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Effects of removal of non-network protein on the rheological properties of heat-induced soy protein gels

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ABSTRACT

Effects of removal of non-network proteins by diffusion process on the storage modulus and loss modulus of soy protein isolate (SPI) gels and glycinin (11S) gels as a function of heating temperature and ionic strength were investigated. The composition of non-network proteins was determined by native-PAGE, non-reducing SDS-PAGE and reducing diagonal SDS–PAGE. Results showed that non-network proteins were composed of a majority of acid polypeptides (A), small amounts of AB subunits, soybean agglutinin, Bowman – Birk trypsin inhibitor, and lower amounts of α′, α and A_3 polypeptides. The results further revealed that 11S gels had higher ratios of non-network proteins than SPI gels, due to the increased ratio of A polypeptides in 11S gel. In addition, the removal of non-network proteins from the gel was found to have no effect on the storage modulus, but on the other hand resulted in a decrease in the loss modulus, suggesting that the loss modulus of the gel network is closely related to non-network proteins. This study presents an approach to investigate the changes of storage modulus and loss modulus of globular gels in relation to the removal of non-network proteins, and provides valuable information on the composition and content of these proteins in gel network formed at various conditions.

1. Introduction

Soy proteins are composed of two main storage globulins, β-conglycinin (7S), and glycinin (11S), and a small amount of soy whey proteins which make up 9–15.3 g/100 g of soy seed protein (Li et al., 2014). The β-conglycinin consists of three subunits namely: α′, α and β. These three subunits exist in various combinations linked by non-covalent interaction, particularly hydrophobic interaction. Another minor γ subunit had also been isolated as 7S fraction (Thanh & Shibasaki, 1977). The 11S fraction is a hexamer of six subunits of acidic A and basic B polypeptides dimers linked by disulfide bond, and five kinds of subunits had been identified, including $A_{1a}B_{1b}$, A_2B_{1a}, $A_{1b}B_2$, $A_5A_4B_3$, and A_3B_4. Soy whey proteins are composed of Bowman – Birk trypsin inhibitor (BBI), Kunitz trypsin inhibitor, soybean agglutinin (SBA), β-amylase, and lipoxygenase. Soy proteins have been extensively utilized for food processing. The ability to form a gel upon heating makes soy proteins suitable for improving texture of food products, such as sausages (Ahmad, Rizawi, & Srivastava, 2010; Muguruma et al., 2003) and meat ball (Ulu, 2004). Heat-induced denaturation of proteins is considered to be a prerequisite for soy gel formation (Nagano, Hirotsuka, Mori, Kohyama, & Nishinari, 1992; Puppo & Anon, 1998; Renkema, Gruppen, & Van Vliet, 2002; Wang et al., 2016; Wu, Hua, Chen, Kong, & Zhang, 2017), which induces disassociation and aggregation of proteins, leading to the formation of submicrometer-size particle (Mori, Nakamura, & Utsumi, 1982) and, above a critical protein concentration, to gel formation (Phan-Xuan et al., 2013). The three-dimensional network of gel is formed by cross-linked soy proteins and their aggregates. However, the participation of 7S and 11S subunits in the gel network is different, proteins which are not engaged in the gel network and entrapped in the gel matrix as soluble components after gelation are denoted as non-network proteins (Wu, Hua, Chen, Kong, & Zhang, 2015), and these non-network protein fractions in gels and its effect on gel storage modulus and loss modulus are poorly studied. After gelling, a small amount of non-network proteins reside in the pores of gel network formed at pH 7.0, while all protein were present as network proteins in gels formed at pH 3.8 (Renkema et al., 2002). The content of non-network proteins in soy protein gels decreased when the protein used for gelling had been cross-linked by transglutaminase (Gan,

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Aishaha, Cheng, & Azharmat, 2009).

It has been experimentally studied that different rheological properties of soy protein gels could be obtained by varying pH (Puppo, Lupano, & Anon, 1995; Renkema, Lakemond, De Jongh, Gruppen, & Van Vliet, 2000), ionic strength (Lakemond, De Jongh, Hessing, Gruppen, & Voragen, 2000; Renkema et al., 2002), heating temperature (Nagano, Mori, & Nishinari, 1994; Renkema & Van Vliet, 2002), and protein composition (Renkema, Knabben, & Van Vliet, 2001; Wu et al., 2017). A higher gel storage modulus (G′) was found at pH 3.8 than at pH 7.6 and 5.2 by Renkema et al. (2002), due to changes in the content of network proteins in the gel network, where more proteins seemed to participate in the network at pH 3.8 than at pH 7.6 and 5.2. Renkema & Van Vliet, 2002 also investigated the relationship between storage modulus of soy protein gels and the degree of protein denaturation by increasing heating temperatures from 76 to 94 °C, and found a higher G′ when more proteins became denatured. Puppo and Anon. (1998) reported that coarser and stiffer gel networks were formed with increasing NaCl concentration from 0 to 0.25 mol/L, possibly due to the increase of protein aggregation because of increasing ionic strength. Glycinin-rich gels were confirmed with higher storage modulus than β-conglycinin-rich gels (Kohyama & Nishinari, 1993; Nagano et al., 1994). Compared with aggregates formed by β-conglycinin which were of smaller size and less compact conformation, heat-induced aggregates by glycinin composed of a denser core and a less dense outer shell (Guo et al., 2012). Higher gel stiffness was also found with increasing protein concentration (Chen, Zhao, Niepceron, Nicolai, & Chassenieux, 2017). However, to the authors' knowledge, little is known about the effect of the removal of non-network proteins on the changes of storage modulus and loss modulus of soy protein gels.

The objective of this study was to obtain knowledge on the effect of the removal of non-network proteins on the rheological properties of heat-induced soy protein gels. Non-network proteins were separated from the gel by a diffusion process. The protein compositions of non-network proteins and their existing forms were detected by native PAGE or non-reducing and reducing diagonal SDS–PAGE. Finally, the effects of removal of non-network proteins from gel network on storage modulus and loss modulus of heat-induced soy protein gels and glycinin gels were discussed.

2. Materials and methods

2.1. Materials

Soy protein isolates (SPI) were prepared following the method of Wu et al. (2015). Briefly, defatted soybean flakes (Shandong Wonderful Industrial and Commercial Co. Ltd.), which had been prewashed by using aqueous alcohol (85 mL/100 mL and 95 mL/100 mL), were suspended in distilled water (1:10, w/v) and the pH was adjusted to 7.0 by 2 mol/L NaOH. After stirring for 1 h, the suspension was centrifuged (15800g, 30 min, 4 °C), then the supernatant was collected and pH was adjusted to 4.5 by dropwise addition of 2 mol/L HCl. The precipitates obtained by centrifugation (10000g, 30 min, 4 °C) were suspended in distilled water and solubilized by adjusting pH to 7.0 by using 2 mol/L NaOH. A small amount of insoluble substances were removed by centrifugation (10000g, 30 min, 4 °C). Protein solution was freeze-dried and stored at −18 °C. 11S protein was separated from defatted soy flakes (Shandong Wonderful Industrial and Commercial Co. Ltd.) according to Nagano et al. (1992). Defatted soybean flakes were mixed with 10-fold (w/w) distilled water and adjusted to pH 7.0 by 2 mol/L NaOH. After stirring for 1 h at 25 °C, the suspension was centrifuged (15800g, 30 min and 4 °C) to obtain the supernatants. Sodium bisulfite (0.98 g/L) was added to the supernatants, after mixed thoroughly, the pH was adjusted to 6.4 with 2 mol/L HCl. This suspension was stored at 4 °C overnight. Then it was centrifuged at 10,000g for 20 min. The precipitates were dissolved in distilled water and the pH was ajusted to 7.0 by adding 2 mol/L NaOH, and lyophilized; and this fraction was 11S protein. The protein contents of soy protein isolates and 11S protein, determined by the micro-Kjeldahl method, were 91.3 ± 0.2 g/100 g and 98.1 ± 0.3 g/100 g, respectively.

All other reagents were of analytical grade and used without further purification.

2.2. Preparation of protein gels

Protein dispersions were prepared by suspending SPI or 11S protein in distilled water at 18 g/100 mL with desired ionic strength (Gels generated at temperature lower than 95 °C or at ionic strength higher than 0.25 mol/L were too weak to handle, thus these two temperatures and NaCl concentrations were selected in this research.). The suspension was mixed thoroughly by stirring for 3 h, then centrifuged at 4000 g for 20 min to remove air and carefully transferred into a cylindrical Teflon vessel with a height of 6.5 cm and internal diameter of 3.0 cm and sealed securely. The gelation was carried out at 95 or 100 °C for 30 min after the protein solution reaching the target temperature. The vessel was immediately cooled by ice water and stored in a refrigerator at 4 °C overnight.

2.3. Removal of non-network proteins

2.3.1. Determination of the non-network proteins ratio in gels

The determination of non-network protein ratio was carried out according to the method by Wu et al. (2015) with some modifications. The gel was cut into slices of 1.5 mm thickness with the aid of a razor and a paperboard filled with horizontal lines of 1.5 mm (± 0.05 mm, obtained by measuring samples in six repeated trials) distance, and then 20 g slices were transferred into a 500 mL glass bottle (Biocolor Co., Ltd., Shanghai, China) with tenfold (w/v) phosphate buffer (0.01 mol/L, pH 7.0). NaN_3 at 0.02 g/100 mL was added as an anti-bacteria agent. The bottle was gently shaken in shaker incubator at 25 °C for 54 h. The time was chosen in order to enable proteins within and outside the gel network attain equilibrium state. The protein concentration in sodium phosphate buffer was determined by using BCA method (Smith et al., 1985). Non-network proteins ratio (R_{non}) in gels was calculated according to Eq. (1) by Wu et al. (2015), which was expressed as:

$$R_{non} = \frac{C_{non} \cdot V_{buffer}}{1000 C_p \cdot M_{gel}} \cdot 100 \quad {}^{g}/_{100g} \tag{1}$$

where C_{non}, V_{buffer}, C_p and M_{gel} were the protein concentration (mg/mL) at final stage (after diffusing of 54 h) in the buffer, the buffer volume (mL), the protein concentration used for gelation(g/g) and the gel slices weight (g) at initial stage, respectively.

2.3.2. Removal of non-network proteins from gel

One gel slice (about 2 g) was weighed by Mettler–Toledo analytical balance, and transferred to a 50 mL beaker. Tenfold excess phosphate buffer (0.01 mol/L, pH 7.0) and 0.02 g/100 mL NaN_3 were added into the beaker. The bottles were shaken in a shaker incubator as described in the previous section for 54 h in order to enable the released of non-network proteins. The gel slice was taken out. The excess buffer on the surface of the slice was removed carefully by filter paper, while the excess water absorbed by gel slice was dehydrated by using Poly (ethylene glycol) 6000 (PEG 6000) powder. PEG is of strong hygroscopicity (Baird, Olayo-Valles, Rinaldi, & Taylor, 2010). To block the transporting of PEG 6000 into gel slice, a RC Dialysis membrane with 3.0 kDa cut off (Sangon Biotech (Shanghai) Co., Ltd.) was placed in between the gel slice and PEG powder. The gel slice was weighed from the beginning until its mass reached the target value ($M_{gel}(1\text{-}R_{non})$). All samples were measured in triplicate.