state of oil droplets in fresh emulsions, and coalescence, flocculation and creaming stability were evaluated. To help understand the mechanism for the emulsion stability, some physicochemical parameters including zeta-potential and amount (and concentration) of adsorbed interfacial proteins of droplets, as well as microstructure of the fresh emulsions were also evaluated. On the other hand, oxidative stability of oils in the SPI emulsions upon storage was also evaluated, due to the consideration that lipid oxidation can occur rapidly in oil-in-water emulsions due to their large surface area that facilitates

lipid oxidation and subsequent rancidity of the emulsions (Waraho, McClements, & Decker, 2010).

2. Materials and methods

2.1. Materials

SPI was prepared from defatted soy flour, provided by Shandong Yuwang Industrial and Commercial Co. Ltd. (China), according to the same process described in the previous work (Tang, Chen, & Foegeding, 2011). The protein content was 91.5% on the wet basis, as determined by the Kjeldahl method ($N \times 6.25$). Nile Blue A and Nile Red was obtained from Sigma–Aldrich (Sigma Chemical Co., St. Louis, MO, USA). Sodium dodecyl sulfate (SDS), isooctane, isopropanol, butanol, methanol, trichloroacetic acid, thiobarbituric acid, ammonium thiocyanate, cumene hydroperoxide, and 1, 1, 3, 3-tetraethoxypropane were purchased from Aladdin Reagent Corporation (Shanghai, China). Bovine serum albumin (BSA) was obtained from Fitzgerald Industries International Inc. (Concord, MA, USA). Soy oil was purchased from a local supermarket in Guangzhou (China). All other chemicals, used with no further purification, were of analytical grade.

2.2. Preparation of oil-in-water emulsions

All the emulsions, stabilized by native or preheated SPI, were prepared at an oil volume fraction of 0.2, with varying c values of 0.5–4.0% (w/v) in the continuous phase. Two sets of the unheated SPI dispersions at a specific c value were prepared by dissolving the SPI powder in de-ionized water and stirring using a magnetic stirrer for 2 h at room temperature. The dispersions were then stored overnight at 4 °C to allow complete hydration. Sodium azide (0.02%, w/v) was used as an antimicrobial agent. The preheated SPI dispersions were prepared by heating one set of the above unheated SPI dispersions, in a water bath at 95 °C for 15 min, and then immediately cooling in ice bath to room temperature. After that, each set of the SPI dispersions (unheated and preheated) were further divided into two subsets, with one subset of SPI dispersions added with 300 mM NaCl. The salt was added by mixing the NaCl powder with the dispersions under stirred conditions, little by little. Finally, the pH of all the SPI dispersions was adjusted to 7.0 using 1 M NaOH or 1 M HCl, if necessary.

For the emulsion formation, any SPI dispersion with a constant volume (about 50 mL) was mixed with soy oil at $\phi = 0.2$. The mixtures were pre-homogenized using a high-speed dispersing and emulsifying unit (model IKA-ULTRA-TURRAX T25 basic, IKA Works, Inc., Wilmington, NC) at 10,000 rpm for 2 min, to produce coarse emulsions. Then, the obtained coarse emulsions were further homogenized through a Microfluidizer (M110EH model, Microfluidics International Corporation, Newton, MA) for one pass at a pressure level of 40 MPa. To help identification, the obtained emulsions from the four kinds of SPI dispersions were denoted as the emulsions I–IV as follows: I, native SPI without 300 mM NaCl; II, native SPI with 300 mM NaCl; III, preheated SPI without 300 mM NaCl; IV, preheated SPI with 300 mM NaCl. All the obtained emulsions were directly subject to analysis.

2.3. Determination of volume-average droplet size ($d_{4,3}$)

The volume-average droplet size ($d_{4,3}$) of freshly prepared or stored (24 h) SPI emulsions were determined using a Malvern MasterSizer 2000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK). Distilled water, or 1% (w/v) SDS solution was used as the dispersant. The relative refractive index of the emulsion was taken as 1.095, that is, the ratio of the refractive index of soy oil

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