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PREPARATION AND CHARACTERISTICS OF PACIFIC CODFISH (*Gadus macrocephalus*) MYOFIBRIL FOR SURIMI

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ABSTRACT

Myofibril is contributing to gel-forming. Every species of fish have different myofibril concentration. Pacific codfish has white flesh which is expected to make surimi. The objective of this research was to analyze the characteristics of surimi prepared from Pacific codfish myofibril (SPM). The method of this research was used ionic strength by using NaCl. The observe parameters of this research were protein solubility, color, microstructure, molecular weight,and texture.the results showed that SPM have 3-dimensional network with rigid and porous structure than other surimi gels. The major molecular weights were 150 kDa (zetalin) and 40 kDa (tropomyosin). The hardness, cohesiveness and adhesiveness of SPM were 0.071338 N/cm^2, 0.259 gf/sec and 116 gf.mm respectively. These results were shown that Pacific codfish was suitable to be used as surimi raw material because it can make a good gel to form surimi.

Keywords: Codfish myofibrillar protein, molecular weigh, mirostructure, texture

INTRODUCTION

Fishes are one of the food resources with high protein content. Fresh fish compositions per 100 gram are moisture (76%), protein (17%), fat (4.5%), mineral and vitamin (2.52–4,5%). Fish is excellent food material because of the high nutritive value of its muscle protein. Fish proteins are classified by sarcoplasmic (20–30%), myofibrillar (65–75%), and stroma (1–3%) (Suzuki, 1981).

Myofibril is a basic rod-like unit of a muscle cell. Myofibrils are composed of long proteins including actin, myosin, and titin, and other proteins that hold them together. Myofibril protein dissolves in the salt solution with medium ionic strength. Myofibril is contributing to gel forming. Every species of fish have different myofibril concentration.

The previous research, surimi made from Alaska pollock. This study compared the semi-gel system at a low temperature with a heat-induced gel system. The result showed that the formation process of heat-induced gel is intertwined while it is temporally separated in cold storage. Overall, myosin was selected as the starting point for establishing a schema chart to characterize the gelation processes of the cold semi-gel and heat-induced gel (Liu, et.al., 2019). Black mouth croaker of surimi have the proximate composition contains protein (14.77±0.506%), lipid (0.94±0.081%), ash (0.58±0.007%) contents and yield rate (36.56±0.732%). It was known that the surimi from Black mouth croaker was an appropriate raw material for surimi production althought it was a pellagic fish (Shekarabi, et.al., 2014). On the other hand, surimi from silver carps showed that the variations in chemical interactions were strongly correlated to gel properties across different washing processes, especially for hydrogen bonds. In terms of the enhancement of gel properties, washing with 0.2% CaCl2 could bring high value when applied to the aquatic industry. (Zhang, et. al., 2018).

Pacific codfish (Gadus macrocephalus) is demersal fish found in huge schools confined to temperate waters in the northern hemisphere. The Pacific codfish is found in both eastern and western regions of

the Pacific. This fish can grow up to 49cm and weigh up to 15kg. Production of Pacific codfish in Japan was 6,610,000 tonnes (FAO, 2014).

Pacific codfish has been an important economic commodity in international markets and popular as a food fish with a mild flavor, low-fat content and a dense white flesh. The majority, Pacific codfish was sold in fillet flesh form. Whereas, Pacific codfish has white flesh and potential use of gelling product raw material.

White flesh is better using for product that have gelling ability product such as surimi. But every white flesh has different characteristics. So it's important to know the characteristic of surimi from Pacific codfish. The objective of the research was to know the characteristics of myofibrillar Pacific codfish myofibril (SPM) for surimi.

MATERIALS AND METHODS

Raw material

Fresh Pacific codfish (*Gadus macrocephalus*) with an average weight of 400–500 g were purchased from the local supermarket (Hiroshima, Japan). Upon the arrival, fish were immediately cut in small size and minced to uniformity by using a grinder.

Preparation of fish myofibrillar protein

Fish myofibrillar protein from Pacific cod mince was prepared according to the method of Subagio, Windrati, Fauzi, and Witono (2004) with some modification. Fish mince was added with 3 volumes of 0.5 % NaCl in 0.1 M phosphate buffer pH 7 and stirred at 4 oC for 3 min, followed by centrifugation at a speed of 3,000 g 4 oC for 10 min. The residue was added with 3 volumes of 0.5 % NaCl in 0.1 M phosphate buffer pH 7 and stirred at 4 oC for 3 min, followed by centrifugation at a speed of 3,000 rpm 4 oC for 10 min.

The residue was filtered by using filter cloth 4 layers. The filtrate was added with 3 volumes of 0.5 % NaCl in 0.1 M phosphate buffer pH 7 and followed by centrifugation at a speed of 3,000 g 4 oC for 10 min. The residue is myofibrillar protein. Myofibrillar protein was added with 5 % sucrose (w/w) and dried by using freeze-drying and kept in an airtight chamber.

Preparation of SPM

Myofibril powder was added in 4% NaCl solution with ratio 1:3 (myofibril powder: NaCl solution) and followed by blend it's all by using pounder. After all material was blended, the mixture was heated at 90 ^{o}C for 30 min. SPM was kept at room temperature for 1hr to decrease the temperature. After that kept SPM in 4 ^{o}C before until analyzed time.

Protein solubility

The soluble content of myofibrillar gel protein was determined using the Folin-Lowry method (Najafian and Babji, 2015) with some modification. Preparation of the sample was started by made sample concentration 2.5 mg/mL and stirred by using magnetic stirrer for 1 hr and 24 hr at 4 oC and 25 oC, followed by centrifugation at a speed of 3000 g for 5 min.

The supernatant of 0.5 mL of the sample was mixed with 2.5 mL of an alkaline-copper reagent and incubated for 10min at room temperature. The mixture was added to 0.25 mL of Folin-Ciocalteu's phenol reagent at 2 times dilution with deionized water and left for 30 min at room temperature. The absorbance at 750 nm was measured with a spectrophotometer (Model U-2001, Hitachi, Japan). The soluble protein content was quantified using bovine serum albumin as the standard with absorbance value were 0.128, 0.159, 0.292, 0.387, 0.500, 0.604. Equation of BSA standard curve was Y = 0.0022x + 0.0655.

Color

The color of myofibrillar gel protein was determined using a colorimeter (Konica Minolta CM-700d/600d). L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) were measured, and whiteness was calculated as described by Lertwittayanon, et al. (2013) as follows:

Whiteness = 100 – [(100 – L*)2 + a*2 + b*2]1/2

Microstructure of protein

The microstructure of myofibrillar gel protein was determined using a scanning electron microscope (Arfat and Benjakul, 2012) with some modification. Samples with a thickness of 0.5x0.5 mm were fixed with 2.5 % (v/v) glutaraldehyde in 0.2 M phosphate buffer

(pH 7.2) for 2 hr. The samples were rinsed in distilled water before being dehydrated in graded ethanol with serial concentrations of 50%, 70%, 80%, 90%, 95%, and 100% (v/v). Dehydration was conducted for 5 min in each solution. Dried samples were mounted on a bronze stub and sputter-coated with gold. The specimens were visualized with a Scanning Electron Microscopy (Hitachi TM 3000).

Molecular weight (SDS-PAGE)

Myofibrillar gel protein was examined for protein patterns based on their molecular weight according to the method of Fowler and Park (2015) with some modification. To prepare the protein sample, 3.75 mL of 20 mM Tris-HCl buffer pH 8 containing 8 M urea, 2 % SDS, and 2 % 2- mercaptoethanol was added to the sample (0.2 g). The mixture was boiled at 99 oC for 2 min and shook more than 20 hr at 30 oC for dissolve the sample. The mixture was taken 200 µL and added 50 µL of 50 mM Tris-HCl buffer pH 8 containing 5 % SDS, 5 % 2-mercaptoethanol, and 50 % glycerol. The sample was boiled at 99 oC for 1 min.

Sample (2.5 µL, 5 µL, 7.5 µL, 10 µL) were loaded onto the polyacrylamide gels comprising a 12.5 % running gel and subjected to electrophoresis at a constant current of 20 mA by using an electrophoresis unit (AE 6530 serial number 5009405) for 75 min. Gels were fixed and stained in 0.125 g/100 mL Coomassie brilliant blue R-250, Followed by rinsed with distilled water 3 times. Molecular weights of bands were determined by comparison to a molecular weight standard (XL-Lader Broad Range SP-2110).

Texture

The texture of myofibrillar gel protein was determined according to Lertwittayanon, et. al. (2013) with some modification. Gels were equilibrated and evaluated at room temperature. One cylinder-shaped sample with a length of 3.0 cm was prepared and subjected to determination. Hardness, cohesiveness, and adhesiveness were measured using the Rheometer (Fudoh Rheometer) equipped with a spherical plunger with diameter 7.0 mm. The result was will be plotting in the equation.

Hardness (N/cm2) = force (g) X 0.0098 X cross-section area of probe

Cohesiveness (gf/sec) = A2 (peak area of second time) / A1 (peak area of first time)

Adhesiveness (gf.mm) = peak area drawn at Y-axis negative direction; A3

RESULT AND DISCUSSION

Protein solubility

Solubility is an important property in the utilization of proteins. Protein solubility of SPM in water was investigated. In all the conditions tested, protein in water phase was not detected. It was suggested that SPM was stabled in water even at room temperature for 24 hr. Another hand, the preparation of fish myofibrillar protein by using 0.5 % NaCl in 0.1 M phosphate buffer pH 7 made the SPM stabled.

According to Kim, Y. S. (2002), protein solubility in aqueous solution was dependent on pH. The isoelectric point was the pH at which a protein has zero net charge in solution. For most of proteins, minimum solubility occurs at the isoelectric pH. Solubility of Pacific whiting (Merluccius productus) protein was the lowest at pH 5.5. The pH more than 5.5 made the solubility was stabled. Gao, et. al. (2018) reported that myofibrillar protein of surimi gel was stabled in 0 day. But it would be decreased after 3 days.

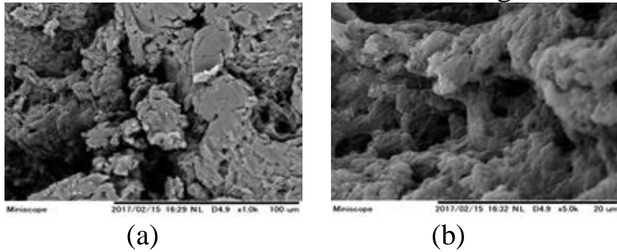
Color

Lightness (L*) value of SPM was 71.097, a* value was -2.947(small redness), and b* value was 4.733 (small yellowness). Whiteness value of SPM was 70.563. Pacific codfish has white flesh so whiteness value is higher. Whiteness value of SPM was lower than yellowtail barracuda surimi (78.24) (Lertwittayanon, et. al., 2013) although both of them have the same type of flesh. It caused by different comparison of white and red flesh every fish.

Another hand, whiteness affected by the type of cryoprotectant. Cryoprotectant from sugar reduction cause browning reaction. The combination of sucrose, sorbitol, and STPP can reduced the browning reaction (Susilo, 2010). SPM only use sucrose as cryoprotectant so whiteness value was not high.

Microstructure of protein

The 3-dimensional network structure of gel is an important determinant of texture and functional properties, such as water and fat holding capacity (Chen, et. al., 2007). The microstructure of SPM was shown in Figure 1.



(a) (b)

Figure 1. Scanning electron microscope image of SPM. (a) = magnification 1000X; (b) = magnification 5000x

Figure 1 (a) was shown that 3-dimensional network structure formed closely. Fig. 1 (b) was shown that 3-dimensional network structure of SPM more rigidly. Every protein attached mighty. This result was similar with the research of Gao, *et. al*. (2018). They reported that the microstructure of surimi was made 3-dimensional network structure rigidly. It is caused by ionic strength. Higher ionic strength will make aggregation gel structure rigidly. Hermansson, *et. al*. (1986) indicated that ionic strength also affects the microstructure of myofibrillar gels. They found that, at low ionic strength (0.25 M KCl), fine-stranded gel structures were formed, whereas, at high ionic strength (0.6 M KCl), coarsely aggregated gel structures were formed in the pH range 5.5 to 6. A higher rigidity was reached by the fine-stranded structure than that of the coarsely aggregated structure.

Molecular weight

The molecular weight of Pacific cod fish was different with the other fish. Protein pattern of SPM in different concentration was shown in Figure 2. According to the Figure 2, myofibrillar gel proteins of Pacific codfish have 2 main bands with molecular weight 150 kDa and 40 kDa respectively. Other bands were 30–40 kDa and 15–20 kDa.

According to Vigoreaux (2005), zetalin have molecular weight 107–210 kDa and tropomyosin has molecular weight 40 kDa. Molecular weight of protein myosin regulatory light chain, Glutathione-S transferase 2 and Troponin I were 24–30 kDa, 32–35 kDa, and 25–29–35 kDa respectively. Molecular weight ADP/ATP transcolase and flightin were 33 kDa and 20 kDa. Troponin C and myosin essential light chain have the same molecular weight 18 kDa.

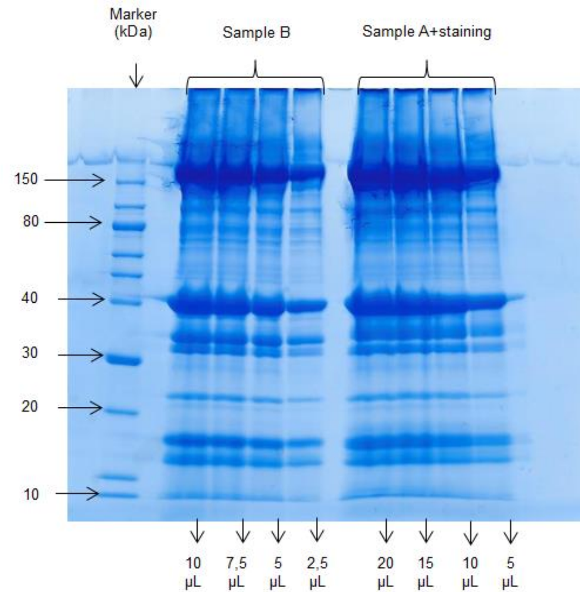


Figure 2. Protein pattern of SPM in different concentration. Sample B: sample after add 50 µL of 50 mM Tris-HCl buffer pH 8 (containing 5% SDS, 5% 2-mercaptoethanol, and 50% glycerol) without staining; Sample A+staining: sample after add 3.75 ml of 20 mM Tris-HCl buffer pH 8 (containing 8 M Urea, 2% 2-mercaptoethanol, 2% SDS), boiled and shake + staining

Texture

Rheological properties of myofibrillar gel protein were calculated as hardness, cohesiveness, and adhesiveness. The hardness, cohesiveness and adhesiveness of myofibrillar gel protein of Pacific codfish were 0.071338 N/cm2, 0.259 and 116 respectively. The hardness affected by ionic strength. Higher ionic strength makes myofibrillar gel more rigidly.

Myosin in low ionic strength conditions (0.2 mol/L KCl) existed in the form of filaments, while in high ionic strength conditions (0.6 mol/L KCl), myosin usually existed in a monomeric or dimeric form (Boyer, et. al., 1996). In high ionic strength solutions, protein swelled, unfolded and became flexible upon absorbing solvent. The swelling and unfolding of actomyosin, in turn, increased its effective volume and shortened the distance between the protein molecules (Liu, et. al., 2008), which was beneficial for the crosslinking of protein during heat-induced gelation.

Samejima, et al. (1981) reported that the rigidity of rabbit myosin gel increased with the increase of ionic strength from 0.2 to 0.6. Laure, et. al. (2014) also found the increase of brine salt content had a positive impact on breaking stress of heat-induced pork gels.

CONCLUSION

Acccording to the research, SPM have 3-dimensional network with rigid and porous structure. The major molecular weights were 150 kDa (zetalin) and 40 kDa (tropomyosin). The hardness, cohesiveness and adhesiveness of SPM were 0.071338 N/cm^2, 0.259 gf/sec and 116 gf.mm respectively. These results were shown that Pacific codfish was suitable to be used as surimi raw material because it can make a good gel to form surimi.

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REFERENCES

Arfat, Y.A. and Benjakul, S. 2012. Gelling characteristics of surimi from yellow stripe trevally (*Selaroides Leptolepis*). International Aquatic Research 4(5): 1-13.

Boyer, C., Joandel, S., Ouali, A. and Culioli, J. 1996. Ionic strength effects on heat-induced gelation of myofibrils and myosin from fast and slow-twitch rabbit muscles. Journal Food Science. 61: 1143-1148

Chen, H.H., Xu, S.Y. and Wang, Z. 2007. Interaction between flaxseed gum and meat protein. Journal Food Engineering. 80: 1051-1059.

Food and Agriculture Organization of the United Nations. Fisheries and Aquaculture Department. 2014. Fishery and Aquaculture Statistics Summary Tables. Retrieved on March 25, 2017 from the website: http://www.fao.org/fishery/statistics/en.

Fowler, M.R. and Park, J.W. 2015. Effect of salmon plasma protein on Pacific whiting surimi gelation under various ohmic heating conditions. Food Science and Technology 61: 309-315.

Gao, Y., Fukushima, H., Deng, S., Jia, R., Osako, K., and Okazaki, E. 2018. Effect of emulsifying stability of myofibrillar protein on the gel properties of emulsified surimi gel. Food Sci Nutr. Vol. 6: 129–1237.

Hermansson, A.M., Harbitz, O. and Langton, M. 1986. Formation of two types of gels from bovine myosin. Journal Science Food Agriculture 37: 69-84.

Kim, Y. S. 2002. Physicochemical Characteristics of Fish Myofibrillar and Sarcoplasmic Proteins Treated at Various pH Conditions. Thesis. Oregon State University.

Laure, B., Philippe, G., Martine, C. and Alain, K. 2014. The effects of low salt concentrations on the mechanism of adhesion between two pieces of pork semimembranosus muscle following tumbling and cooking. Meat Science. 96: 5-13.

Lertwittayanon, K., Benjakul, S., Maqsood, S., and Encarnacion, A.B. 2013. Effect of different salts on dewatering and properties of yellowtail barracuda surimi. International Aquatic Research 5(10): 1-12.

Liu, R., Zhao, S.M., Xiong, S.B., Qiu, C.G. and Xie, B.J. 2008. Rheological properties of fish actomyosin and pork actomyosin solutions. Journal Food Engineering. 85: 173-179.

Liu, X., Zhang, T., Xue, Y., and Xue, C. 2019. Changes of Structural and Physical Properties of Semi-Gel From Alaska Pollock Surimi During 4 °C Storage. Food Hydrocolloids. Vol. 87: 772–782.

Najafian, L. and Babji, A. S. 2015. Isolation, purification, and identification of three novel antioxidative peptides from patin (Pangasius sutchi) myofibrillar protein hydrolysates. Food Science and Technology 60: 452-461.

Samejima, K., Ishioroshi, M. and Yasui, T. 1981. Relative molecule roles of the head and tail portions of the molecule in heat-induced gelation of myosin. Journal Food Science. 46: 1412-1418.

Shekarabi, S. P. H., Hosseini, S. E., Soltani, M., Kamali, A., and Valinassab, T. 2014. A Comparative Study on

Physicochemical and Sensory Characteristics of Minced Fish and Surimi from Black Mouth Croaker (Atrobucca nibe). J. Agr. Sci. Tech. Vol. 16: 1289-1300

Subagio, A., Windrati, W.S., Fauzi, M. and Witono, Y. 2004. Characterization of myofibrillar protein from gold band goatfish (Upeneus moluccensis) and bigeye scad fish (Selar crumenophthalmus). Jurnal Teknol dan Industri Pangan XV(1): 70-78.

Susilo, I. 2010. Influence of Cryoprotectant to Surimi Quality in Frozen Storage. Surabaya: Faculty of Agriculture Technology. University of Catholic Widya Mandala.

Suzuki, T. 1981. Fish and krill protein: Processing technology. London: Applied Science Publishers Ltd.

Vigoreaux, J. O. 2005. Molecular basic of muscle structure. Vermont: Department of Biology. University of Vermont. USA.

Zhang, L., Li, Q., Shi, J., Zhu, B., Luo, Y. 2018. Changes in chemical interactions and gel properties of heat-induced surimi gels from silver carp (*Hypophthalmichthys molitrix*) fillets during setting and heating: Effects of different washing solutions. Food Hydrocolloids. Vol. 75: 116–124.