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Enhanced CaSO₄-induced gelation properties of soy protein isolate emulsion by pre-aggregation



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ABSTRACT

The effects of $CaSO_4$ -induced pre-aggregation on the rheological and structural properties of soy protein isolate (SPI) emulsion gels were investigated. As the Ca^{2+} concentration during pre-aggregation increased (from 0 mM to 7.5 mM), the elastic modulus of the gels showed substantial increase, indicating stiffer gel structures. Large-deformation rheology suggested stronger but more brittle networks formed at higher Ca^{2+} concentration during pre-aggregation. However, when the pre-aggregated Ca^{2+} concentration reached 10 mM, the corresponding gel became weaker. Water-holding capacity (WHC) of the gels were significantly improved via the pre-aggregation process. The differences in rheological properties and WHC among the gels were consistent with the variation in their microstructures. Pre-aggregation helped to form denser and more uniform structures with thicker strands, whereas over aggregation made the gel network coarser.

1. Introduction

Soy cheese, also called "tofu" in Asian countries, has attracted increasing attention in recent years because of its bland taste and nutritional advantages with low levels of saturated fat and cholesterol (Ono, 2003). Traditional soy cheese is made from soy milk in a process that usually involves soaking, grinding beans in water, filtering, heating, coagulating, breaking the curd, and finally pressing and reforming the gel (Hou, Chang, & Shih, 1997). The process is complex and time consuming (Kamizake, Silva, & Prudencio, 2016). Soy protein isolate (SPI) is widely used in the food industry. During SPI extraction, most components such as lipids, soybean oligosaccharides, and isoflavones, which have been assumed to be the source of soy off-flavors, allergens, and flatulence factors, respectively, to some people, are washed out (Visser & Thomas, 1987). Hence, using SPI as a raw material for soy cheese has gained considerable attention because it makes the cheesemaking process simpler, cleaner, and more controllable compared with traditional methods (Murekatete, Hua, Chamba, Djakpo, & Zhang,

Acid-induced and salt-induced are the two major methods involved in the formation of soy cheese. Previous studies have found that GDL and CaSO₄ can produce more uniform soy curds with much smoother structure than those produced from other coagulates (e.g., MgCl₂, MgSO₄, and CaCl₂) (Kao, Su, & Lee, 2003), but GDL was not suitable for

Chinese-style tofu because of its sour flavor. In the formation of soy cheese, coagulation of soymilk is the most important step because it directly determines the quality of the product (Hou et al., 1997). It has been proposed that in a Ca²⁺-induced gelation process, the coagulation can be divided into two steps: (1) the soy protein forms particles (aggregates) at low calcium concentration and (2) the soluble proteins are bonded to the network by further Ca²⁺ addition (Guo & Ono, 2005; Ono, Katho, & Mothizuki, 1993). The aggregates formed in the first step could therefore define the final gel properties.

In fact, numerous studies have been conducted to understand the relationships between the structural and textural properties of soy cheese and have suggested that the microstructure of soy cheese is affected by the protein aggregate properties (e.g., size and content) because protein aggregates are related to the thickness of gel strands and the density of the network (Doi, 1993; Lakemond et al., 2003; Lu, Lu, Yin, Cheng, & Li, 2010). Our previous study also demonstrated that larger and/or more protein aggregates helped to formed stronger gels with denser and more compact structures (Wang et al., 2017). However, most previous research has focused on investigating the factors that influence the heat-induced protein aggregation or gelation, such as preheating conditions (i.e., preheating methods, heating temperature, and holding time), pH, and ionic strength (Lu et al., 2010; Renkema, Gruppen, & Van Vliet, 2002; Zhao, Li, Qin, & Chen, 2015). Whereas for salt-induced gelation, very few studies concerning the effects of protein

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aggregation induced by the coagulant itself on the gel properties are reported. The addition of Ca²⁺ screens electrostatic interactions between charged protein molecules and promotes the protein aggregation (Li et al., 2009). Additionally, the combination of Ca²⁺ and soy protein can be described as H⁺/Ca²⁺ exchanges (Canabady-Rochelle, Sanchez, Mellema, & Banon, 2009), which also neutralize electrostatic repulsion and form salt bridges, thus allowing protein molecules to form aggregates and eventually leading to the formation of three-dimensional network (Lu et 2010; al.. Maltais. Remondetto. Gonzalez, & Subirade, 2005).

In the present study, a two-step addition of the coagulate method is proposed, with a traditional one-step control, to estimate the impact of ${\rm Ca}^{2+}$ -induced pre-aggregation of SPI emulsion on its gelation properties. In the first step, a small amount of ${\rm Ca}^{2+}$ was added into the SPI emulsion to make the emulsion partially aggregated (pre-aggregation). In the second step, the remaining ${\rm Ca}^{2+}$ was further added to form the final gel network (gelation). The effects of the ${\rm Ca}^{2+}$ concentration during pre-aggregation on the microstructure, rheological and physiochemical properties of the gels were investigated.

2. Materials and methods

2.1. Materials

SPI was extracted from defatted soybean meals (Taiwan 292, harvested in 2015) according to the method described by Guo et al. (2015). The protein content of the SPI was 92.3% (dry basis) determined by the Kjeldahl method. Soy oil and nail oil was purchased from a local supermarket; Nile red, and Rhodamin B were obtained from Sigma-Aldrich (St. Louis, Mo, USA). All other chemicals in the study were of analytical grade.

2.2. Preparation of SPI emulsions

A 60 mg/mL SPI dispersion (pH 7.0, adjusted with 0.1 N NaOH or HCl) was prepared by dispersing SPI powder into deionized water, stirring mechanically at room temperature for at least 2 h and centrifuging at 10,000g for 10 min to remove insoluble materials. The dispersion was subjected to heat treatment at 95 °C for 15 min and then cooled to room temperature in an ice bath. The pretreated SPI dispersion was mixed with 5% (v/v) soy oil and pre-homogenized using a disperser homogenizer (T 18 basic ULTRA-TURRAX*, IKA Corp., Staufen, Germany) at 13,500 rpm for 2 min, followed by homogenization through a homogenizer (AH-BASIC, ATS Engineering Inc., Canada) at 40 MPa for one pass.

2.3. Gel preparation

2.3.1. Pre-aggregation of SPI emulsion

The pre-aggregation treatment was conducted before the gelation process. To do this, the SPI emulsion was mixed with a stock $CaSO_4$ dispersion to Ca^{2+} concentrations of 0, 2.5, 5, 7.5 and 10 mM. The emulsion/ $CaSO_4$ mixtures were mechanically stirred at 300 rpm at room temperature and allowed to aggregate for 1 h.

2.3.2. Gelation of SPI emulsion

After the pre-aggregation process, additional $CaSO_4$ was added to the samples to reach the total Ca^{2+} concentration of 35 mM. The mixtures were heated to 80 °C and allowed to coagulate for 30 min in a water bath. After coagulation, the gels were immediately cooled to room temperature in an ice bath and stored at 4 °C.

2.4. Evaluation of emulsion characteristics after pre-aggregation

2.4.1. Zeta (ζ) potential

The emulsions were diluted to a protein concentration of 5 mg/mL

of with 0.05 M phosphate buffer (pH 7.0). One milliliter of each diluted sample was put in an electrophoresis cell (DTS 1060, Malvern Instruments Ltd.) and the ζ -potential was measured using a Malvern Zetasizer Nano ZS90 (Nano ZS, Malvern Instruments, Worcestershire, UK). All measurements were replicated at least 3 times under room temperature.

2.4.2. Oil droplet size

The oil droplet size of the SPI emulsions was determined by a particle size analyzer (Microtrac S3500, Microtrac Inc., North Largo, FL, USA). Distilled water was used as the dispersant. The relative refractive index of the emulsion was taken as 1.095 (Tang, Chen, & Foegeding, 2011). The volume-average diameter $d_{4,3} (\sum_i^n d_i^4 / \sum_i^n d_i^3)$, where n_i is the number of particles with diameter d_i) was recorded.

2.5. Dynamic oscillatory measurements

2.5.1. Small amplitude oscillation

The viscoelastic properties of the SPI emulsion gels were characterized by a controlled-stress rheometer (HAAKE MARS III, Thermo Fisher Scientific, Karlsruhe, Germany) with a parallel plate (d = 35.002 mm, gap = 1 mm), using temperature sweep and frequency sweep mode. The emulsions were immediately loaded between the plates of the rheometer after the addition of CaSO₄. Low-viscosity silicon oil was used to prevent water evaporation. The gels were oscillated at 1% strain (within the linear viscoelastic region, LVR) and a frequency of 1 Hz. The temperature was heated from 25 °C to 80 °C at 5 °C per minute, followed by incubation at this temperature for 30 min before cooling to 25 °C at 5 °C per minute. The storage modulus (G') and loss modulus (G'') were recorded. After the gelling process was completed, frequency sweep tests were carried out at 25 °C using an angular frequency (ω) of 1–100 rad/s at a constant strain of 1%.

2.5.2. Large-scale deformation

Large-scale deformation tests were performed at 25 $^{\circ}$ C using the same rheometer in an oscillatory amplitude sweep mode. The strain was increased from 0.1% up to the fracture point when the stress began to decrease at a frequency of 1 Hz. The shear stress was recorded as a function of strain.

2.6. Water-holding capacity (WHC)

The WHC of the gels was determined according to the method of Wu, Xiong, Chen, Tang, and Zhou (2009), Approximately 5 g of gel (each sample) was transferred to 50 mL centrifuge tubes and centrifuged at 10,000g for 15 min at 4 °C. WHC (%) was defined as the ratio of the water weight in the pellet to the water weight in the original gel multiplied by 100.

2.7. Confocal laser scanning microscopy (CLSM)

Samples for CLSM analysis were prepared in single concave slides (Sail Brand, Jinliu Instrument Co., Ltd., Nanjing, China) covered with nail oil to prevent water evaporation. Rhodamine B and Nile red were used as fluorescence dyes for protein and oil phases (5 mL of stock emulsion + 0.05 mL of 0.1% (w/w) fluorescence dye), with excitation wavelengths at 552 and 488 nm, respectively. The gelation process was carried out as mentioned Section 2.3. The CLSM images were obtained by sequential scan (TCS SP8, Leica Microsystems Inc., Heidelberg, Germany) with a $63 \times$ magnification lens.

2.8. Scanning electron microscope (SEM)

The microstructure of the SPI emulsion gels were also examined by SEM (FEI Quanta 200, FEI Company, Hillsboro, OR, USA). The samples were prepared according to the method described in our previous study

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