Food Chemistry 112 (2009) 112–119

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process

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ARTICLE INFO

Article history:
Received 2 February 2008
Received in revised form 6 April 2008
Accepted 15 May 2008

Keywords:
Gelation
pH-shift process
Solubilisation
Surimi
Tilapia
Washing

ABSTRACT

The biochemical and gel properties of tilapia surimi prepared by a conventional washing method and protein isolated using alkaline-acid-aided processes were studied. Solubility and recovery of protein was found to be highest by using a conventional method, followed by an alkaline- and acid-aided process, respectively. Decreases in myoglobin and lipid contents were found in alkaline- or acid-aided process when compared to the conventional process ($p < 0.05$). The highest breaking force and deformation of kamaboko and modori gels was found in the gels prepared by the conventional washing method. Higher expressible water and whiteness were found in modori gels when compared to kamaboko gels. TCA-soluble peptide contents of conventional surimi gels were lower than those of acid- and alkaline-recovered protein gels. Degradation of myofibrillar protein was observed in acid-isolated protein. Microstructure of kamaboko gels showed more compact network than in modori gels in both conventional surimi and protein recovered using the pH-shift process.

1. Introduction

Surimi technology has been widely developed. Surimi is produced by repeatedly washing mechanically separated fish flesh with chilled water (5–10 °C) until most of the water-soluble protein is removed. The washing procedure is of great importance for surimi quality – not only for removing fat and undesirable materials, such as blood, pigments and odorous substances – but, more importantly, for increasing the concentration of myofibrillar protein, thereby improving gel-forming ability (Lanier & Lee, 1992). The gel-forming ability of surimi varies with the function of the myofibrillar proteins. A process that has been met with some success in recovering fish proteins is the production of surimi; however, yields are low because the process involves several washing steps (Kristinsson, Theodoure, Demir, & Ingadottir, 2005).

Acid- and alkaline-aided solubilisation has shown significant potential as a new method for maximal protein recovery from muscle food. The extraction mechanism of the two processes is to solubilise the muscle protein at low and high pH to separate soluble proteins, bone, skin, connective tissue, cellular membranes, and neutral storage lipids through the centrifugation. The solubilised proteins are collected and recovered by isoelectric precipitation to give a highly functional and stable protein isolate (Kristinsson & Ingadottir, 2006). The proteins recovered by this process have good functionality and in some cases better gelation properties than have proteins recovered by conventional surimi processing (Kristinsson et al., 2005).

Tilapia is one of the important economic freshwater fish of Thailand, constituting about 76% of the total aquaculture production of tilapia worldwide. Chiang Rai is the second largest tilapia production area in the northern part of Thailand and supports other provinces in that area. Tilapia muscle has a high content of pigments and non-structural lipids which can cause a high intensity of muddy and fishy odour. The presence of these components can strongly affect the flavour and colour of processed tilapia meat during storage and also affect consumer acceptability. Removal of the soluble sarcoplasmic proteins, lipids, fish blood, and other water-soluble materials from the flesh, as well as concentration the myofibrillar proteins by washing, have been investigated (Park, Lin, & Yongsawatdigul, 1997). This process can improve the functionalities and sensory characteristics of fish meat. However, a low yield is obtained with this process. The new approach of recovering protein by the pH-shift process can be used to encounter this problem. However, no information regarding the biochemical and gelling properties of muscle from tilapia cultured in Thailand has been reported. Therefore, the objective of this study was to investigate the effects of the pH-shift process and conventional washing on biochemical and gelling properties of tilapia muscle.

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doi:10.1016/j.foodchem.2008.05.047

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), acrylamide, Tris, sodium dithionite were obtained from Fluka Chemika-BioChemika (Buchs, Switzerland). *L*-tyrosine, β-mercaptoethanol (βME), glutaraldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium dodecyl sulphate (SDS), *N,N,N′, N′*-tetramethyl ethylene diamine (TEMED) and Coomassie Blue R-250 were procured from Bio-Rad Laboratories (Hercules, CA, USA). Sodium chloride, tricholoroacetic acid, calcium chloride, ethanol were obtained from Merck (Darmstadt, Germany).

2.2. Effect of processing conditions on biochemical properties of tilapia muscle

2.2.1. Fish sample

Fresh tilapia (weight 500–600 g/fish) was obtained from Pla Thong fish farm, Phan District, Chiang Rai, Thailand, and transported on ice to the Food Technology Department, Mae Fah Luang University. Fish were headed, eviscerated, and thoroughly washed before being subjected to mincing using a meat mincer (32 EI/80 Tre Spade, Torino, Italy) and kept in a freezer (−18 °C) until used.

2.2.2. Washed mince preparation

2.2.2.1. Conventional washing method. Washed mince (by conventional method) was prepared according to the method of Chaijan, Benjakul, Visessanguan, and Faustman (2006) with slight modifications. The washing was performed with a 1:3 (w/w) ratio of mince to cold distilled water (4 °C), and continuously stirred for 10 min in a cold room. The washed mince was filtered through four layers of cheese-cloth and then subsequently dewatered by using a hydraulic pressing machine (Owner Foods Machinery, Bangkok, Thailand). Washing was performed twice. The third washing step was carried out using 0.5% NaCl solution with a mince to NaCl solution ratio of 1:3 (w/w). Sample was referred to as the "*conventional washed mince: Con*" after dewatering.

2.2.2.2. Alkaline-aided process. The alkaline-aided process was carried out as described by Hultin and Kelleher (2000) with slight modifications. Minced fish was homogenised (IKA Labortechnik homogenizer, Selangor, Malaysia) at a 1:9 (w/v) ratio with cold distilled water for 60 s. The pH of the homogenates was adjusted to 11.2 by using 2N sodium carbonate. Homogenates was centrifuged at 10,000*g* for 20 min at 4 °C. The alkaline-soluble fraction was collected and adjusted to the isoelectric point of muscle proteins (pH 5.5) by using 2N HCl. The precipitate was then filtered through 4-folded cheese-cloth and was dewatered by centrifugation at 12,000*g* for 20 min at 4 °C. The final pH of the sample was adjusted to pH 7.0 using 2N NaOH. The sample was referred to as the "*alkaline washed mince: Alk*"

2.2.2.3. Acid-aided process. The acid-aided process was done using the method of Hultin and Kelleher (2000) with slight modifications, as described previously, except that the pH of sample was adjusted to 3.0 with 2N acetic acid. Soluble proteins were recovered by isoelectric precipitation at pH 5.5 and collected as described above. The precipitate was adjusted to pH 7.0 by using 2N NaOH. The sample was referred to as the "*acid washed mince: Acid*".

2.2.3. Physicochemical properties of minced fish

2.2.3.1. Protein solubility. The solubility of protein obtained from different processes was measured according to the method of Choi and Park (2002) with slight modifications. Samples (2 g) were homogenised with 18 ml of 0.5 M borate buffer solution, pH 11.0, for 60 s and stirred for 30 min at 4 °C. The homogenates were centrifuged at 8000*g* for 5 min at 4 °C, and the protein concentration of the supernatant was measured by the Biuret method. Protein solubility (%) was defined as the fraction of protein remaining soluble after centrifugation and calculated as follows:

$$\text{Protein solubility}\ (\%) = \frac{\text{protein concentration in supernatant}}{\text{protein concentration in homogenate}} \times 100$$

2.2.3.2. Protein recovery. Protein recovery (% yield) of the washed mince from different washing methods was determined according to the method of Kim, Park, and Choi (2003). The recovery was expressed as the weight of recovered protein divided by the weight of the minced fish (at the same moisture content). After a acid-aided, alkaline-aided or conventional washing process, the moisture content of washed mince and protein isolates was equally adjusted to 79% moisture (the initial moisture content of fish muscle); the weight of recovered protein at the same moisture content was recorded. The recovery of protein was calculated as follows:

$$\text{Protein recovery}\ (\%\,\text{yield}) = \frac{\text{weight of recovered washed mince}}{\text{weight of initial minced sample}} \times 100$$

2.2.3.3. SDS-Polyacrylamide gel electrophoresis (SDS–PAGE). The pattern of protein obtained from different methods was determined by using electrophoresis according to the method of Laemmli (1970). To 3 g of sample were added with 27 ml of 5% SDS (85°C). The mixtures were homogenised at a speed of 11,000 rpm for 1 min using an IKA Labortechnik homogenizer (Selangor, Malaysia). The homogenate was incubated at 85°C for 60 min, followed by centrifugation at 8000*g* for 5 min at room temperature, using a Mikro 20 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). The supernatants were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4% SDS, 20% glycerol and 10% BME), and boiled for 3 min. The samples (20 μg protein) were loaded into the polyacrylamide gel (10% running and 4% stacking gel) and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protean II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.2.4. Total pigment and myoglobin analysis

2.2.4.1. Total pigment determination. The total pigment content was determined according to the method of Lee, Hendricks, and Cornforth (1999). Washed mince (1 g) was mixed with 9 ml of acid-acetone (90% acetone, 8% deionised water and 2% HCl). The mixture was stirred with a glass rod and allowed to stand for 1 h at room temperature. The extract was filtered with a Whatman No. 1 filter paper, and the absorbance was read at 640 nm against an acid-acetone blank. The total pigment was calculated as hematin using the following formula:

$$\text{Total pigment content}\ (\text{ppm}) = A_{640} \times 680$$

2.2.4.2. Myoglobin content determination. The myoglobin content was determined by direct spectrophotometric measurement, as described by Chaijan et al. (2006). Two grams of washed mince were weighed into a 50 ml centrifuge tube and 20 ml of 40 mM phosphate buffer, pH 6.8, were added. The mixture was homogen-