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Thermal stability and gel quality of myofibrillar protein as affected by soy protein isolates subjected to an acidic pH and mild heating

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ABSTRACT

Thermal stability and gel quality of myofibrillar protein were evaluated with regard to the addition of native soy protein isolates (SPI) and SPI subjected to acidic pH and mild heating (modified SPI). Compared with the control, the addition of modified SPI increased the compression force of the protein gel and decreased water loss ($P < 0.05$). Differential scanning calorimetry results showed that an addition of 0.75% native SPI decreased the first transition temperature ($P < 0.05$), and addition of 0.5% and 0.75% modified SPI exhibited no appreciable changes on it ($P > 0.05$), indicating that a higher concentration of modified SPI would not damage the protein thermal stability. Moreover, the addition of modified SPI enhanced hydrogen bonding and disulphide linkages. Atomic force microscopy analysis revealed that the addition of modified SPI decreased the roughness of the mixed myofibrillar protein gels. Overall, modified SPI has the potential to improve myofibrillar protein gel texture and water holding capacity.

1. Introduction

Myofibrillar proteins (MP) have excellent gelation ability and play important roles in forming cohesive structures (Xiong, 1997). To improve the nutritional and functional properties of the meat and meat products, soy protein isolate (SPI) has been widely used as a nonmeat protein additive in the meat processing industry (Pietrasik & Li-Chan, 2002). However, several studies have reported that native SPI (N-SPI) has negative effects on the gelation properties of meat proteins (Petruccelli & Anon, 1994; Scilingo & Anon, 1996; Wang et al., 2015).

Soy protein is composed of two main globular proteins; namely, soy β-conglycinin (7S) and glycinin (11S) (Herreroa, Carmonab, Cofradesc, & Jiménez-Colmenero, 2008). The denaturation temperatures of 7S and 11S are around 75 °C and 90 °C, respectively, so N-SPI should not exhibit any structural changes under normal meat processing conditions (pH 5.5–6.0 and temperatures up to 75 °C). The lack of structural changes for 7S and 11S globulins under normal meat processing conditions can limit the interaction between N-SPI and meat proteins (Feng & Xiong, 2002) and result in less functionality of N-SPI in MP gels (Jiang & Xiong, 2013).

There are many methods to modify the structure of N-SPIs, among which heat treatment is a conveniently effective method. Recently, an acidic pH treatment has been reported as a convenient energy-efficient method to modify the protein structure and improve the functionality of SPI (Liang & Kristinsson, 2007; Matsudomi, Sasaki, Kato, & Kobayashi, 1985; Wagner & Gué guen, 1999; Wagner, Sorgentini, & Añón, 1996). Jiang, Chen, and Xiong (2009) indicated that an extreme acidic pH of 1.5 enhanced the emulsifying activity, solubility and thermal stability of SPI. In a previous study, we demonstrated that an acidic treatment of pH 1.5, combined with heating at 60 °C, induced SPI into a "molten globular" structure and increased the particle size, surface hydrophobicity, and sulfhydryl content of SPI (Liu, Geng, Zhao, Chen, & Kong, 2015). The reason for this phenomenon induced by the shifted pH treatment might be related to the connection and interaction among protein subunits which led to the changes in secondary and tertiary structures of SPI. Jiang et al. (2009) proved that proteins showed improved functional properties when they were in this molten globular state. Furthermore, we showed that with a pH 1.5 treatment at 60 °C, SPI (AH-SPI) improved the gelation and rheological properties of MP upon heating (Niu, Li, Han, Liu, & Kong, 2017). Moreover, Jiang et al. (2009) reported the tertiary structure of globular proteins might be partly unfolded in the circumstance of extreme pH conditions. Huang et al. (2010) reported that the interactions between N-SPI and other food components could be enhanced by thermal unfolding or denaturation of N-SPI. Therefore, the structural modification of SPI induced by an acidic pH treatment combined with mild heating may offer an alternative method to improve the functional properties of MP.

The objective of this study is to investigate the gelation and thermal properties of myofibrillar protein as affected by the addition of SPI that was subjected to an acidic pH treatment combined with mild heating.

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The gel surface morphology was observed by atomic force microscopy (AFM) and the possible interactions between the AH-SPI and MP were investigated by identifying the molecular forces in the MP gels.

2. Materials and methods

2.1. Materials

Defatted soy flakes were obtained from Harbin HighTech Group (Harbin, Heilongjiang, China). Pork longissimus muscle (24 h post-mortem) was purchased from a commercial local store (Harbin, Heilongjiang, China). The samples were kept on ice and used on the same day. Piperazine-N and *N*′-bis (2-hydroxypropanesulfonic acid) (PIPES) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Reagent-grade chemicals were used throughout.

2.2. Preparation of N-SPI and AH-SPI

Defatted soybean meals were used to prepare N-SPI according to a method previously described by Jiang et al. (2009). AH-SPI was prepared according to a method described by Jiang and Xiong (2013) and Liu et al. (2015). In brief, N-SPI (30 mg/mL protein) was adjusted to pH 1.5 with 2 M HCl, and then heated at 60 °C in a water bath for 5 h. The pH of 1.5 was maintained during heating to account for any pH drift due to sample dissolution. After that, the solution was adjusted to pH 7.0 with 2 M NaOH, and maintained for 1 h to allow partial refolding. To remove any introduced salts during pH adjustments, the treated proteins were precipitated at pH 4.5, washed three times, and then re-solubilized at pH 7.0. The N-SPI and AH-SPI samples were subsequently freeze-dried and sealed in polyethylene bags. The protein content of the prepared N-SPI and AH-SPI were 94.76% and 93.35% (w/w) as determined by the Kjeldahl method.

2.3. Preparation of myofibrillar protein

MP was prepared as described previously by Xia, Kong, Liu, and Liu (2009). Biuret method was used to measure the MP concentration, and bovine serum albumin was used as the standard protein (Gornall, Bardawill, & David, 1949). The MP was stored in a tightly capped bottle, kept on ice, and utilized within 48 h.

2.4. Preparation of mixed protein solutions

MP suspensions (final protein concentration of 40 mg/mL) were prepared with 50 mM PIPES buffer containing 0.6 M NaCl (pH 6.25). N-SPI and AH-SPI were added to the suspensions to obtain final SPI concentrations of 0.25, 0.5, and 0.75% (w/v) after mixing. These mixed protein solutions were stored at 4 °C for at least 12 h to allow maximum protein dissolution.

2.5. Compression tests

Aliquots (15 g) of mixed protein solutions were transferred into glass vials (25 mm length × 40 mm diameter), and heated in a 75 °C water bath for 20 min. After heating, the gels were immediately chilled in ice water for 30 min and then equilibrated at room temperature for 1 h. A compression test of the formed gels was carried out with a Model TA-XT2 texture analyser (Stable Micro Systems Ltd., Godalming, UK) according to Kong, Tashiro, and Ogawa (2001) with some modifications. The formed gels were taken out and were cut into slices (2.0 cm thick with a 2.6 cm diameter). The gels were axially compressed with a P36 cylinder probe (36 mm in diameter) to 30% of their original height. The trigger force was 5 g, and the test speed was 1.5 mm/s. The maximum force (kg) required to achieve the given deformation of the gels, was defined as compression force.

2.6. Water loss

Water loss was evaluated by cooking loss and total water loss as described by Wang, Luo, and Ertbjerg (2017) with some modifications. Briefly, aliquots (10 g, W_0) of mixed solutions were transferred into glass vials (25 mm length × 40 mm diameter), and heated in a 75 °C water bath for 20 min. After cooling in ice water for 30 min and room temperature for 1 h to equilibrate, the formed gels were taken out, wiped dry with a filter paper and weighed as W_1. Then the weighed gels were placed in tubes and centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was discarded and the gels were taken out, wiped dry with a filter paper again and weighed as W_2. Cooking loss and total water loss were calculated using the following equation:

$$Cooking\ loss\ (\%) = \frac{W_0 - W_1}{W_0} \times 100\%$$

$$Total\ water\ loss\ (\%) = \frac{W_0 - W_2}{W_0} \times 100\%$$

2.7. Gel whiteness

The mixed gels were prepared as described in Section 2.5. Gel whiteness was determined using a ZE-6000 color meter (Nippon Denshoku, Inc., Tokyo, Japan). Gel whiteness was calculated according to the following formula on the basis of L^* (brightness), a^* (red), and b^* (yellow). The instrument was calibrated using a standard white reflector plate. For each sample, measurements were repeated 6 times, and the average values of data were obtained.

$$Whiteness = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

2.8. Differential scanning calorimetry (DSC)

The effect of N-SPI and AH-SPI on the thermal stability of MP was measured using a Model Q20 DSC machine (TA Instruments, Inc., New Castle, DE, USA). The mixed protein solutions that were prepared as described in Section 2.4 (9–10 mg) were hermetically sealed in aluminium pans and were equilibrated to 30 °C in the instrument for 2 min. A thermal scan was conducted from 30 to 100 °C at a constant heating rate of 10 °C/min. An empty pan was used as reference. The transition temperatures (T_{max}) were recorded.

2.9. Molecular forces in the MP gels

The molecular forces were evaluated by the method of Sun and Arntfield (2012) with some modifications. Three denaturing solutions were used: 8 M urea in 50 mM sodium phosphate (pH 7.0) to detect hydrogen bonds, 0.5% SDS in 50 mM sodium phosphate (pH 7.0) to detect the total non-covalent forces, and 0.25% β-mercaptoethanol in 50 mM sodium phosphate (pH 7.0) to detect disulphide bonds. Aliquots of 5 g from the protein gels were mixed with 45 mL of each denaturing agent and homogenized with an IKA T18 basic, (IKA-Werke GmbH & Co., Staufen, Germany) at a speed setting of 6 (13,500 rpm) for 20 s. The mixed solutions were then heated at 80 °C for 1 h in a water bath and chilled to room temperature before centrifugation at 10,000*g* for 15 min. The protein content in the supernatant was determined according to the Biuret method using bovine serum albumin as a standard. The gel solubility was calculated by the relative protein content in the supernatants divided by the protein content in the original suspension that was needed to prepare the protein gel.

2.10. Surface morphology of the gel

The surface morphology of the gel was analysed using an AFM (Multimode 8, Bruker AXS, Santa Barbara, USA) according to the