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Effect of setting conditions on proteolysis and gelling properties of spotted featherback *(Chitala ornata)* muscle

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ABSTRACT

Gels from spotted featherback (SF) muscle were prepared by different setting conditions including 4 °C/18 h, 25 °C/30 min, 25 °C/2 h, 40 °C/30 min and 60 °C/30 min followed by cooking at 90 °C/20 min. Directly cooked gel was used as a control. SF gels set at 4 °C/18 h and 60 °C/30 min exhibited higher proteolytic degradation than did by other setting temperatures and control ($P < 0.05$) as evidenced by TCA-soluble peptides and the marked decrease in myosin heavy chain (MHC) under SDS-PAGE. For gelling properties, setting at 60 °C/30 min showed the gel with lowest breaking force and highest expressible drip whereas setting at 25 °C/30 min rendered the gel with highest breaking force ($P < 0.05$). Indeed, the higher the setting temperature applied the lower the a* value was observed ($P < 0.05$). However, the L* and b* values were varied among setting conditions. A finer structure of SF gel correlated well with the breaking force, particularly at medium setting temperature (25 °C/30 min). Therefore, the setting regimes strongly influenced the proteolysis and gel properties of SF muscle. Setting at 25 °C for 30 min was the best suit to prevent the proteolysis and hence strengthen the gel of SF muscle.

1. Introduction

Gelation is one of important functional properties of fish mince, surimi (washed fish mince) and ground meat. Basically, gelation is the cross-linking of randomly dispersed polymer chains to form a three-dimensional network (Smith, 1991) which including initial denaturation to cause protein unfolding, protein–protein interactions and aggregation giving rise to matrices capable of holding water, fat or other components through physico-chemical forces (Mulvihill & Kinsella, 1987; Sikorski, 2001). However, protein–protein interaction, known as association, aggregation and polymerization, are dependent upon temperatures, pH, and the type of muscle proteins used (Deng, Andrews, & Laursen, 1997). The three-dimensional structure is responsible for the elasticity and the textural strength of the gel (Sikorski, 2001). Myofibrillar proteins, particularly myosin and actomyosin, which are composed of multiple corporative domains, are able to form highly viscoelastic and rigid gels (Xiong, 1997). In general, protein gelation has been traditionally achieved by heating (Totosaus, Montejano, Salazar, & Guerrero, 2002). Upon heating, the denaturation and degradation of muscle proteins can occur with varying degrees depending on temperature and time. The influence of setting phenomenon on gel properties of surimi has been reported (Benjakul, Visessanguan, Riebroy, Ishizaki, & Tanaka, 2002). The setting temperature can affect enzymatic activities, protein degradation and gelation (Huang, Seguro, Motoki, & Tawada, 1992). In the case of surimi, incubation the sol at 0–40 °C prior to heating is generally used for gel strengthening (Lanier, 1992). Setting can be performed at low temperatures (0–4 °C), medium temperatures (25 °C) and high temperatures (40 °C) (Lanier, 1992), whereas protein degradation caused by proteinases, commonly found at 50–60 °C (Lee et al., 1990; Kamath, Lanier, Foegeding, & Hamann, 1992) leading to gel structure disintegration or softening (Benjakul, Visessanguan, & Chantarasuwan, 2004; Jiang, 2000). Benjakul, Chantarasuwan, & Visessanguan, (2003) suggested that setting at different temperatures produces different gel characteristics and setting at low temperature usually takes a longer time.

Spotted featherback (*Chitala ornata*, SF) is an important freshwater fish in economic value of Thailand. In general, SF meat has been used to produce many kinds of foods such as fish ball, fried curry-fish cake and som-fug (fermented fish sausage). SF is commonly sold in the form of scraped meat in plastic bags and kept on ice during transportation and distribution. For nutritional value,

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spotted featherback meat is a good source of protein (Puwastein et al., 1999).

Generally, heating is usually applied for SF meat processing. SF meat can be mixed with other adjunct ingredients (salt, sugar, chili paste, etc.) and kept on ice overnight (18–24 h) before cooking. This preparation can be considered as low temperature setting of SF meat. In this traditional preparation, proteolysis and biochemical changes of muscle can be taken place to some degrees during iced storage. Those changes would associate with poorer gelling characteristics of final products. However, many products and recipes from SF mince were directly cooked (without setting). For instance, Thai green curry with fish ball (Kang-Keaw-Wan) production, the SF meat is knead with a pinch of salt (about 0.33%) and the ball-shaped of mixture is boiled without setting. From point of view, SF meat is popularly used as a raw material for production of food product with high quality in textural properties. However, no information regarding the degradation of muscle proteins and gel-forming ability of SF meat as affected by different setting conditions has been reported. Therefore, the objective of this study was to investigate the effect of setting conditions on proteolysis and gel properties of SF meat.

2. Materials and methods

2.1. Chemicals

Sodium chloride (NaCl) and bromophenol blue were obtained from Carlo Erba (Milan, Italy). Trichloroacetic acid (TCA), methanol and bis-acrylamide were purchased from Merck (Darmstadt, Germany). Acetic acid, glutaraldehyde, ethanol, and β-mercaptoethanol (βME) was obtained from Sigma (Steinheim, Germany). N,N,N′,N′- tetramethylethylenediamine (TEMED) was obtained from AMRESCO (Solon, OH, USA). Coomassie brilliant blue R-250 was obtained from Panreac (Barcelona, Spain).

2.2. Fish samples and preparation

Fresh SF (body length about 60–65 cm and an average weight of 1.5–2.0 kg) were obtained from Bangpakong River, Prachin Buri, Thailand. After capture within 12 h, fish were transported in ice with a fish/ice ratio of 1:2 (w/w) to Food and Nutrition Laboratory, Faculty of Agriculture, Kasetsart University within 1 h. The whole fish were immediately washed, filleted and manually scraped into meat. The meat was kept on ice (2 h) during preparation and analysis. The pH value of SF meat was measured as described by the method of Benjakul, Seymour, Morrissey, and An (1997).

2.3. Gel preparation

Before gel preparation, SF meat was manually mixed to uniformity. Moisture content of sample was measured according to the method of AOAC (2000) and adjusted to 80%. Then, the sample were added with 2.5 g/100 g NaCl and chopped for 5 min to obtain the homogenous sol. The sol was stuffed into polyvinylidine casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. To prepare gel, the sols were incubated at several conditions including 4 °C/18 h, 25 °C/30 min, 25 °C/2 h, 40 °C/30 min, 60 °C/30 min prior heating at 90 °C/20 min. The gel without setting was heated directly and used as the control. The gels were cooled in ice-water for 30 min and stored for 24 h at 4 °C prior to analyses.

2.4. Determination of TCA-soluble peptides

TCA-soluble peptides were determined according to the method of Morrissey, Wu, Lin, & An (1993). Gel samples (3 g) were homogenised with 27 ml of TCA (5 g/100 ml) at speed no.2 (11,000 rpm) using a homogeniser (T18 basic, IKA, Staufen, Germany). The homogenate were kept on ice for 1 h and centrifuged at 8000 ×*g* for 5 min using a refrigerated centrifuge (Allegra X-15R centrifuge, Beckman Coulter, California, USA). The soluble peptides in the supernatant were measured by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and expressed as μmol tyrosine/g dry weight.

2.5. SDS–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to the method of Leammli (1970) using 10 g/100 ml running gel and 4 g/100 ml stacking gel. Gel sample (3 g) were mixed with 27 ml of 5 g/100 ml SDS and homogenised for 1 min. The homogenate were incubated at 85 °C for 1 h to dissolve the proteins, followed by centrifuged at 8500 ×*g* for 5 min at room temperature using a centrifuge (Allegra X-15R centrifuge, Beckman Coulter, USA). Protein concentrations were determined according to the Biuret method (Robinson & Hodgen, 1940) using bovine serum albumin as a standard. Solubilized samples were mixed at a 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4 g/100 ml SDS and 20 g/100 ml glycerol) in the presence of 10 ml/100 ml βME. Samples (10 μg protein) were loaded onto polyacrylamide gels. The electrophoresis was carried out at 15 mA per gel using a vertical gel electrophoresis unit (Mini-Protean II; Bio-Rad Laboratories, Richmond, California, USA). After separation, protein bands were stained with 0.05% (w/v) Coomassie Blue R-250 in 15 ml/100 ml methanol and 5 ml/100 ml acetic acid and destained with 30 ml/100 ml methanol and 10 ml/100 ml acetic acid.

2.6. Determination of expressible drip

Expressible drip was measured according to the method of Ng (1978). A gel sample with a thickness of 0.5 cm were weighed and placed between two pieces of Whatman filter paper no. 1 at the top and three pieces of the same type of filter paper at the bottom. The standard weight (5 kg) were placed on the top of the sample and maintained for 2 min. The sample were then removed and weighed again. Expressible drip were calculated and expressed as percentage of sample weight.

2.7. Determination of textural properties

Texture analysis, breaking force and deformation, of the gel were performed using a Texture Analyzer (TA-XT plus, Stable Micro System, Surrey, UK). Gels were equilibrated and evaluated at room temperature (25–28 °C). Five cylinder-shaped samples with a length of 2.5 cm were prepared and subjected to determination. Breaking force (gel strength) and deformation (elasticity and deformability) were measured using the texture analyser equipped with a spherical plunger (diameter 5 mm, depression speed of 60 mm/min).

2.8. Determination of colour

The colour of sample was measured in L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) the using a ColorFlex (Colorflex, HunterLab, USA).

2.9. Determination of microstructure

Microstructure of gels with different setting temperatures and direct heating were determined as described by Jones and Mandigo (1982). Samples with a thickness of 2–3 mm were fixed with