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Modification of gel properties of soy protein isolate by freeze-thaw cycles are associated with changes of molecular force involved in the gelation

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ABSTRACT

The current study investigated the effects of various freeze-thaw (F-T) cycles on gel properties and molecular forces involved in gelation of soy protein isolate (SPI). The gel strength of SPI submitted to 4 and 5 freeze-thaw (F-T) cycles increased 1.95- and 1.98 folds compare to control, respectively ($P < 0.05$). The observation from scanning electron microscope and atomic force microscopy indicated that the SPI submitted to 4 and 5 F-T cycles exhibited smoother, flatter and denser gel surface morphology images than the control samples. G' and G'' values of the sample submitted to 4 and 5 F-T cycles dramatically increased during the cooling period in dynamic rheological testing and the final G' value increased 32.6-folds for the samples submitted to 5 F-T cycles compare to control. In addition, the results of particle size distribution provided direct evidence that the aggregation occurred in SPI was accelerated by multiple F-T cycles. The results of molecular force measurement implied that these effects could be primarily attributed to the stronger molecular interactions in SPI gel, with the main forces being the disulfide bond, the hydrogen bond and the hydrophobic interactions. Overall, multiple F-T treatments offered a new way to improve the gel properties of SPI.

1. Introduction

Soy protein is being increasingly used worldwide as an important functional ingredient to obtain desirable quality properties in many food products [1]. The ability of soy protein to form thermally induced gel is generally considered to be one of its most important functional properties and is also of great significance in generating texture in food [2]. However, primarily due to its structural compactness and the lack of reactive groups exposure, the gel properties of untreated (native) soy protein is rather limited [3]. Thus, it is worthwhile to modify soy protein isolate (SPI) to improve its gel properties and to study the relationship between the internal molecular forces involved in gel formation and gel properties of modified soy protein [4].

There are various ways to analyze and observe the structure of protein gel, such as scanning electron microscopy (SEM), dynamic viscoelasticity measurements and atomic force microscopy (AFM) [5]. AFM has provided a new insight into the structure of biological macromolecules in recent years because of its high resolution [6]. It can also be used to quantitatively measure the particle size and intuitively reveal the 3D structure of sample surface [7]. Tay et al. [8] used AFM to observe the aggregation process of 11S and 7S soy protein before and after the addition of glucono-δ-lactone. The results showed that the particles of 11S became a cluster of aggregates in just one minute upon the addition of GDL, however, the cluster of aggregates of 7S protein did not change at the same length of time.

The heat-induced soy protein gelation was caused by cross-linking its polypeptide chains, thus forming a 3D network. The cross-linking of soy proteins is caused by various molecular forces including disulphide bonds, hydrogen bonds, hydrophobic interactions and ionic attractions [9]. Gulseren et al. [10] suggested that the gel properties of soy protein are influenced by different molecular forces including disulphide bonds, hydrogen bonds,

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hydrophobic interactions, and electrostatic interactions or a combination of the above. Some chemical reagents can be used to disrupt special chemical bonds and to analysis molecular forces involved in the protein gelation. To ascertain the impact of these covalent and non-covalent forces on gel properties of protein, several researchers determined that these covalent and non-covalent molecular forces could be deduced by dispersing the protein gels into solution with various denaturing reagents (such as guanidine hydrochloride, propylene glycol, and urea) [9,11,12]. By determining the gel solubility in the solutions containing various added reagents, the disruptions in the molecular forces caused by certain denaturing reagents would be known.

Freezing and storing frozen-food can maintain chemical and microbiological stability, while extending the shelf life of food products [13]. It has been noted that for soy proteins the surface hydrophobicity increased with increasing frozen storage time [14]. During freezing, soy proteins in solution become partially insoluble due to the formation of intermolecular disulfide bonds [15]. Noh et al. [16] noted that the soy protein solution prepared from frozen sopped soybean (soaked in tap water for 10 h, removed excess water and then frozen) produced a more uniformly structured gel than that prepared from unfrozen sopped soybeans. In a previous study, we have confirmed that total sulfhydryl groups in the SPI decreased with the number of increased freeze-thaw (F-T) cycles [17]. Additionally, F-T cycles were found to significantly increase surface hydrophobicity after being submitted to 1–3 F-T cycles. In addition, the results of UV–vis spectra, circular dichroism spectra, high-performance size exclusion chromotography and intrinsic fluorescence spectroscopy provided some evidence that the secondary, tertiary and quaternary structures of the protein were modified by the F-T cycles [17]. Because structural modifications are influenced by freezing, the subsequent thawing steps and the numbers of cycles [18], multiple F-T cycles could therefore be implemented as a strategy to modify SPI gel properties, thereby producing a more viscoelastic and interactive soy protein gel network.

This study evaluated the gel properties of SPI submitted to multiple F-T cycles by penetration testing, water-holding capacity (WHC) and dynamic rheological measurement. The microstructure of SPI gels was observed by SEM, the gel surface microcosmic structure was observed by AFM, the particle size distribution and the molecular forces involved in soy protein gel were also determined. The purpose was to investigate the effect of F-T cycles as a new way to modify gel network structure and improve the gel properties of soy protein.

2. Materials and methods

2.1. Preparation of soy protein isolate

SPI was prepared from soy flour by alkali extraction and subsequent acid precipitation according to the modified method of Zhao et al. [17]. Deionized water (3 L) was added to 200 g of dehulled, defatted, and milled soybean flour which was obtained from Harbin High Technology Group (Harbin, Heilongjiang, China). The pH value of water solution of soybean flour was adjusted to pH 7.8 with 2.0 M of NaOH and the mixture was stirred for 2 h at 25 °C. Next, the suspension was centrifuged for 20 min at 8000*g*. Subsequently, the pH of the supernatant was adjusted to the soy proteins isoelectric point (pH 4.5) by using 2.0 M HCl. The precipitate, which was collected by centrifugation for 10 min at 8000*g*, was washed twice with 5-fold (w/v) deionized water and was re-centrifuged at 8000*g* for 10 min. Thereafter, the precipitate was mixed with deionized water at a ratio of 1:5 (w/v) of precipitate to water and the pH of the mixture was adjusted to 7.0 with 2.0 M NaOH. The protein concentration was measured according to the Biuret method.

2.2. Freeze-thaw treatment

F-T cycle treatments of SPI were carried out according to our previous method [17]. After the protein extraction, SPI was dissolved in 400 mL of deionized water to obtain 50 mg/mL protein solution and later placed in a well-sealed polyethylene bottle. A total of 50 bottles of SPI solutions were used for F-T treatments. All the bottles of frozen SPI solutions were stored at −18 °C for 2 days then thawed at 25 °C for 12 h. Aliquots of 10 bottles from 50 bottles were randomly selected as 1 F-T cycle. The other 40 thawed bottles of SPI solution were frozen and stored at −18 °C for another 2 days. The F-T procedures were repeated five times. After each F-T cycle treatment, the obtained samples were lyophilized immediately. The sample with no F-T treatment was considered as control or native SPI which was directly lyophilized after the protein extraction. All the lyophilized samples were stored at 4 °C in sealed containers and used for following analysis.

2.3. Gel preparation, gel strength and water-holding capacity measurement

The SPI were suspended in deionized water to a protein concentration of 120 mg/mL. Aliquots of 15 mL of the SPI samples were placed into glass vials with an inner diameter of 25 mm and a height of 50 mm. Protein gels were formed by heating at 90 °C in a water bath for 30 min. The heated samples were cooled immediately in an ice slurry and stored at 4 °C for 12 h.

Before the measurement of gel strength, the SPI gels in vials were equilibrated at 25 °C for 1 h. The gel strength of SPI samples were analyzed according to the modified method of Jian et al. [3] with a TA-XT2 texture analyzer (Stable Micro Systems Ltd., Godalming, UK) attached to a 5 kg load cell. The gels in the vials were tested by a stainless steel flat-surface cylindrical probe (Model P/0.5, 12 mm in diameter) and axially compressed at a penetration rate of 1 mm/s. The penetration force, corresponding to the peak force, was defined as the rupture force of the gels and was also expressed as gel strength.

The gel WHC was determined using a centrifugation method [19,20]. The gels (10 g) were placed in a 50 mL centrifuge tube and centrifuged at 3000*g* for 15 min at 4 °C. WHC was expressed as the percentage of water remaining in the gel after centrifugation.

2.4. Scanning electron microscopy

The morphology of the SPI gels was observed using a scanning electron microscope (SEM, Model S-3400 N, Hitachi Corp., Tokyo, Japan) according to Jiang and Xiong [12]. The protein gels were prepared as above described in Section 2.3. Cubic sample gels (27 mm^3) were fixed in 2% glutaraldehyde for 24 h at 4 °C, then thoroughly dehydrated in the following graded series of ethanol solutions: 50, 70, 80, and 90% ethanol once each for 10 min, twice in 100% ethanol for 10 min, and then air-dried for 15 min. Dehydrated samples were mounted on an aluminum mount with adhesive, conductive carbon disk and sputtercoated with gold (Sputter Coater SPI-Module, PA, USA). The images were captured at a 10 kV electron velocity of the SEM.

2.5. Atomic force microscopy

The surface structure of the gel samples were imaged by atomic force microscopy (AFM) according to the method of Tay et al. [8] with some modifications. The SPI suspension (120 mg/mL) treated with or without F-T cycles was prepared in a scale glass vial and

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