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The effect of fish bone collagens in improving food quality

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Abstract

The aim of this study was to evaluate the effect of addition of 6% collagen extracted from various sources of fish bones on the quality of myofibril proteins of fish. For this purpose, fish bones were collected from 9 different fish species : seawater catfish (*Arius thalasinus*), threadfin bream (*Nemipterus nematophorus*), stingrayfish (*Dasyatis sephen*), big eye snapper (*Lutjanus lutjanus*), catfish (*Clarias batrachus*), shark (*Charcarius* sp), eastern little tuna (*Euthynnus affinis*), lizardfish (*Saurida tumbil*) and purple-spotted bigeye (*Priacanthus tayenus*). Quality of myofibril was evaluated for its water sorption isotherm, Ca-ATPase activity, water holding capacity, gel strength, folding test and viscosity of protein, as well as proximate composition, phosphorus and calcium contents as supporting quality parameters. The result showed that the effect of collagen addition on the phosphorus and calcium contents in myofibril proteins varies according to fish sources species. The addition of collagen can also retard the decrease of Ca-ATPase activity, viscosity, gel strength, folding test and water holding capacity in myofibril proteins. High value of gel strength, water holding capacity and folding test shows a high quality of myofibril protein-based product, especially for collagen added from snapper collagen with the value of 1436.2 gr/cm, 41.2 ± 0.04, A, respectively and threadfin bream collagen with the value of 1596.10 gr/cm, 52.27 ± 0.02, A, respectively. The addition of fish bone collagens did not show significant difference in water sorption isotherm profile of myofibril protein, but all collagens has an effect on retarding the denaturation rate of myofibril protein. This research delivered a conclusion that collagen extracted from various source of fish bones have influences on food quality especially in altering the rate of protein denaturation.

Introduction

Indonesian production of catfish, snapper, and eastern little tuna are about 114,371 tonnes; 4,371 tonnes and 421,905 tonnes respectively. While the production of lizard fish (*Saurida tumbil*), purple-spotted bigeye (*Priacanthus tayenus*), stingray fish (*Dasyatis sephen*), seawater catfish (*Arius thalasinus*) and other marine fishes are in total amount of 243,376 tonnes (Marine and Fsiheries Affair, 2009). Besides for being exported, the fish production has been shared for domestic market which is mainly in the form of fillets. The increase of fish fillet production leads to an increase of waste such as: bones, skin and viscera of the fish and so far these wastes have not been efficiently utilized and only converted into fishmeal products. The utilization of fish skin wastes have been investigated by other researchers including utilization of giant catfish skin (Wang *et al.*, 2008; Rawdkuen *et al*., 2010), shark skin (Kittiphattanabawon *et al*., 2010), yellowfin tuna dorsal skin (Yoon *et al*., 2008) as gelatin products. However, the study of using fish bones for added value product is still limited. The study conducted by Darmanto *et al.* (1997) and Kim and Park (2004) showed potential use of fish waste as collagen products.

Collagen is the main structural component of connective tissue proteins making up to 30% of the total protein in the body tissue of vertebrates and invertebrates containing the following amino-acids such as glutamine and aspargine (Balian and Bowes, 1977), alanine, arginine, lysine, glycine, and proline (Poppe, 1997). Collagen molecules composed of three α – chains intertwined in the so-called collagen triple-helix (Te Nijenhuis, 1977). The triple-helix is approximately 300 nm in length, and the chain has molecular weight of approximately 105 kDa (Papon *et al.*, 2007). Collagen denaturation cause separation of the rods and total or even partial separation of the chain due to destruction of hydrogen bonds, causing the loss of triple-helix conformation, and following the denaturation, polimer will exist in a coiled form (Karim and Bhat, 2009).

Collagens can be added to food products to

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improve their nutritional and functional quality. In mammals, collagen is found in skin, cartilage, and connective tissue (Johns, 1977; Karim and Bhat, 2009). In addition, Schrieber and Gareis (2007), stated that collagen can be classified into 4 types, in which one of them primarily found in connective tissue such as skin, bone and tendons. In addition Kasankala *et al.* (2007) said that collagen can be found in the skin and bone of animals and fish, producing high quality of gelatin when it undergoes thermal denaturing. As the composition of collagen is unique, and collagen can be considered nutritionally useful as an aid to building tissue in human body.

In the last decade, there has been an increase of protein-based fish products such as: fish sausage, fish nuggets, fish cake, kamaboko, chikuwa, fish balls, etc. The quality of these products depend on raw materials used in their manufacture. Thus, various proteinl-based fish products will depend on the amount of myofibril proteins that are present in the fish species. Suzuki (1981) reported that about 65-75% protein in fish consists of myofibril protein, and therefore study on myofibril protein is very important in terms of protein-based fish product in order to get the optimal condition relates to the quality of product and consumer acceptance.

Myofibril protein is partly in the form of colloid, gel or sol and mostly it is unstable and easily to have denaturation (Suzuki, 1981). Proteins denaturation can occur during dehydration and freezing process (Yamashita *et al*., 2003; Asako *et al*., 2005; Wang *et al*., 2009). Darmanto (1999) stated that denaturation of myofibril protein results in decrease on water holding capacity, lower gel strength, and reduce fat bond. There are many methods to determine protein denaturation during dehydration, such as solubility of myofibril protein, viscosity, Ca-ATPase activity and electron microscopy (Cleland *et al*., 1986, Yamashita *et al*., 2003). Among these methods, Ca-ATPase activity shows the most preferable method as its simple and can perform reliable condition of early process on protein denaturation. Nozaki *et al.* (1993) used the Ca-ATPase activity as an indicator of quality of myofibril protein during frozen storage and dehydration.

In order to examine the effectiveness of collagen in keeping chemical and physicochemical properties of myofibril protein of fish the application of collagen in fish products is necessarily to be conducted. The collagen made from various source of fish bones were used as the basic ingredient of fish products made from white fish myofibril protein. By adding collagen in protein-based fish products, it is expected that collagen would have an important role in maintaining shelf life and quality of the product and improve its nutrient. Therefore, it is important to observe the effect of various sources of fish bone collagens to retard the denaturation rate of myofibril protein. In previous study, Darmanto (1999) revealed that the addition of Chitin and Chitosan in myofibril protein caused changes of myofibril protein which led to prolong the shelf life of the product.

The aim of this study was to observe the effects of collagen addition from different fish sources on quality of myofibril proteins during dehydration process and examine for water sorption isotherm, Ca-ATPase activity, viscosity, gel strength, folding test and water holding capacity (WHC), as well as the position changes of water on the myofibril proteins from the various treatments.

Materials and Methods

Source of fish bones

The samples of collagen were extracted from nine different fish bones sources: seawater catfish (*Arhius thalasinus*), threadfin bream (*Nemipterus nematophorus*), stingrayfish (*Dasyatis sephen*), big eye snapper (*Lutjanus lutjanus*), catfish (*Clarias batrachus*), shark (*Charcarius* sp), eastern little tuna (*Euthynnus affinis*), lizardfish (*Saurida tumbil*), and purple-spotted bigeye (*Priacanthus tayenus*). All fishes samples were obtained from Northern coastal area of Java sea. The fishes were then transported to laboratory for further analysis by using a chilling box to maintain the freshness

Preparation of collagen and myofibril protein

Fish bone collected from fish processing enterprises in the Northern coast area of Java island were then processed into collagen at the laboratory of Fish Processing, Diponegoro University. Fish bones, after being washed and dried, were then soaked with 4% (w/v) HCl 2N for 6 days (Ward and Courts, 1977). The collagen was then analyzed for proximate, phosphor and calcium based on AOAC methods (AOAC, 2000). Myofibril protein was prepared by isolating thread bream fish meat according to the method of Katoh *et al.* (1977) with slight modifications by Nozaki *et al*. (1991).

Application of collagen in Myofibrillar protein

The concentration of collagen used was 6% (w/w) based on preliminary research (using different concentration of collagen). The preliminary research showed that addition of 6% collagen gave the highest gel strength on myofibril protein, therefore it was used for the study.

Moisture content

The moisture content was determined as the loss of water in a sample after oven drying at 105°C for 16 to 18 hours based on SNI 01-2891-2001 (Indonesian National Standard, 2001).

Water activity

Water activity of the samples were measured by using aw meter (Rotronic Hygropalm AW DIO, Serie Number 265 22.013 CE). Preparation of sample with different moisture content was conducted by introducing the sample to desiccators containing silica gel. The water activity and moisture content were simultaneously in a certain interval time in order to perform sorption isotherm.

Ca-ATPase activity

Effect of collagen on denaturation of myofibril protein during dehydration process was studied by analyzing the change of Ca-ATPase activity. The activity was indicated by micro moles per minute of the inorganic phosphate in the presence of 1 mM ATP, 100 mM KCl and 5 mM $CaCl_2$ at pH 7,0 with 25 mM tris-Maleate buffer. The phosphate was determined by using Fishe-Subbarow's method (1925), while myofibril protein concentration was determined by the Biuret method with bovine serum albumin as standard.

Preparation of Myofibril protein gel for gel strength, WHC and folding test

Myofibril protein with size of 3 cm diameter and 1 cm thickness were heated at two different heating treatments (40°C for 30 minutes and 90°C for 15 minutes) then stored at 5°C.

Gel strength

A slice of myofibril protein with 3 cm diameter and 2.5 cm thickness was plunged by a texture analyzer type TA Plus LLOYD 2 with the ball probe.

Water holding capacity (WHC)

A slice of myofibril protein with 1 cm diameter and 1.5 cm thickness was placed between two filter paper, and pressed by a small oil compressor under a pressure 10 kg/cm^2 for 2 min. The WHC was determined as ratio of weight differences before and after weighing to the initial weight before pressing (Shimidzu *et al*., 1981) based on formula belows

$$\% \text{ WHC} = \frac{\text{volume (ml) of water absorbed}}{\text{sample weight (g)}}$$

Folding test

A slice of myofibril protein with 2 cm diameter and 0.5 cm thickness was folded once into a semicircle or twice into quadrant. The samples were assigned as follows: AA: No crack when it was folded into a quadrant, A: No crack when it was folded into a semicircle, B: Cracks when it was folded into a semicircle, C: Breaks into two pieces when it was folded into a semicircle.

Analysis of water sorption isotherm

Moisture contents of myofibrils during dehydration were plotted against water activity from sorption isotherm experiment. Each of the isotherm was divided into three sections as demarcated by M_1 and M_2 which signed as a flexing point on the isotherm. The water content up to M_1 is regarded as in a state of monolayer, then the one between M_1 and M_2 is multilayer, and above M_2 is capillary, respectively. The monolayer water (M_1) was determined using the Brunauer *et al.* (1968) formula as below.

$$\frac{1}{V} x \frac{Aw}{1-Aw} = \frac{1}{Vm \, x \, C} + \frac{C-1}{Vm \, x \, C}$$

A_w : Water activity
Vm : Monolayer water content (g/g of dried matter)
V : Volume of adsorbed water (mL)
C : Constanta (1)

$$M_1 (\%) = \frac{100 \, x \, Vm}{1+Vm}$$

M_1 : Moisture content (%) for upper limit of monolayer water.

The Multilayer water (M_2) was determined using Bull's method (1944).

Analysis of myofibrillar Ca-ATPase activity

Myofibrils, one of the main muscle components, have an enzymatic activity to split ATP. Therefore, by measuring this activity during dehydration process, it was expected to provide information on protein denaturation (Nozaki *et al*., 1989). The Ca-ATPase activity was analyzed by the method of Katoh *et al*., (1977) using the formula as follows:

$$\text{Ca-ATPase activity} = L_n \{(1\text{-Pi}) \times (6/5) \times (1/31) \times (1/5) \times 1 (5/A)\}$$

Pi : Acquired by the equation of the regression of phosphorous standard solution.
A : Acquired by the equation of the regression of bovine serum albumin as standard.

Statistical analysis

Three analytical determinations were carried out on each independent replication for every parameter. Three independent replicates (n = 3) were obtained from each treatment and the results presented in

tables and graphs are reported as means ± standard deviation (SD). Fish collagens effect were evaluated by means of One-way anova followed by Tukey's HSD post hoc comparison test at $P \leq 0.05$ (Steel and Torrie, 1991)

Result and Discussion

The results of chemical composition of collagen from various fish bones (g/100 g of dried matter) is shown in Table 1. The composition of protein, lipid, and ash from different types of collagen showed small differences. However, Schoeninger and Deniro (1983) reported that nitrogen content in bone collagen from animals that fed exclusively in the marine environment showed 9% more than those in the terrestrial environment. There were significant different ($P < 0.05$) on phosphorus and calcium contents for each collagen. Snapper had calcium and phosphorus contents of 16.77% and 2.63%, respectively followed by eastern little tuna : 14.22% and 1.30%; purple spotted big eye: 12.8% and 1.13%,and seawater catfish : 11.55% and 1.78%. For other fishes, for example lizard fish had a calcium and phosphorus level of 9.60% and 2.03%, followed seawater catfish, stingray, threadfin bream and shark that are equal to 8.97% and 1.88%; 3.06% and 1.36%; 3.03% and 0.66% and 2.25% and 0.63%, respectivelly. It was noticed that when the calcium and phosphor in high concentration, the quality of collagen is low. According to Ward and Courts (1977), the purpose of bone demineralization is to remove calcium and other salt, in order to release the collageneous producing substance so called "ossein". Therefore the high amount of calcium and phosphorus should be reduced to achieve good quality of collagen. Fat and ash contents were higher than those of collagen powder from skin accounted for 0.35% and 3.38% (Shon *et al.*, 2010).

The results of M_1, M_2, WHC and Folding Test of myofibril are given in Table 2. The Ca-ATPase activity for different fish bone collagens showed similar pattern againts water activity. Increasing water activity was followed by increasing Ca-ATPase activity (Figure 1). The relation between moisture content and Aw of each fish bone collagen is shown in Figure 2.

Myofibril protein quality decreases when it is dehydrated and frozen. Shon *et al.* (2010) explained that the degree of conversion of collagen into gelatin is related to the severity of both the pre-treatment and the extraction processes, wich depends on pH, temperature and extractions time. It may be observed that each species of fish has different Ca-ATPase

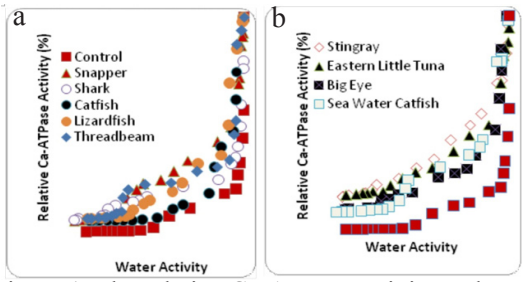

Figure 1. The relative Ca-ATPase activity and water activity of various fish bone collagen

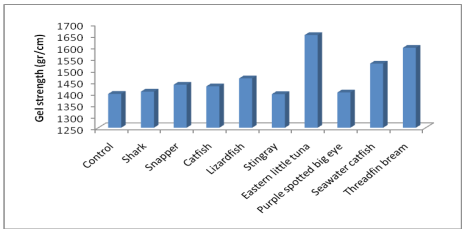

Figure 2. The gel strength value of myofibril protein with various fish bone collagen

activity, as indicated in Figure 1a and 1b. The effect of fish bone collagen on the denaturation of protein was evaluated by measuring Ca-ATPase during dehydration. Figure 1a shows the values of relative Ca-ATPase against water activity. During desorption, Ca-ATPase activity sharply decreased in water activity between 1.0 - 0.8, and almost 30% decreased was achieved. Between aw 0.4 - 0.8, Ca-ATPase was gradually decreased, and then below A_w 0.4 there is no significant decrease until it was stabilized at the end. From Figure 1 we can observe that the collagen added to the myofibrillar protein has resulted in higher relative Ca-ATPase activity than the one without adding the collagen (control). The collagen derived from different fish bone did not show any differences except to the control. These result indicated that collagen could inhibit the denaturation processes of myofibril proteins. The early rapid denaturation phase would probably caused by the myosin detachment from F-actin, and the following slow denaturation phase might be implied by the denaturation of actomyosin (Osako *et al*., 2005).

The desorption isotherm curve for myofibril protein-based product with various type of collagen added is shown in Figure 1b. All the curves are sigmoidal in shape with slight decreasing point on the aw between 1.0 - 0.8, and below 0.2 whereas between aw 0.2 and 0.8 the decreasing process of moisture content is not significant. Regardless of the types of collagen, the shape of the curve is almost similar. It is obviously showed that desorption isotherm curve of myofibril protein-based product without collagen (control) always below the one with collagen. In particular, between aw 0.2 to 0.8 moisture content of control is always lower than myofibril protein-based

Table 1. Chemical composition of the collagen from various fish bones (g/100 g of dried matter)

Source of Fish Bone Collagen	Crude Protein	Crude Lipid	Crude Ash	Water Content	Phosphor	Calcium
Cat Fish	25.59±1.00	0.54±0.14	55.21±0.99	6.05±0.57	1.78±0.04	11.55±0.17
Threadfin bream	27.48±0.82	0.61±0.49	57.72±2.14	6.28±0.64	0.66±0.13	3.03±0.54
Snapper	24.50±0.27	0.72±0.12	52.43±0.16	5.49±0.13	2.63±0.22	16.77±0.17
Shark	31.82±2.72	0.58±0.14	51.67±1.90	6.99±0.82	0.63±0.11	2.25±0.25
Stingray	28.52±1.10	0.67±0.09	52.14±2.20	5.28±0.21	1.36±0.15	3.06±0.12
Eastern little tuna	28.57±0.19	0.75±0.15	53.38±0.23	6.35±0.04	1.30±0.01	14.22±0.17
Seawater Catfish	26.64±0.31	0.47±0.01	50.98±0.25	6.67±0.61	1.88±0.03	8.97±0.11
Lizardfish	29.82±1.69	0.51±0.37	51.64±2.01	6.28±0.22	2.03±0.41	9.60±0.17
Purple spotted Big eye	30.27±0.17	0.56±0.15	53.08±0.22	5.15±0.34	1.13±0.21	12.80±0.19

Table 2. M_1, M_2, WHC and Folding Test of myofibril protein

Type of Collagen	M_1	M_2	Water Holding Capacity (%)	Folding Test
Shark	6.803	19.074	40.21± 0.03	B
Snapper	10.275	13.071	41.2±0.04	A
Catfish	5.085	13.12	56.01±0.01	B
Lizardfish	4.653	14.162	41.07±0.03	B
Stingray	5.123	17.024	47.77±0.02	B
Eastern Little Tuna	8.676	18.18	46.98±0.01	B
Purple spotted Big eye	10.85	11.272	36.56±0.02	B
Seawater Catfish	8.467	15.26	31.37±0.01	B
Threadfin bream	5.979	19.32	52.27±0.02	A
Control	3.382	9.808	27.57±0.01	B

product with collagen. Labuza (1968) reported that desorption isotherm tests are designed to determine the end point of a food dehydration process. It represents the relationship between A_w and moisture content of sample at constant temperature.

The aim of the collagen addition to the myofibril protein was to evaluate the characteristic of the state of water in myofibril protein-based product from water sorption isotherm, the amout of Monolayer Water (M_1) and Multilayer Water (M_2). As shown in Table 2, M_1 and M_2 from myofibril protein-based product with collagen is greater than that of myofibril protein-based product without addition of collagen. It means that the composition of collagen influences the water holding capacity (WHC) of myofibril protein-based product, and collagen gives an effect to the state of water contained therein. This founding is in accordance to Nozaki *et al.* (1991) who reported that various amino acids gave different effect on monolayer water and multilayer water during dehidration.

The gel strength or gel forming ability is one of the most important factors for quality evaluation. The result for gel strength of myofibril protein-based product added with different collagens are shown in Figure 2. It shows that myofibril protein-based product with added collagen gives a higher value of gel strength. All measurements of gel strength products with collagen are higher than without collagen. The highest value of gel strength was obtained from myofibril protein with added collagen from eastern little tuna with the value of 1651.26 gr/cm followed by threadfin bream (1596.10 gr/cm), seawater catfish (1527.67 gr/cm) and snapper (1436.20 gr/cm). The result of folding test showed that there was no significant different between myofibril protein-based product added by collagen and without added collagen. The highest value of folding test was performed by myofibril protein-based product added collagen from snapper and threadfin bream. Darmanto (1999) explained that protein denaturation of myofibril protein-based product will reduce water holding capacity, which resulted in reducing gel strength and more lost in lipid chain.

Water holding capacity (WHC) is an ability of myofibril protein to bind molecules of water. WHC values shows the quality of protein, it means that the higher the value of WHC, the better the quality of protein. Water holding capacity can also be defined as the ability of a protein gel to retain water against a gravitational force. The level of water retained in a gel is affected by the same factors that affect the formation of a good protein gel. Myofibril protein-based product added by collagen from catfish shows the highest WHC value.

Conclusions

Addition of collagens derived from different fish bones have shown an impact on food quality especially in altering the rate of protein denaturation which had been performed by retaining on Ca-ATPase activity, WHC, gel strength and folding test. The addition of collagen to the myofibril protein has resulted higher relative Ca-ATPase activity than without adding the collagen (control). The increase of Ca-ATPase activity was significant when aw is between 0.8 and 1. The effect of different collagen addition to the myofibril protein-based product showed that collagen containing high calcium and phosphorous contents will reduce quality. Collagen from snapper and threadfin beam provided the most quality of myofibril protein-based products.

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