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Biochemical and physical characterizations of fish protein isolate and surimi prepared from fresh and frozen whole fish

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ABSTRACT

The effect of frozen storage on the biochemical and physical characterization of fish protein isolate (FPI) and surimi made from tilapia was elucidated: tilapia frozen for 3 mo can be used like fresh fish in the processing of FPI and surimi. The Ca^{2+}ATPase activity continuously reduced throughout the frozen storage up to 3 mo; however, the diminishing trend of Ca^{2+}ATPase activity was slow. According to storage modulus (G′), storing whole fish frozen for 3 mo did not affect the gelling ability of FPI and surimi with 0% salt. The comparable results were observed by surface hydrophobicity, surface reactive sulfhydryl content, and differential scanning calorimetry. The addition of salt into FPI induced higher degrees of denaturation before gelation compared to surimi. Our results suggested that frozen tilapia, if stored up to 3 mo, can be used to make good quality FPI and surimi.

1. Introduction

Two procedures have been successfully utilized to refine fish proteins: fish protein isolate (FPI) and surimi. However, the processes of each are quite different. The FPI method induces chemical unfolding of fish proteins to solubilize both myofibrillar and sarcoplasmic proteins, recovers these soluble proteins at the isoelectric point by centrifugation, and then the unfolded proteins are refolded by neutralization (Hultin & Kelleher, 2000). The conventional surimi process, however avoids or minimizes chemical and physical denaturation to obtain good surimi quality and primarily recovers myofibrillar proteins.

In general, fresh fish are used for FPI and surimi production. Freshness of fish is considered one of the decisive factors for the quality and stability of the fish protein gel. Storing fish in a freezer for extended periods of time can negatively affect the gel-forming ability of muscle proteins (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2005; Okazaki & Kimura, 2013). The quality of surimi gels made from frozen hoki, Alaska pollock, and lizardfish determined by MacDonald, Lelievre, and Wilson (1992); Scott, Porter, Kudo, Miller, and Koury (1988); and Kurokawa (1979), respectively, demonstrated a declining trend as frozen storage induced denaturation.

Factors affecting gel-forming ability during frozen storage are fish species, freezing methods, storage temperature, and enzymatic degradation (Ang & Hultin, 1989; Benjakul, Visessanguan, Thongkeaw, & Tanaka, 2003; Hsieh & Regenstein, 1989). Benjakul et al. (2005) found that, under extended frozen storage, lizardfish was most susceptible to denaturation and loss in gelation compared to threadfin bream, bigeye snapper, and croaker. Results indicated the formaldehyde level in lizardfish muscle was the highest among these four tropical species, which contributed to increased protein denaturation.

Denaturation of fish proteins during frozen storage is affected by various factors: partial dehydration of protein during freezing; an increase in inorganic salts in the frozen phase; interaction of lipids, free fatty acids, and/or products from lipid oxidation with proteins; and trimethylamine oxide demethylase (TMAOase) activity (Benjakul & Visessanguan, 2010). These reactions lead to significant reduction in gel-forming ability. However, frozen seafood products are imperative since fish products are one of the most highly traded food commodities. If frozen fish could be used for FPI or surimi production, logistics for raw fish handling would be much easier. No information exists on fish protein gels made from FPI and surimi using fresh and frozen whole fish of the same species. The purpose of this study was to evaluate the relationship of the biochemical and physical properties of fish protein gels prepared using differently refined proteins (FPI and surimi) from fresh and frozen tilapia.

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2. Materials and methods

2.1. Materials

Live tilapia (*Oreochromis niloticus*) with a size of 0.9–1.4 kg were obtained from Green Hill Gardens Inc. (Eugene, OR, USA). Whole fish were packed in ice for 120 h in a cold room (4 °C). A half was used as fresh fish and the other half was stored in a −18 °C freezer for 1 and 3 mo and used as frozen fish. To process frozen fish, the fish were thawed in the cold room for 36 h, filleted, and ground using a meat grinder (Electric 2.6 HP 2000 Watt Industrial Meat Grinder) with 4.5 mm perforations before subjecting to two refining processes. Ingredients used for gel preparation of surimi or FPI were NaCl (Morton Iodized Salt, Morton International, Inc., Chicago, IL, USA), phosphate blend (SD BNI CO., LTD., Hwaseong-shi, Kyeonggi-do, Korea), sugar (Pure Cane Sugar, C&H Sugar Company, Inc., Crockett, CA, USA), and sorbitol (sorbitol NF/FCC, Roquette American Inc., Gurnee, IL, USA). Bradford reagent for protein analysis was purchased from Bio-Rad Laboratory (Hercules, CA, USA). All other chemicals were reagent grade.

2.2. Measurement of Ca-ATPase activity

Actomyosin (AM) was extracted according to the method of Benjakul et al. (2005) with slight modifications. A filleted sample (8 g) was mixed with 80 mL of 0.6 M KCl (4 °C, pH 7.0) and homogenized at speed 5 for 4 min (Tissue Tearor Homogenizer, Bio-Spec Products Inc., Bartlesville, OK, USA). The homogenized solution was centrifuged at 5000×*g* at 0 °C for 30 min. The supernatant was filtered through two layers of cheesecloth and three volumes of chilled deionized water were added. Then, AM was collected by centrifuging at 9000×*g* at 0 °C for 20 min. The pellet was recovered and stirred in an equal volume of 1.2 M KCl (4 °C, pH 7.0) for 20 min. After stirring, the suspension was centrifuged at 9000×*g* at 0 °C for 20 min.

ATPase activity was measured using the AM solution at a concentration of 2.0 mg/mL in 0.6 M KCl (pH 7.0) (Benjakul, Seymour, Morrissey, & An, 1997). One quarter mL of 0.5 M Tris-maleate (pH 7.0), 0.25 mL of 0.1 M $CaCl_2$, and 3.75 mL of deionized water were added to the 0.5 mL of AM solution. To this mixture, 0.25 mL of 20 mM ATP solution was added to start the reaction and then incubated for 8 min at 25 °C. The reaction was stopped by the addition of 2.5 mL chilled 15% trichloroacetic acid. The concentration of inorganic phosphate released during incubation was measured using a UV–VIS spectrophotometer (UV–2401PC spectrophotometer, Shimadzu Corp., Kyoto, Japan) described by Fiske and Subbarow (1925). The ATPase specific activity was presented as μ mole inorganic phosphate (Pi) released/mg protein/min. Both pH and ATPase activity were measured in triplicate from three different fish. All steps in the preparation of samples were performed in a 4 °C cold room or by keeping samples in ice in order to maintain temperature below 5 °C.

2.3. Preparation of fish protein isolates

Fish mince, prepared above (pH 6.9 at 0 mo, 6.8 at 1 mo and 6.7 at 3 mo frozen storage), 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 pH of the homogenates was adjusted to 11.0 ± 0.01 using 2 N NaOH. Samples were then centrifuged at 8000×*g* at 4 °C for 20 min (Sorvall RC-5B, Newtown, CT, USA and/or Beckman Coulter, Fullerton, CA, USA). After centrifugation, two layers of cheesecloth were used to filter the supernatant from neutral lipids, skin, bone, and connective tissues. The pH of the filtered supernatant was adjusted to the isoelectric point (pH 5.5) using 2 N HCl. Once the pH was adjusted, aggregated precipitates were wrapped in four layers of cheesecloth and one layer of screen mesh (2 mm) before being dewatered by 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 2 N NaOH.

2.4. Preparation of surimi

Ground meat was washed by manually stirring at a 1:2 ratio (mince:cold water) for 10 min. Washed mince was filtered using a screen mesh and dewatering was manually performed by repeated pendulum swings. These washing-dewatering steps were repeated once. Then, two layers of cheesecloth and the above mentioned screen mesh was used to dewater the washed mince in the washing machine as described above for FPI.

2.5. Freezing FPI and surimi with cryoprotectants

Fish protein isolates and surimi, independently, were mixed with cryoprotectants (5 g/100 g sorbitol, 4 g/100 g sugar, and 0.3 g/100 g of a mixture (50:50) of sodium tripolyphosphate and tetrasodium pyrophosphate), and placed in a plate freezer overnight (−25 °C). Samples were vacuum-packed and stored at −18 °C until tested. All steps were conducted in a 4 °C cold room or in ice in order to maintain a temperature below 5 °C. Two batches of FPI and surimi were made using whole fish frozen for 0, 1, or 3 mo.

2.6. Paste preparation

A frozen FPI or surimi block (approximately 50 g) was partially thawed at room temperature for 10 min and cut into small pieces. The sample was chopped using a blender (Osterizer 4172, Sunbeam-Oster Co., Inc., Fort Lauderdale, FL, USA) at speed level 5 for 1 min. Once the sample was chopped for 1 min, 0, 2, and 3 g/100 g salt for three treatments, respectively, were added before chopping for an additional 1 min. Before continuing to chop for another 1 min, moisture content was adjusted to 79% (g/100 g) using ice/cold water. The sample was then chopped until the total chopping time reached 5 min. The final temperature of the FPI or surimi paste was less than 10 °C. The paste was used for biochemical and physical analyses, such as surface hydrophobicity, surface reactive sulfhydryl content, oscillatory dynamic measurement, and differential scanning calorimetry.

2.7. Surface hydrophobicity

Protein surface hydrophobicity (S_0) of the supernatant from the paste was measured according to the method described by Alizadeh-Pasdar and Li-Chan (2000) with slight modification. 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 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. ANS stock solution (20 μL) was then added to four milliliters of diluted samples and incubated at room temperature for 10 min. A wavelength of 390 nm and 470 nm ($\lambda_{excitation}$, $\lambda_{emission}$), respectively, was used to measure the samples by a luminescence spectrophotometer (Perkin Elmer LS-50B, Norwalk, Conn., USA). The initial slope (S_0) of relative fluorescence intensity versus protein concentration was calculated by linear regression analysis and used as the protein surface hydrophobicity.