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Effects of fish sarcoplasmic proteins on the properties of myofibrillar protein gels mediated by microbial transglutaminase

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ABSTRACT

Fish sarcoplasmic protein (SP) was extracted and lyophilized to obtain the SP powder. Fish myofibrillar protein (MP) was mixed with SP powder (0, 0.1, 0.5, and 1.0 g/100 g) in 1.8 and 2.6 g/100 g NaCl in the presence of 0.5 g/100 g microbial transglutaminase (MTG) at 4 °C for 6 h. Shear stress of MP mixture decreased with increasing SP concentrations. High thermal stability of MP mixture, assessed by differential scanning calorimetry, at either 1.8 or 2.6 g/100 g NaCl, was observed when SP (1 g/100 g) was added. The myosin heavy chain partially disappeared, suggesting the formation of cross-linked proteins. The gel strength of MP was not affected by the addition of 0.1 g/100 g SP ($P > 0.05$) whereas it started to decrease when SP was added up to 0.5 g/100 g, regardless of the NaCl concentration. The cooking loss of the MP gel was reduced efficiently when SP was added, even at a low concentration (0.1 g/100 g). A further reduction of cooking loss was observed when SP concentration was increased. The smooth microstructure of the gel surface was observed in samples containing SP, showing the lower cooking loss of fish myofibrillar protein gel.

1. Introduction

Fish sarcoplasmic proteins (SP) are a large family of proteins that include myogens and enzymes, and are soluble in water or low ionic strength solution. These components are normally washed out from surimi processing since they can lead to deterioration during surimi storage. The washing step is necessary for improving the quality of surimi gel, especially gel strength and whiteness. In the washing process, a high amount of water is used and vast quantities of washed water are generated as waste, which contains soluble SP. Lin, Park, and Morrissey (1995) reported that 1.7 kg of protein could be recovered for every 100 kg of surimi produced. The utilization of SP from washed water generated by surimi plants has been investigated to utilize the by-product as food ingredients. In addition, it would be an alternative way to reduce waste.

SP have been reported to inhibit the gelation process of the myofibrillar protein (MP) counterpart (Hashimoto, Katoh, Nozaki, & Arai, 1985). On the other hand, the gel strength of mackerel proteins increases with the addition of SP (Morioka & Shimizu, 1990). SP from threadfin bream improved textural properties of lizard fish surimi (Piyadhammaviboon & Yongsawatdigiul, 2009). The gel weakening effect, modori, of myofibrillar protein from milkfish was restricted by the addition of its SP (Ko & Hwang, 1995). The gel strength of threadfin bream surimi improved by the addition of SP extracted from carp (Jafarpour & Gorczyca, 2009). SP from rockfish positively contributed to the gelation of MP (Kim, Yongsawatdigul, Park, & Thawornchinsombut, 2005). There was no absolute agreement on the role of SP for the gelation of MP. However, the effects of SP on protein gelation mediated by microbial transglutaminase (MTG), the cross-linking enzyme, have not yet been investigated.

MTG has been isolated from *Streptoverticillium* spp. and is a Ca^{2+}-independent enzyme (Ando et al., 1989). It catalyzes the acyl transfer reaction between the glutamine-bound peptides/proteins to the primary amine or lysine-bound peptides, resulting in the formation of the ε-(γ-glutamyl)-lysyl isopeptide bond. MTG has been widely used to improve textural properties of food protein gel including surimi gel (Cardoso, Mendes, Vaz-Pires, & Nunes, 2010). However, high cooking loss of meat protein gels was observed when MTG was applied (Hong & Chin, 2010). Non-meat proteins from legume and milk (soy proteins isolate, sodium caseinate, and whey protein concentrate) have been used successfully to overcome this problem (Uresti, Tellez-Luis, Ramirez, & Vazquez, 2004). For the water holding ability of SP powder from threadfin bream, it has been reported a value of 0.43 g/g sample (Yongsawatdigul & Hemung, 2010). However, application of SP from fish as water

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holding agent has not been performed. Therefore, the objective of this study was to investigate the effects of fish SP on the properties of MP paste/gel mediated by MTG.

2. Materials and methods

2.1. Materials

The farmed fish (red sea bream, *Pagrus major*) of 0.030–0.035 m length from Gunnae-ri, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea, were killed and transported to the Meat Science Laboratory, Chonnam National University, Korea, in foam box covered with ice within 20 min. Fish fillets were prepared after manual eviscerating and filleting. All samples were packed under vacuum and stored at −70 °C until required. MTG (TG-S) containing 1 g/100 g of crude MTG was obtained from Ajinomoto (Seoul, South Korea) and activity was about 100 units of MTG/g. All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Sarcoplasmic protein (SP) preparation

Frozen fish fillets were allowed to thaw in the cold room overnight before homogenizing with 3 volumes of de-ionized water (DI-water) in a blender for 2 min. The homogenate was centrifuged at 1000 × *g* for 15 min before supernatant collection. This process was repeated once more. The collected supernatant (SP solution) was used to determine for its patterns using sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) and the left solution was kept overnight in the freezer (−70 °C) before lyophilizing using a freeze-dryer (IlShin Lab. Gyeonggi-do, South Korea). The obtained powder was used as SP powder sample. The protein content in the SP powder was estimated by Kjeldahl method according to AOAC (2000) procedure. The protein content was about 74.4 ± 0.22 g/100 g sample. The same batch of SP preparation was used throughout the experiment.

2.2.2. Myofibrillar protein (MP) extraction

Fish fillets were thawed overnight at 4 °C before performing MP extraction according to the method described by Xiong (1993), with slight modifications. Briefly, fish samples were blended with 4 volumes of washing buffer (100 mmol L^{-1}, NaCl, 50 mmol L^{-1} phosphate buffer, pH 6.25) for 2 min. The homogenate was centrifuged for 15 min at 1000 × *g* and only pellets were collected. The process was repeated twice. The obtained pellet was mixed with 8 volumes of 100 mmol L^{-1} NaCl (adjusted pH to 6.25) before filtering through 2 layers of gauze to remove connective tissue. The filtrate was centrifuged at 1000 × *g* for 15 min and the pellet was used as MP. The protein concentration in pellet was determined by Biuret method using bovine serum albumin as a standard. The protein pattern of isolated MP was determined using SDS-PAGE technique. In addition, the moisture content in the MP was also determined by AOAC method in order to calculate the total solid content.

2.2.3. MP gel preparation

The MP gel was prepared at 4 g/100 g (based on total solid and protein content using Biuret method) and the reaction mixture was controlled at 1.8 and 2.6 g/100 g NaCl, pH 6.25. MTG was added at 0.5 g/100 g, while the concentration of SP was varied (0, 0.1, 0.5, and 1.0 g/100 g). The MP mixture was poured in to a glass vial with a diameter of 12 mm and incubated for 6 h at 4 °C. All samples were heated in the water bath heating (WB-22, Daihan Scientific Co., Seoul, Korea) at the rate of about 3 °C/min from 5 to 80 °C (≈40 min). The cooked samples were cooled rapidly on ice and stored overnight at 4 °C (cold room).

2.2.4. Rheological analysis

The rheological properties of fish MP mixtures incubated at 4 °C for 6 h were tested by a rotational rheometer (RC30, Rheotec Messtechnik GmbH, Berlin, Germany). The bob (120°) and cup with radii of 7 and 7.59 mm, respectively, were used as a probe. Shear stress values were recorded, while the chilled sample was sheared up to 1000 s^{-1}.

2.2.5. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (DSC S-650, Scinco Co. Ltd., Seoul, Korea) calibrated with indium standard was used to test for thermal properties of MP paste. The sample (≈15 μg) was encapsulated in the aluminum pan before heating at the rate of 10 °C/min from 25 to 100 °C. A reference pan was prepared by encapsulating the empty pan. The endothermic peaks of MTG mediated MP mixture incubated with and without SP (1 g/100 g) was recorded.

2.2.6. SDS-PAGE

MP isolated from red sea bream was dissolved in NaCl solution (3.5 g/100 g) at the ratio of MP:NaCl solution of (1:9) in order to obtain the soluble MP. The soluble MP was determined for protein concentration by Biuret method before mixing with treatment buffer, containing SDS and β-mercaptoethanol (BME) prior determination of protein pattern using SDS-PAGE according to Laemmli (1970). The protein sample (20 μg) was loaded onto the gel made from 4 g/100 g acrylamide as stacking gel and 10 g/100 g acrylamide as running gel. The samples were separated using a mini Protean II unit (Bio-Rad Laboratories Inc., Richmond, Calif., USA). The protein patterns of SP were also analyzed as above except the running gel was 12.5 g/100 g acrylamide.

MP mixture (incubated at 4 °C for 6 h) was mixed with 10 times of SDS (5 g/100 g) solution before boiling for 10 min. The solution was centrifuged at 2000 × *g* for 15 min before centrifuging and collected the supernatant as the soluble protein extract. Protein concentration in the supernatant was estimated by the Biuret method. The soluble protein was mixed with treatment buffer containing BME in order to break down the disulfide linkages. The pattern of soluble protein was analyzed by SDS-PAGE.

2.2.7. MP gel characterization

2.2.7.1. Cooking loss. The MP gels were brought from the cold room to incubate at room temperature for approximately 4 h. The exudates from the gel were recorded. The cooking loss of the MP gel was expressed as a percentage of the original weight, which was considered 100%.

2.2.7.2. Color measurement. The color of MTG mediated fish MP gel prepared with different SP and NaCl contents were measured using colorimeter (CR-10, Minolta Co. Ltd., Japan). The color value were reported as hunter *L*, *a*, and *b* values.

2.2.7.3. Breaking force. A puncture test was applied to determine the breaking force, representing the gel strength, using an Instron Universal testing machine (Instron Corporation, Canton, MA, USA). The puncture probe with a diameter of 9 mm was used. The fish MP gels were compressed with the head speed of 50 mm/min and the penetration length was controlled at 12 mm. The first peak was recorded as the breaking force, which can be represented the gel strength.

2.2.7.4. Microstructure. The cubic shape fish MP gels were prepared before fixing with 2.5 g glutaraldehyde/100 g of 100 mmol L^{-1} sodium phosphate buffer (pH 7) overnight at 4 °C. Thereafter, the samples were washed with washing buffer (100 mmol L^{-1} sodium phosphate buffer pH 7) for 10 min and post