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Gelation properties of tilapia fish protein isolate and surimi pre- and post-rigor
Rigor condition of tilapia FPI and surimi

Yuka Kobayashi[a], Steven G. Mayer[b], Jae W. Park[a,*]

[a] Oregon State University Seafood Research and Education Center, USA
[b] Chemistry Department, University of Portland, USA

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ABSTRACT

The structural changes and rheological properties of tilapia proteins prepared using two refining methods (pH shift processing and surimi processing, respectively) with pre- and post-rigor muscle were investigated. Higher storage modulus (G′) and better gel texture were observed in surimi produced from pre-rigor fish than surimi made from post-rigor fish, but no rigor effect was noted on the gel-forming ability of fish protein isolate (FPI). The effect of salt on the gelation of tilapia muscle with different rigor states or refining method was also determined. The addition of salt appeared to readily unfold protein structures in FPI as measured by surface hydrophobicity, surface reactive sulfhydryl content, dynamic rheology, and Raman spectroscopy. However, the effect of salt on the degree of unfolding in surimi was not as sensitive as in FPI.

1. Introduction

Two successful methods are currently being used to refine fish muscle proteins: surimi and fish protein isolate (FPI). During the production of surimi, both chemical and physical denaturation of proteins must be avoided to obtain good quality surimi (Park, Graves, Draves, & $2 Yongsawatdigul, 2014). On the other hand, FPI is produced through chemical-induced denaturation by pH shift. The pH shift procedure first maximizes the solubility of fish muscles and recovers most of soluble proteins subsequently acquired at the isoelectric point by centrifugation. FPI is different compared to surimi, since sarcoplasmic proteins remain along with the myofibrillar proteins (Hultin & $2 Kelleher, 2000).

The gel-forming ability of FPI or surimi is a complex physicochemical process involving structural and functional changes of myofibrillar proteins. The rheological and biochemical characteristics of the gel can be affected intrinsically (species, freshness, and degree of stress before harvest) and extrinsically (protein concentration, pH, ionic strength, and temperature). In addition, the functional properties of the muscle proteins can be affected by the biochemical changes that occur during rigor mortis (Park et al., 2014).

Early research has reported that the functional properties of muscle proteins can be affected by different species and processing methods during rigor mortis. Extractability of salt soluble protein (SSP) in pre-rigor beef and pork was higher compared to SSP in post-rigor beef and pork (Saffle & $2 Galbreath, 1964). However, extractability of SSP from chicken white tissue between pre- and post-rigor did not show significant differences (Sayre, 1968).

Xiong and Brekke (1991) found that chicken myofibrils extracted from post-rigor breast showed greater protein extractability and stronger gel than myofibrils extracted from pre-rigor breast, however the reverse was found for leg myofibrils. One possible reason for improved gel formation of myofibrils from post-rigor chicken breast was the slow denaturation of the protein until proteolysis was increased (Yongsawatdigul, Hemung, & $2 Choi, 2014).

Park, Korhonen, and Lanier (1990) reported surimi produced from pre-rigor tilapia had significantly higher protein content and yield, reduced cook loss, stronger gel, and improved gel-forming ability. The higher amount of protein recovery possibly occurred due to the compact particles in pre-rigor muscle. This may have helped reduce the release of fine particles and swelling during the water leaching process. Moreover, the results of differential scanning calorimetry showed greater enthalpy of denaturation in surimi prepared from pre-rigor fillets, indicating the protein from pre-rigor surimi was more “native” protein and needed higher energy to denature the protein structure (Park & $2 Lanier, 1989). Xiong and Brekke (1991) and Park et al. (1990) obtained different results for the gel-forming ability by rigor using chicken and tilapia muscle, respectively. Different species

* Correspondence to: 2001 Marine Drive #253, Astoria, OR 97103, USA.
E-mail address: jae.park@oregonstate.edu (J.W. Park).

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may have different rates and ways of protein denaturation during rigor mortis. The sample preparations to refine functional proteins using washing and pH shift are different, which likely affect the results. Thus, it is necessary to find the optimum processing stage (pre- and post-rigor) and further develop their relationship with FPI and surimi. No information regarding the biochemical and gelling properties of FPI prepared using pre- and post-rigor fish has been reported. The objectives of this study were to investigate the gelation behavior of FPI and surimi made from tilapia processed during pre- and post-rigor stages and further to understand how the two protein refining methods contribute differently to the formation of the fish protein gel.

2. Materials and methods

2.1. Materials

Live tilapia (*Oreochromis niloticus*), 130 fish with a size of 0.9–1.4 kg, were obtained from a fish farm (Green Hill Gardens Inc., Eugene, OR, USA), and kept in aerated holding tanks (24–26 °C) for 36 h. The fish were removed from the tank and batch-weighed on an electronic scale to determine total weight, and then chill-killed using an ice-water bath. The fish were packed in ice and filleted at different times: immediately after harvest or 120 h after harvest for the pre- and post-rigor stage, respectively. The fillets were ground using a meat grinder (Electric 2.6 HP 2000 W Industrial Meat Grinder, City of Industry, CA, USA) with 4.5 mm perforations. Ingredients used for gel preparation of surimi or FPI were NaCl (Morton Iodized Salt, Morton International, Inc., Chicago, IL, USA), sugar (Pure Cane Sugar, C & $2H Sugar Company, Inc., Crockett, CA, USA), and oil (PAM canola oil, ConAgra Food Inc., Omaha, NE, USA). Bradford reagent for protein analysis was purchased from Bio-Rad Laboratory (Hercules, CA, USA). All other chemicals were reagent grade (GR).

2.2. Preparation of fish protein isolates

Tilapia mince was homogenized (model GLH-115, PG700, Fisher Scientific, Pittsburgh, PA, USA) with chilled tap water (1:9 ratio) for 1 min at speed level 3. The homogenates were adjusted to pH 11.0 ± 0.01 using 2N HCl and/or 2N NaOH. Samples were then centrifuged at 8,000×*g* for 20 min at 4 °C (Sorvall RC-5B, Newtown, CT, USA and Beckman Coulter, Fullerton, CA, USA). The supernatant, separated from neutral lipids, skin, bone, and collagen, was filtered using two layers of cheesecloth. The pH of the filtered supernatant was adjusted to the nominal isoelectric point (pH 5.5) (Ingadottir & $2 Kristinsson, 2010). Aggregated precipitates in four layers of cheesecloth and one layer of screen mesh (2 mm) were dewatered using a washing machine (Kenmore 20022, Kenmore, Chicago, IL, USA) at 700 rpm for 12 min. The pH of the protein isolates was adjusted to approximately 7.0 using 2N NaOH. The protein isolates were mixed with cryoprotectants (5% sorbitol, 4% sugar, and 0.3% sodium tripolyphosphate) using a mixer (VCM 40, Hobart Corp, Troy, OH, USA) for 2 min, placed in a plate freezer overnight (−25 °C), vacuum-packed, and stored at −18 °C until tested within 2 month. Temperature was maintained below 5 °C during processing. Two batches of FPI were made using pre-rigor fish, and two additional batches were made using post-rigor fish.

2.3. Preparation of surimi

Ground meat was washed at a 1:2 ratio (mince: cold water) with manual stirring for 10 min. Washed mince was filtered using the screen mesh (2 mm) and dewatering was done manually by repeated pendulum swings. These washing-dewatering steps were repeated once more. The washed mince was then wrapped in two layers of cheesecloth and one layer of screen mesh before dewatering in the washing machine as described above for preparation of FPI. Lastly, the dewatered meat was mixed with cryoprotectants using the mixer as explained above for preparation of FPI before placing in a plate freezer overnight. Samples were vacuum-packed and stored at −18 °C until tested within 2 months. All steps in the preparation of surimi were done in a 4 °C cold room or on ice to maintain the temperature below 5 °C. Two batches of surimi were made using pre-rigor fish, and two additional batches were made using post-rigor fish.

2.4. Paste and gel preparation

Approximately 40 g of frozen surimi or FPI were partially thawed at room temperature for 10 min and cut into small pieces. The sample was chopped at speed level 5 for 1 min with a blender (Osterizer 4172, Sunbeam-Oster Co., Inc., Fort Lauderdale, FL, USA). Chopping continued for 1 min after the addition of 0%, 2%, and 3% salt, respectively. Before continuing to chop for another 1 min, moisture content was adjusted to 79% using ice based on the moisture content of original FPI and surimi. The sample was then chopped for an additional 2 min for a total chopping time of 5 min. The final temperature of the surimi or FPI paste was less than 10 °C. Paste (28.0 g) was molded into a thin sheet using a homemade stainless sheet molding frame (7.5 x, 25.5×0.1 cm thick) positioned on aluminum foil sprayed lightly with a lecithin anti-stick coating. The sheet was cooked on a wire rack under steam at 90 °C for 20 min. The sheet of gel was held at a room temperature (approximately 20 °C) for 5 min and stored overnight at 4 °C. Gel preparation was done twice for each treatment at 3 different salt levels.

2.5. Surface hydrophobicity

Using the procedure described by Alizadeh-Pasdar and Li-Chan (2000), protein surface hydrophobicity (S_0) of the supernatant from the paste was determined. The supernatant was collected by homogenizing 3 g of paste and 27 ml of 0.6 M KCl in 20 mM Tris-HCl buffer pH 7.0 for 1 min and centrifuging the mixture at 20,000 x g for 30 min at 4 °C (Beckman Coulter). An ANS (1-anilinonaphathalene-8-sulfonate) probe was used with the ANS stock solution containing 8 mM ANS in 0.1 M phosphate buffer pH 7.4. Protein concentration was determined by the Bradford dye-binding method with bovine serum albumin (BSA) as purchased. The protein concentration of the supernatant was adjusted to 0.05, 0.1, 0.2, and 0.4 mg/ml using 0.6 M KCl in 20 mM Tris-HCl buffer pH 7.0. Then, 20 μl of ANS stock solution was added into 4 ml of samples and held at room temperature for 10 min. The samples were measured at a wavelength of 390 nm and 470 nm ($\lambda_{excitation}$, $\lambda_{emission}$), respectively, using a luminescence spectrophotometer (PerkinElmer LS-50B, Norwalk, CT, USA). The initial slope (S_0) of the net relative fluorescence intensity versus the protein concentration was used as the protein surface hydrophobicity. The greater slope indicates more unfolded protein structures.

2.6. Surface reactive sulfhydryl content

Surface reactive sulfhydryl (SRSH) content was determined using Ellman's reagent (5, 5′-dithiobis (2-nitrobenzoic acid); DTNB) according to the method of Hamada, Ishizaki, and Nagai (1994). Salt soluble protein was extracted by homogenizing 3 g of paste with 27 ml of 0.6 M KCl in 20 mM Tris-HCl buffer pH 7.0 for 1 min and centrifuging them at 20,000 x g at 4 °C for 30 min (Beckman Coulter). The protein concentration of salt soluble protein from the paste was diluted to 1 mg/ml protein with 0.6 M KCl in 20 mM Tris-HCl buffer pH 7.0. Diluted sample (0.5 ml) was mixed with 2 ml of 0.6 M KCl in 20 mM Tris-HCl buffer pH 7.0, and 50 μl of 0.1 M sodium phosphate buffer pH 7.2 containing 10 mM DTNB and 0.2 mM EDTA. The sample was Vortexed (Vortex Genie 2, Scientific Industries, INC., Bohemia, NY, USA) and incubated at room temperature for 15 min before reading the absorbance at 412 nm (UV–VIS Spectrophotometer, UV 2401PC, Shimadzu Co., Kyoto, Japan). The SRSH content was calculated based on absorbance using a molar extinction coefficient of 13,600 $M^{-1}cm^{-1}$

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