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Molecular forces involved in heat-induced freshwater surimi gel: Effects of various bond disrupting agents on the gel properties and protein conformation changes

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ABSTRACT

Urea, sodium dodecyl sulfate (SDS) and N-ethylmaleimide (NEM) were employed to monitor the contributions of hydrogen bonds, hydrophobic interactions and disulfide bonds in heat-induced surimi gel. Surimi gels were prepared with the bond disrupting agents followed by texture profile, gel strength, rheological and Raman analysis. The rheological analysis demonstrated that remarkable changes of storage moduli (G′) were presented below the critical temperature, especially for SDS treatments. Gel strength significantly decreased as the concentration of urea and NEM increased, while it was slightly improved by 1% SDS. With the involvement of bond disrupting agents, hardness and gumminess of surimi gel presented a dramatical decrease. The Raman results indicated that protein secondary structure tended to transform to random coil, with content of α-helix and β-sheet decreasing. Textural properties were in correlation with protein conformation. Less of random coil led to improvement of gel strength, and springiness was mainly contributed by β-sheet and β-turn. The results suggested that different bond disrupting agents induced various changes in surimi gel properties and protein secondary structure.

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1. Introduction

Surimi-based product has unique gelling properties. It is convenient, low-cholesterol, low-fat and suitable to produce ready-to-eat (RTE) food, which makes the demand for surimi product increasing. Gelation of protein is an important step for forming desired texture. During the heating process, a three-dimensional network of myofibrillar proteins was formed due to aggregation and cross-linking of exposed hydrophobic parts (Ni et al., 2014). The network formation was associated with several molecular forces, including hydrophobic interaction, hydrogen bonding, disulfide bonding, or a combination of them (Otte, Schumacher, Ipsen, Ju, & Qvist, 1999; Sun & Arntfield, 2012).

Bond disrupting agents can be used to destroy the main molecular forces in protein gels. They destabilized native structure of protein and induced irreversible molecular rearrangement, leading to variation of protein gel properties. Urea denatures protein molecule in a way of binding to the exposed protein surface by forming hydrogen bonds. It replaces the protein-protein and protein-water contacts, which results in more and more hydrophobic residues getting exposed to the denaturant solution (Das & Mukhopadhyay, 2008). Shan et al. (2014) found that urea prevented network formation of soy protein gel and induced dramatical decreases of gel firmness by breaking down hydrogen bonds and hydrophobic interactions. Sun et al. (2015) also found that the storage moduli (G′) value of corn germ proteins was decreased by urea. In the study reported by Jin et al. (2014), addition of urea reduced the firmness, cohesiveness and adhesive force of scallop (*P. yessoensis*) male gonad hydrolysates (SMGHs). Sodium dodecyl sulfate (SDS) is an amphipathic surface-active molecule, containing a polar group with a long hydrophobic carbon tail (Wang & Wu, 2012). It damages hydrophobic and electrostatic interactions in protein, promoting the disruption of the inter-molecular bonds. As a result, the protein conformation is dissociated, unfolded and denatured (Schmidt, Giacomelli, & Soldi, 2005). Nieto, Wang, Ozimek, and Chen (2016) found that oat protein gel was hardened as treated with SDS. Huang and Sun (2000) also found that shear strength of soy protein containing SDS was different with the unmodified sample. Covalent disulfide bonding also impacts the formation of surimi gel (Liu, Bao, Xi, & Miao, 2014). N-ethylmaleimide (NEM) reacts with sulfhydryl groups to form a stable

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alkyl derivative, preventing the formation of disulfide bonds between protein molecules. Involvement of sulfhydryl/disulfide interchange during gelation has been inhibited by preparing protein gels with NEM (Sun & Arntfield, 2012). In the study reported by Mession, Sok, Assifaoui, and Saurel (2013), the aggregation route of pea globulins was slightly modified in the present of N-ethylmaleimide.

Gel properties were also associated with the protein secondary structural changes. Herrero, Cambero, Ordonez, de la Hoz, and Carmona (2008b) found a significant correlations ($p < 0.05$) between secondary structural changes and the textural properties of meat systems. Shao, Zou, Xu, Wu, and Zhou (2011) also demonstrated that a positive significant correlation ($p < 0.05$) between textural properties and β-sheet structure was found in meat batters prepared with different lipids by Raman spectroscopy.

However, to our knowledge, no investigation has been carried out to examine the structural changes and protein secondary structure of surimi gels prepared with different bond disrupting agents. As a result, the objective of our current research was to evaluate the effect of various bond disrupting agents (urea, SDS and NEM) on the textural properties and protein conformation of heat-set surimi gels. The investigation allowed us to understand the relationship between molecular forces and textural properties and the corresponding changes of protein secondary structure.

2. Material and methods

2.1. Materials

Frozen silver carp surimi grade AAA was purchased from Hubei Jingli food co., LTD, The surimi was maintained at −18 °C until use. All chemicals used in this work were analytical grade. Urea and SDS were obtained from Sinopharm Chemical Regent Co., LTD; NEM was obtained from Sigma-Aldrich Co. According to the manufacturer, moisture content of surimi grade AAA is less than 77.0 ± 0.5%, pH is in the range of 6.8–7.4, whiteness is among 50–53, and the gel strength is in the range of 400–550 g cm.

2.2. Preparation of bond disrupting agent solution

Based on Wang and Arntfield (2016), bond disrupting agent solutions were prepared separately and then added into surimi at a specific ratio. Chemical stock solutions, including 2, 4, 6, 8 M urea, 0.5%, 1%, 2%, 4% SDS and 20 mM, 40 mM, 60 mM, 80 mM NEM were prepared in distilled water separately.

2.3. Preparation of surimi gel with bond disrupting agent

According to the method of Zhang, Xue, Li, Wang, and Xue (2015), frozen surimi was thawed at 4 °C for 12 h followed by cutting into small pieces (about $2 \times 2 \times 2$ cm^3) and then placed in a mixer (Joyoung Co., Ltd., Shandong, China). The surimi was chopped for 3 min, followed by addition of 2% NaCl, then chopped for another 3 min. Then bond disrupting agent solutions were added into the surimi at a 1:4 ratio (w/w), so that the final moisture content of mixture was adjusted to 80%. Chopping was continued for additional 3 min. The control was prepared without bond disrupting agent. Temperature was maintained at below 10 °C during chopping. The sol was stuffed into a polyvinylidene chloride casing with a diameter of 2.5 cm and both ends were sealed tightly. Surimi gels were prepared by incubating the sol at 40 °C for 30 min, followed by heating at 90 °C for 20 min. Subsequently, all gels were cooled in iced water for 30 min and stored at 4 °C overnight prior to analyses.

2.4. Texture profile analysis (TPA)

Texture profile analysis was carried out as described by Xu, Xia, Yang, Kim, and Nie (2010), using a Universal TA.XTPlus texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a cylindrical P/36R probe. Nine slices of surimi gels (2.5 cm thick and 2.5 cm diameter) were allowed to equilibrate to room temperature and then compressed twice to 50% of their original height. A time of 5 s was allowed to elapse between the two compression cycles. Force–time deformation curves were obtained with a 25 kg load cell applied at a cross-head speed of 1 mm/s. Hardness, springiness, cohesiveness, gumminess and resilience were calculated using Texture Expert software version 1.22 (Stable Micro Systems).

2.5. Gel strength

Gel strength was determined as described by Oujifard, Benjakul, Ahmad, and Seyfabadi (2012). The surimi gels were cut into 2.5-cm-high cylindrical specimens and equilibrated at room temperature (25 °C) for 2 h before analysis. The breaking force and distance to rupture were determined with a 5.0-mm-diameter spherical head plunger to press into one end of each specimen by a texture analyzer (TA.XTPlus, Stable Micro System, Surrey, UK). The speed of the sample table was maintained in an upper direction at a rate of 1 mm/s and 50% compression. Each measurement was replicated 9 times.

2.6. Rheological analysis

Rheological properties were performed according to the method of Hu et al. (2016) with minor modification. The storage (G′) and loss (G″) modulus were measured by dynamic viscoelastic measurements using a stress controlled rheometer (DHR-3, TA, USA). Parallel plate geometries were used (diameter 20 mm, gap 1 mm). The dynamic temperature sweep measurements were conducted from 25 to 90 °C with heating rate of 2 °C/min. Measurements were made at 1% strain and 1 Hz frequency. A strain sweep test revealed that 1% strain at 1 Hz frequency was within the linear viscoelastic region (LVR) for the samples. Samples were poured onto the bottom plate of the rheometer directly and excess sample was removed. In order to avoid evaporation, the applied sample was covered with silicone oil prior to measurement. All the experimental dynamic rheological data were obtained directly from the TA Rheology Advantage Data Analysis software.

2.7. Raman spectral analysis of surimi gels

Protein structures were analyzed for surimi gels containing bond disrupting agents. Raman spectra were recorded using the method described by Yang et al. (2016) with minor modifications. Sample was put on glass slides with tin foil paper. A microscope (Horiba Jobin Yvon S.A.S., France) equipped with a 50 × lens was used to focus the excitation laser beam (632.8 nm excitation line of a Spectra Physics Ar-laser) on the sample, and Raman signals were collected in the back scattered direction. For measurement, the sample was recorded in the range of 400–3500 cm^{-1}. The conditions of each spectrum were as follows: 1 scans, 30 s exposure times, 2 cm^{-1} resolution, 2 min per spectrum and a sampling speed of 120 cm^{-1}/min, with data collected every 1 cm^{-1}. The spectra obtained were baseline-corrected and smoothed using Labspec version 5.0. All analyses were performed in triplicate, and averaged for the reported results.

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