



# Freeze-thaw stability of oil-in-water emulsions stabilized by soy protein isolate-dextran conjugates



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## ABSTRACT

The soy protein isolate-dextran (SPI-D) conjugates were prepared by ultrasound (power output 500 W for 40 min) and microwave (power output 800 W for 2 min) assisted glycosylation to improve the freeze-thaw stability of soy protein isolate stabilized oil-in-water (o/w) emulsions. Fourier transform infrared and fluorescence emission spectroscopy analyses confirmed that covalent bonds were formed between soy protein isolate (SPI) and dextran molecules through the Maillard reaction. The stability of SPI, SPI+D mixture, ultrasound SPI-D conjugates (SPI-D<sub>U</sub>) and microwave SPI-D conjugates (SPI-D<sub>M</sub>) emulsions subjected to from one to three freeze-thaw cycles was investigated. In comparison with SPI and SPI+D emulsions, SPI-D<sub>U</sub> and SPI-D<sub>M</sub> emulsions exhibited smaller creaming index, oiling off, droplet diameters, flocculation degree (FD) and coalescence degree (CD) after each freeze-thaw cycle. In addition, the zeta potential and specific surface area (SSA) were greater. Appearance and microstructure indicated that SPI-D<sub>U</sub> and SPI-D<sub>M</sub> emulsions exhibited a relative stable state after three freeze-thaw cycles.

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## 1. Introduction

Soy protein isolate (SPI) has been widely utilized as an emulsifier to stabilize an oil-in-water (o/w) emulsion due to the surface-active properties of  $\beta$ -conglycinin and glycinin (Palazolo, Sobral, & Wagner, 2011). Nevertheless, most of emulsions prepared with native protein became physically unstable and even completely broken down into oil and aqueous phase after freezing and thawing. When the emulsions are stored at  $-18\text{ }^{\circ}\text{C}$ , all kinds of physicochemical processes could occur, including freeze concentration, ice formation, fat crystallization, biopolymer conformational changes and interfacial phase transitions (McClements, 2004; Ghosh & Coupland, 2008; Donsì, Wang, & Huang, 2011). These changes may lead to creaming, sedimentation, oiling off, Ostwald ripening, flocculation, coalescence and phase separation of the emulsions (Magnusson, Rosén, & Nilsson, 2011), which limit the

utilization of SPI in frozen food. In recent years, there emerges increasing potential applications for emulsions that can be frozen and then thawed prior to use, such as ice cream, mayonnaise and frozen food industry (Thanasukarn, Pongsawatmanit, & McClements, 2006). Freezing storage can prevent microorganisms growth, maintain the chemical stability while extending the shelf life of foods. Hence, it is of great significance to improve the freeze-thaw stability of SPI emulsions.

Palazolo et al. (2011) reported that increasing protein concentration could improve the freeze-thaw stability of emulsions by decreasing initial flocculation and coalescence. Ice crystal growth could be inhibited by adding pectin,  $\iota$ -carrageenan (Mun, Cho, Decker, & McClements, 2008), sucrose (Gu, Decker, & McClements, 2007) and maltodextrin (Mao, Roos, & Miao, 2015) to the protein emulsions. Thus, increase the amount of unfrozen water and decrease the freezing point of the aqueous phase in the freezing conditions. Additionally, the Maillard reaction products of soy protein-dextran (Diftis & Kiosseoglou, 2006) and soy protein-soluble polysaccharide (Yang et al., 2015) used as emulsifiers could improve emulsifying properties and physical stability of emulsions, especially in the reduction of droplet diameters and emulsions stabilization against creaming. The protein-sugar conjugates could adsorb to the interface together with un-reacted protein constituents, enhancing steric stabilization forces among oil droplets (Diftis & Kiosseoglou, 2006; Diftis, Biliaderis, &

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Kiosseoglou, 2005). Indeed, it has been demonstrated that sodium caseinate-maltodextrin conjugates (Regan & Mulvihill, 2010) and whey protein isolate-dextran conjugates (Xu et al., 2010) brought about a significant improvement in freeze-thaw stability compared with the control protein and protein-sugar mixture. However, to the best of our knowledge, no studies about the freeze-thaw stability of emulsions stabilized by SPI-dextran conjugates have been reported.

The functional characteristics of SPI can be modified by a variety of methods. Ultrasound and microwave technologies could not only initiate Maillard reaction, but also increase the yield and speed up the rate of reaction. The acceleration effect of ultrasound treatment on the  $\beta$ -conglycinin-maltodextrin (Zhang, Chi, & Li, 2013) and mung bean protein isolate-glucose (Wang, Han, Sui, & Qi, 2015) Maillard reaction have been investigated. The SPI-saccharide (Guan, Qiu, Liu, Hua, & Ma, 2006) and whey protein isolate-lactulose (Nooshkam & Madadlou, 2016) conjugates have also been prepared by microwave-assisted glycosylation.

In the present study, in order to improve the freeze-thaw stability of SPI emulsions, the soy protein isolate-dextran (SPI-D) conjugates were prepared by ultrasound and microwave, respectively, and their structures were characterized. The stability of emulsions stabilized by SPI, SPI+D mixture, ultrasound SPI-D conjugates (SPI-D<sub>U</sub>) and microwave SPI-D conjugates (SPI-D<sub>M</sub>) subjected to 1, 2 or 3 freeze-thaw cycles was studied in this research.

## 2. Materials and methods

### 2.1. Materials

Defatted soy flour was obtained from Harbin High Tech Co., Ltd. (Heilongjiang, China). Dextran (40 kDa) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium dodecyl sulphate (SDS) and Sudan III were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

### 2.2. Preparation of soy protein isolate

SPI was prepared from defatted soy flour according to the method of Qi, Hettiarachchy, and Kalapathy (1997). The SPI had a protein content of 90.45 g/100 g, as determined using the Kjeldahl method.

### 2.3. Preparation of soy protein isolate-dextran conjugates

SPI (4 g/100 mL) and dextran (6 g/100 mL) were dispersed in phosphate buffer solution (0.01 M, pH 8.0), stirred at room temperature for 2 h, then stored overnight at 4 °C to ensure a complete hydration protein and dextran. Then, the dispersions were stirred until reaching room temperature. An ultrasound processor (XH-2008D, XiangHu Science and Technology Development Co., Ltd., Beijing, China) equipped with an 8 mm diameter titanium probe was used to treat 100 mL dispersions in a flat bottom conical flask which was immersed in a water bath device at 80 °C. Sample was treated at 25 kHz at the power output 500 W for 40 min. A flat bottom conical flask contained 100 mL dispersions was placed in a microwave oven (KD23B-DA, Midea Group, Guangdong, China) and irradiated at 2450 MHz, 800 W power output for 2 min. The dispersions were cooled rapidly in an ice-water for 5 min to stop the reaction, and then the SPI+D mixture, ultrasound and microwave Maillard reaction dispersions were dialysed at 4 °C for 24 h. The SPI+D (as the control), SPI-D<sub>U</sub> and SPI-D<sub>M</sub> powders were obtained by freeze-drying and stored at –18 °C until analyzed.

### 2.4. Characterization of soy protein isolate-dextran conjugates

#### 2.4.1. Fourier transform infrared spectroscopy

The freeze-dried samples (2 mg) were blended with 200 mg KBr powder, and then produced by pressing in KBr windows at room temperature on a Carver press at 5–6 T pressure before measurement. The IR spectra were recorded on an 8400S FTIR spectrometer (Shimadzu Corporation, Japan) with 64 scans at 4 cm<sup>-1</sup> resolution from 4000 to 400 cm<sup>-1</sup>.

#### 2.4.2. Intrinsic fluorescence emission spectroscopy

The intrinsic fluorescence emission spectra of the protein dispersions (0.25 g/100 mL) were obtained by a Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Ltd, Tokyo, Japan). The excitation wavelength was 347 nm, slit was 5 nm for both excitation and emission, and the emission spectra were recorded from 375 to 550 nm with a constant scan speed of 240 nm/min.

#### 2.4.3. Scanning electron microscopy

The freeze-dried samples were ground slightly and coated with gold to a thickness of 15 nm using an ion sputter (Hitachi, Ltd, Tokyo, Japan). The morphology of the samples was observed under a S-3400 scanning electron microscope (Hitachi, Ltd, Tokyo, Japan) at an accelerating voltage of 5 kV.

### 2.5. Preparation of oil-in-water emulsions

Protein samples (protein concentration 1.5 g/100 mL) were dissolved in sodium phosphate buffer (0.01 M, pH 7.0) with stirring for 2 h at room temperature to ensure complete dissolution, followed by adding sodium azide (0.02 g/100 mL) to inhibit microorganisms growth. Emulsions were prepared by mixing the protein suspensions and soy oil (volume fraction 0.2) at the speed of 11,000 rpm for 1 min by a T18 Basic high-speed blender (IKA, Staufen, Germany) and followed by using a high pressure homogenizer (Stansted Fluid Power Company, UK) at 60 MPa for 2 passes (Mao et al., 2015; Yang et al., 2015). The emulsions were immediately transferred to glass tubes (diameter 20 mm, height 160 mm).

### 2.6. Freeze-thaw treatment

The emulsions (20 mL) transferred to glass tubes were immediately stored in a freezer at the temperature of –18 °C for 22 h. After freezing, samples were thawed by placing them in a water bath at 20 °C for 2 h (Mun et al., 2008). These freeze-thaw procedures were repeated three times and the freeze-thaw stability of emulsions was measured after each cycle.

### 2.7. Evaluation of the freeze-thaw stability

#### 2.7.1. Creaming index

After each freeze-thaw cycle, the emulsions separated into two layers. The height of the serum layer ( $H_S$ ) at the bottom and the total height ( $H_T$ ) of the emulsion were measured by a ruler. The creaming index (CI) of emulsions was expressed as:

$$CI(\%) = (H_S/H_T) \times 100 \quad (1)$$

#### 2.7.2. Oiling off

The oiling off of the emulsions after freeze-thaw was determined by the method reported by Palanuwech, Ponitani, Roberts, and Coupland (2003).

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