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Improvement of gelation properties of soy protein isolate emulsion induced by calcium cooperated with magnesium

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ABSTRACT

The aim of this study is to investigate the cooperation effects of calcium ($CaSO_4$) and magnesium ($MgCl_2$ and $MgSO_4$) salts on the rheological and microstructural properties, and water holding capacity (WHC) of soy protein isolate (SPI) emulsion-based tofu gels. Small-deformation rheology indicated that the aggregation power of the three coagulants follows the order of $MgCl_2$ > $MgSO_4$ > $CaSO_4$, and the coagulants containing $MgSO_4$ (5 and 10 mM) and $MgCl_2$ (5 mM) promote to produce stronger gels with greater gel firmness and rigidity. When compared with single $CaSO_4$ as the coagulant, a low concentration of Mg^{2+} helped to form compact protein aggregates and facilitated gel homogeneity and resistance to deformation. The WHC of the gels was significantly enhanced ($p < 0.05$) by the action of 5 mM $MgSO_4$. The relationships between aggregates induced by the coagulants and gel properties, as well as the influencing mechanism were discussed. The findings provide valuable information for understanding the coagulant-related gelation mechanism of protein and the application of SPI emulsion-based gels with improved properties in food industry.

1. Introduction

Soybeans have been transformed into various food products, among which tofu received increasing attentions worldwide in recent years (Prabhakaran et al., 2006). Traditional tofu is made from soymilk, and its preparation typically involves soaking and grinding of soybeans, filtering, heating, and coagulation of soymilk; breaking the curd; and pressing to reform gel (Hou et al., 1997). The process is complicated and commonly cause pollution because of the discharge of tofu whey and okara. Soy protein is a versatile, nutritional source of vegetable protein, that is free of cholesterol and contains less saturated fat (Murekatete et al., 2015; Yasir et al., 2007). In our previous studies, we attempted to prepare packed (filled) tofu using soy protein isolate (SPI) as raw material. This process is much simpler and easier to control because it only requires heating, homogenization and gelling. Moreover, SPI-based tofu contains negligible amounts of soybean oligosaccharides (lower than 0.3%) and less isoflavones (60% of which are washed out during SPI preparation), thus lowering the risk of flatulence and allergy, respectively, to some people (Kinsella, 1979; Rickert et al., 2010; Visser and Thomas, 1987; Wang et al., 2017).

In the traditional process, tofu quality is considerably influenced by the type of coagulant. (Prabhakaran et al., 2006) Salt-induced and acid-induced are the two most common methods used in the coagulation of tofu gel. After heating the soymilk, the coagulants, such as Mg^{2+} ($MgCl_2$, $MgSO_4$), Ca^{2+} ($CaCl_2$, $CaSO_4$) or Glucono-δ-Lactone (GDL), is added to form tofu curd. According to Kao et al. (2003), $MgCl_2$, $CaCl_2$ and $MgSO_4$ resulted in soy curd with coarse, hard, and granular texture due to their quick coagulating power. By contrast, GDL and $CaSO_4$ can produce tofu with soft, smooth, and homogeneous texture, however, GDL imparts tofu with a sour flavor, which affects consumer acceptance. The differences in tofu texture can be partially attributed to the protein aggregation rate induced by different coagulants; as fast aggregation leads to the formation of large and coarse protein aggregates. Protein aggregates have been reported to be associated with gel strands (e.g., thickness and bending), thereby affecting the gel structure and strength (Lu et al., 2010). In most studies, heating is usually applied to produce protein aggregates. Authors have investigated the impact of heating temperature, time and mode on the aggregation and subsequent gelation of proteins (Liu et al., 2004; Lu et al., 2010; Zhao et al., 2015). In our previous study, we demonstrated that increasing the protein concentration during heating can induce the formation of larger aggregates and enhance the gel properties of SPI emulsion (Wang et al.,

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2017). Ionic strength is another factor influencing protein aggregation, an increase in ionic strength accelerates protein aggregation and formes large aggregates, which increase the coarseness of the gel structure with decreased water-holding capacity (Li et al., 2009; Urbonaite et al., 2016). However, to our knowledge, very few researches have focused on the aggregate characteristics induced by the coagulant without additional treatment and the mechanisms affecting gel properties.

In our earlier work, we prepared SPI emulsion-based packed tofu using $CaSO_4$ as the coagulant and found that protein aggregates induced by the coagulant itself (pre-aggregation) play an important role in the gelation of SPI emulsion. Sufficient pre-aggregation produced larger and more compact aggregates, thus enhancing the strength and WHC of the emulsion gels, whereas excessive pre-aggregation made the final gel network coarser with weak gel properties (Wang et al., 2018). In other words, controlling protein aggregation using coagulants may be an effective approach to improve the gelation properties of protein or emulsion. It can be speculated that different coagulants may have significant impacts on the aggregation state and even gelation of protein due to different aggregation power of calcium and magnesium salts. In the present study, the cooperation effects of calcium sodium and magnesium ions (primarily $MgCl_2$ and $MgSO_4$) on the rheological and microstructural properties and WHC of SPI emulsion gels were investigated, and the gelation mechanism is discussed.

2. Materials and methods

2.1. Materials

Soybeans (Taiwan 292, harvested in 2017) and soy oil were purchased from a local market. SPI was extracted from defatted soybean meals according to Guo et al. (2015). The protein content of SPI was 91.6 g/100 g (dry basis) determined by the Kjeldahl method using *N* factor 6.25. Rhodamin B was obtained from Sigma-Aldrich (St. Louis, Mo, USA). All other chemical reagents were of analytical grade.

2.2. Preparation of SPI emulsion

SPI powder was dispersed in deionized water with 0.02% (m/v) sodium azide and stirred mechanically at room temperature for at least 2 h. Then the SPI dispersion was centrifuged at 10,000 g for 10 min and adjusted to pH 7.0 with 0.1 M HCl. For emulsion preparation, SPI dispersion was first 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 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. The protein concentration (mg/mL) in continuous phase and oil content (v/v) were 60 mg/mL and 5%, respectively.

2.3. Preparation of SPI emulsion gel

Freshly prepared SPI emulsions were mixed with stock $CaSO_4$ dispersion and $MgCl_2$ or $MgSO_4$ solution following the ratio listed in Table 1, up to a total ion concentration of 35 mM. The mixtures were then heated to 80 °C and allowed to coagulate for 30 min in a water bath to accelerate the gelation process. After coagulation, the gels were immediately cooled to room temperature in an ice bath and stored at 4 °C. The SPI emulsion gels induced by different coagulant mixtures were denominated as gel I to V.

Table 1
The mix ratio of the coagulants.

Coagulants	SPI emulsion gels				
	I	II	III	IV	V
$CaSO_4$ (mM)	35	30	25	30	25
$MgCl_2$ (mM)	0	5	10	0	0
$MgSO_4$ (mM)	0	0	0	5	10

2.4. Rheology properties measurements

2.4.1. Gelation kinetics

The gelation process was measured 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. The emulsions were immediately loaded between the plates of the rheometer after the addition of $CaSO_4$. 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 variation of storage modulus (G′) and loss tangent (tan δ) were recorded.

2.4.2. Creep and recovery

After the formation of SPI emulsion gel, a constant stress of 8 Pa was applied to the gel at 25 °C to evaluate its creep behavior, the change in strain over time (called creep) was recorded for 5 min. This stress was subsequently released, and the recovery response was observed for another 5 min. To identify the deformation pattern of the gels, a four-parameter Burger's model was used to fit the creep data (Steffe, 1996).

$$J(t) = \frac{1}{G_0} + \frac{1}{G_1}\left(1 - e^{-\frac{t}{\lambda}}\right) + \frac{t}{\mu_0} \quad (1)$$

Where J is the creep compliance (1/Pa), G_0 and G_1 represent the instantaneous and retarded elastic modulus (Pa), respectively. λ is the retardation time, and μ_0 is the viscous modulus associated with viscosity flow (Pa s).

2.4.3. Large deformation measurements

Large-scale deformation tests were performed at 25 °C using 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.

2.5. Water-holding capacity (WHC)

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

2.6. Confocal laser scanning microscopy (CLSM)

The gel samples for CLSM observation were prepared in single concave slides (Sail Brand, Jinliu Instrument Co., Ltd., Nanjing, China) covered with nail oil to prevent water evaporation, according to the same procedure described in section 2.3. Rhodamine B was used as the fluorescence dye (5 mL of stock emulsion + 0.05 mL of 0.1% (w/w) fluorescence dye), with excitation wavelengths at 552 nm. The CLSM images were obtained (TCS SP8, Leica Microsystems Inc., Heidelberg, Germany) with a 63 × magnification lens.

2.7. Fractal analysis of the CLSM images

The fractal analysis of the CLSM images was carried out using software ImageJ v1.50i. Micrographs were transformed into 8-bit binary images of 1024 × 1024 pixels with a median grey level