

Characteristics of gelatins extracted from fresh and sun-dried seawater fish skins in Indonesia

¹Pranoto, Y., ¹Marseno, D.W. and ²Rahmawati, H.

¹Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Sosio Yustisia, Bulaksumur, Yogyakarta 55281, Indonesia

²Department of Aquatic Product Technology, Faculty of Fishery and Marine Science, Lambung Mangkurat University, Jl. Ahmad Yani km 36, Simpang Empat, Banjarbaru, South Borneo 70721, Indonesia

Abstract: Sun-drying was carried out to dry fish skins of four species of seawater fish, namely yellowfin tuna, brown stingray, red snapper and white cheek shark, and the gelatins extracted from the skins were characterized in comparison to those of extracted from fresh skins. The fish skins were pretreated using 0.05 M acetic acid followed by extraction at 80°C for 2 h. The gelatin obtained was analyzed for yield, proximate composition, functional properties, and amino acid composition. Results showed that drying on brown stingray and red snapper fish skins significantly decreased gelatin yield. Gelatins from dried fish skins had higher crude protein, lower ash content and lower crude lipid. Drying led to decreased viscosity, increased gel strength, and no significant effect on melting point. Gelatin from dried skins showed a higher turbidity and darker appearance. It was observed that drying did not interfere with amino acid composition. This study showed that sun-drying seems to be a prospective method for preservation of fish skins.

Keywords: Fish gelatin, characteristics, seawater fish, skin, sun-drying

Introduction

Gelatin, an extracted protein from animal collagen, has several functions for food, pharmaceutical, medical, cosmetic and photographic industries. The major gelatin in the world is derived from pigskin and bovine hide. However, Moslem and Jewish do not accept any pig related food products, while Hindu does not consume cow based food. In addition, bovine spongiform encephalopathy (BSE) becomes an issue in consuming products from cow (Baziwane and He, 2003; Gudmundsson, 2002). Therefore, finding an alternative to the mammalian gelatins which is acceptable to these religious groups and overcoming food safety issues is in urgent need. Gelatin from fish is the potential alternative to mammalian gelatins. In recent years, fish gelatin has progressively been studied (Montero and Gómez-Guillén, 2000; Gómez-Guillén and Montero, 2001; Gómez-Guillén *et al.*, 2002; Gudmundsson, 2002; Jamilah and Harvinder, 2002; Muyonga *et al.*, 2004; Cho *et al.*, 2005). The studies covered the extraction and characterization of gelatin properties from several fish skins like megrim, cod, tuna, yellowfin tuna, Nile perch and tilapia. Fish gelatin is now commercially available and it has been used for several applications in place of mammalian gelatins.

The source of fish skin in Indonesia is abundantly available. As the marine country with a large number

of coastal areas, it affords various species of seawater fishes. Now days, fish processing industries are getting popular, like filleting industry that normally result in by-product waste such as fish skin and fish bone. These by-products are usually processed into animal feed. In fact, these materials are potential sources of collagen that further can be converted into gelatin. Until now, there are no significant and reliable gelatin manufacturers in Indonesia (Wahyuni, 2007).

Fresh fish skin is commonly utilized as a source material for gelatin production. However, fresh fish skin is highly susceptible to deterioration, when compared to mammal sources which are more stable and easily preserved. Moreover, after degutting and filleting of fish, skins are often kept together with the rest of by-products, being subject to rapid enzymatic and microbial damage. This problem urges the need of seeking methods for preservation. The best method to preserve fish skins is by freezing. However, gelatin extracted from frozen fish skin performed a bit lower functional properties, especially molecular distribution and rheological characteristics (Fernández-Díaz *et al.*, 2003). Also, this method seems costly in terms of energy consumption and machinery installation. Another method to preserve fish skin is by drying. Drying has been used for long time to stabilize fishery products. Sun-drying is the oldest, conventional and traditional technique to preserve agricultural and fishery products therefore

*Corresponding author.

Email: pranoto@ugm.ac.id

Tel: +62 274549650; Fax: +62 274549650

it can stabilize them from deterioration. Drying of the materials reduces transportation, storage and distribution costs compared to freezing (Moeljanto, 1992). Similarly, drying could be an alternative method to stabilize fish skin before further processing in gelatin manufacturing. Air drying method has been studied to preserve skins of Dover sole fish and channel catfish. The gelatin extracted from Dover sole fish was reported to have gel strength 140-170 Bloom, was similar to that of extracted from the fresh skin. Drying showed a little in lowering of gelling and melting points. In addition, gelatin from dried channel catfish skin exhibited higher gel strength, similar gelling and melting point compared to those of extracted from fresh fish skin (Giménez *et al.*, 2005; Liu *et al.*, 2008).

This study was aimed to investigate the gelatins properties extracted from fresh and sun-dried skins of seawater fish. The gelatins were analyzed for the yield, proximate composition, functional properties, and amino acids composition.

Materials and Methods

Materials

The main materials used were fish skins of four seawater fishes, namely yellowfin tuna (*Thunnus albacares*), brown stingray (*Dasyatis annotatus*), red snapper (*Lutjanus altifrontalis*), and white cheek shark (*Carcharias dussumieri*) obtained from filleting industries in East Java Province, Indonesia. The fresh skins were kept in a cool chamber and directly transported to the laboratory in Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia. For dried skin preparation, the fresh fish skins were cleaned by washing with tap water and then sun-dried outside at temperature of around 30°C for 48 h to obtain moisture content <10% (wb), which is considerably safe and stable against deterioration. All chemical reagents used thorough this study were analytical grade purchased from chemical suppliers in the city.

Gelatin extraction from fish skins

Gelatin extraction employed type A method using acid solution in accordance to Montero and Gómez-Guillén (2000) with a slight modification. Dried fish skins were treated specifically before extraction process. They were initially rehydrated by dipping in water with the ratio of skin and water 1:4 (w/v) for 4 h. This pretreatment was done to create favorable condition for extraction process, as similar condition in the fresh skins. The skin was treated in hot water for about 1 min, and cleaned from remaining fat and

other impurities. The skins were cut into small pieces, and dipped in 0.05 M acetic acid solution with the ratio of fish skin and acid solution was 1:4 (w/v) at ambient temperature for 10 h. After that, the skin was rinsed with abundant tap water until neutral condition. Gelatin extraction was conducted by putting the fish skins in distilled water at 1:3 (w/v) ratio and heated up to 80°C for 2 h. The gelatin liquor was then filtered through a filter paper-layered cloth to obtain gelatin filtrate. The filtrate was put on the pan, and dried in a cabinet drier at 55°C for 48 h to obtain gelatin sheets. The sheets were ground to result in gelatin granules. The gelatin granules were packed in a plastic, and kept in a refrigeration temperature until used for analysis.

Yields and proximate composition analysis

Yield was expressed in a percentage (%), calculated by weighing the resulted gelatin granules divided by the weight of fish skin in dry basis (db), after considering the moisture content of each condition. The proximate compositions of gelatin granules were analyzed in terms of moisture content, crude protein, crude lipid and ash content following the AOAC (1996) method.

Determination of viscosity, melting point, gel strength and turbidity

The viscosity of gelatin solution was expressed in centipoises (cP), measured by using a rotary viscometer (Bohlin Instruments Ltd., Gloucestershire, UK). Gelatin solution (6.67%) was prepared by dissolving gelatin granules in distilled water at 60°C, and the viscosity measurement was conducted at temperature of 40-50°C.

The melting point was determined following the method of Muyonga *et al.* (2004) using thin wall and screw-capped test tubes (12 mm x 75 mm). Gelatin solutions (6.67%) in distilled water were filled in test tubes with some headspace, closed and held in a refrigeration temperature of 7°C for 16-18 h. The samples were transferred into a 10°C water bath in an inverted position, so the headspace was at the bottom. The water bath was warmed gradually at a rate of 1°C/min, and the gel melting temperature was recorded as gas moving up to the headspace.

The gel strength was carried out according to Gómez-Guillén *et al.* (2002), determined by using Universal Testing Machine Instron Model 4510 (Instron Co, Canton, Mass, USA), with load cell 5 kN, cross-head speed 1 mm/s, equipped with a 1.27-cm diameter of flat-faced cylindrical Teflon plunger. Gel sample with 3.3 cm diameter and 6 cm height was prepared by dissolving gelatin into distilled water to

make concentration 6.67%, and then kept at 7°C for 16-18 h for maturation. Gel strength was expressed in Bloom, obtained from maximum force (g) when the plunger had penetrated 4 mm into the gelatin gel.

The turbidity of gelatin solution 6.67% was measured using Spectronic 20D turbidometer (Milton Roy Co, Rochester, NY, USA) with turbidity range 0-2 NTU and standard applied was 0,5 NTU. Turbidity measurement was taken at the gelatin solution at temperature of 40°C.

Color measurement

The surface color of fish gelatin granules were measured using a Color Reader CR-10 (Konika Minolta Sensing Inc., Japan). The parameters measured were **L**, **a** and **b** in a triplicate which correspond to **L** black (0) to white (100), **a** green (-) to red (+) and **b** blue (-) to yellow (+), respectively.

Amino acids analysis

Amino acids composition was analyzed using high performance liquid chromatography (HPLC) type ICE with a column ODS (Ultra Techspere), fluorescence detector, mobile phase of buffer A (Na-acetic pH 6.5, Na-EDTA; methanol: THF) and buffer B (methanol 95%) with flow rate of 1 ml/min. Initially, gelatin sample was hydrolyzed using 6 N and 0.01 N HCl, and filtered through a millipore paper, added with potassium boric buffer pH 10.4 at 1:1 ratio. Then, 10 µl sample was mixed with 25 µl OPA in a vial, left for 1 min and the sample was injected into HPLC column. Running time was carried out for 25 min until amino acid separation was complete.

Statistical analysis

The experiments employed complete randomized design (CRD) using two independent variables; fish species and skin condition. Data were analyzed with analysis of variance (ANOVA) using Statistical Package for Social Science software (SPSS version 15.0). When there were any significant differences between samples, Duncan's multiple range test was used to determine the significance of the average ($p < 0.05$).

Results and Discussion

Yield and proximate composition

The yields of gelatin extracted from different seawater fish skins, with different conditions of skins are presented in Table 1. Yellowfin tuna and brown stingray fish skins resulted in a significant high gelatin yield, both from fresh skin and dried skin. Drying fish skins affected significantly ($p > 0,05$) the gelatin

yield in brown stingray and red snapper, showing lower yields, and was no effect on yellowfin tuna and white cheek shark. This result was comparable to that of reported by Giménez *et al.* (2005), in which the gelatin yield extracted from dry skin was lower than that of extracted from fresh skin. Because, the drying affected the fish skin, although the dry fish skin had been rehydrated in water before extraction process. Rehydration did not bring the dry skin to be fresh-like skin. The drying caused the change in protein structure of the skins, such as denaturation and water molecules release (Suwetja, 1997). Protein denaturation makes the lost of native characteristic of protein structure due to damages of hydrogen bound and other secondary bounds which usually strengthen protein molecule. This characteristic could not be regained back as in the initial condition during rehydration. Giménez *et al.* (2005) also stated that the loss of water in drying may promote protein-protein interactions and induce protein aggregation, which may impairs collagen swelling and gelatin extraction.

Table 1. Yields of gelatin extracted from fresh and dried fish skins

Fish skins	Yield (%)	
	Fresh Skins	Dried Skins
Yellowfin tuna	52.56±6.54 ^{dA}	51.03±8.51 ^{cA}
Brown stingray	63.17±11.46 ^{cA}	23.45±1.32 ^{bB}
Red snapper	22.51±1.32 ^{bA}	12.81±0.49 ^{aB}
White cheek shark	14.43±1.30 ^{aA}	12.09±0.49 ^{aA}

Values were given as mean ± standard deviation. Values with the same superscript letters within a column and value with the same superscript capital letters within a row are not significantly different ($p < 0.05$)

Proximate compositions of fish gelatin extracted from fish skins are summarized in Table 2, consisting of moisture content, crude protein, crude lipid and ash content. The moisture content of gelatin was analyzed to check whether the gelatin follows the standard or not. The moisture content lay down between 8.48 to 10.78% (wb). Different fish species affected slightly moisture content. In general, the different material sources of gelatin (fresh skins and dried skins) did not affect significantly ($p < 0.05$) moisture content. Indonesian National Standard (SNI, 1995) regulates the maximum level of gelatin moisture at 16%, while the British Standard Institution (BSI, 1975) regulates the maximum moisture content at 14%. Therefore, the moisture content of these fish gelatins had fulfilled the standard regulations.

Crude protein of gelatin can be used to evaluate the purity of the gelatin. It was shown that the protein content of gelatin extracted from fresh fish skins ranging from 71.11 to 86.76%. From all fish species, there was a significant ($p > 0.05$) difference in protein content of gelatins extracted from different skin conditions, those extracted from dry skins tend to have higher protein content. Indonesian National Standard (SNI, 1995) does not specifically regulate the level

Table 2. Proximate composition of gelatin extracted from fresh and dried fish skins

Fish skins	Moisture (%)		Crude protein (%)		Crude lipid (%)		Ash content (%)	
	Fresh skins	Dried skins	Fresh skins	Dried skins	Fresh skins	Dried skins	Fresh skins	Dried skins
Yellowfin tuna	10.27±0.29 ^{ba}	10.68±0.30 ^{ba}	81.63±0.09 ^{aA}	96.08±0.71 ^{cB}	1.15±0.03 ^{ba}	0.13±0.05 ^{cB}	3.66±0.11 ^{aA}	1.28±0.22 ^{bB}
Brown stingray	8.48±0.33 ^{aA}	10.78±0.71 ^{bb}	83.86±0.23 ^{aA}	92.25±1.38 ^{bb}	0.95±0.04 ^{ba}	0.91±0.06 ^{ba}	5.65±0.43 ^{ba}	1.66±0.41 ^{bB}
Red snapper	9.91±0.54 ^{ba}	9.34±0.26 ^{aA}	71.11±0.74 ^{aA}	86.95±1.80 ^{bb}	1.58±0.26 ^{aA}	0.40±0.00 ^{bb}	4.02±0.17 ^{aA}	2.88±0.67 ^{bb}
White cheek shark	10.04±0.52 ^{ba}	10.51±0.45 ^{ba}	86.76±0.65 ^{ba}	85.11±2.29 ^{aA}	0.37±0.03 ^{aA}	0.73±0.10 ^{bb}	3.31±0.51 ^{aA}	4.75±0.43 ^{cB}

Values were given as mean + standard deviation. Values with the same superscript letters within a column and value with the same superscript capital letters within a row of each parameter are not significantly different ($p < 0.05$)

of protein content, and the commercial gelatin from bovine hide contains total protein approximately of 85.99%. Therefore, most of the fish gelatins obtained here had met the commercial requirement of the protein content.

Gelatin extracted from fresh skin of white cheek shark exhibited lower crude lipid content (0.37%), and that of extracted from red snapper fish skin exhibited the highest one (1.58%). However, the same trend did not occur in the gelatins from dried skins. In general, gelatin extracted from dried skins tended to have lower lipid content. Lipid content was closely affected by fish species. In addition, sun-drying on fish skins also significantly affected lipid content. There is no specific standard of lipid content in the gelatin. The commercial gelatin from bovine hide is reported to have lipid content of 0.23%, therefore the lipid content in these fish gelatins were higher than commercial requirement.

The ash content of gelatin extracted from fresh fish skins ranged from 3.31 to 5.65%, whereas those from dried fish skins varied from 1.28 to 4.75%. Thus, the ash content of gelatin from dried skins was significantly ($p < 0.05$) lower than those from fresh skins. It was presumed that mineral was partly released out together with the water during sun-drying of fish skins. Indonesian National Standard (SNI, 1995) regulates the ash content of gelatin is below 3.25%, and our results were laid down in this limit range. Commercial gelatin of bovine hide requires lower ash content, around 1.66%. This indicates that the extraction process needs special attention to result in lower ash content toward minimum level.

Viscosity, melting point, gel strength and turbidity

The viscosity of gelatin extracted from fresh fish skins ranged from 6.64 to 8.00 cP, whereas those extracted from dried skins varied 6.29 to 7.52 cP (Table 3). There was a similar trend between gelatins extracted from fresh and dried skins. The gelatin from brown stingray skin performed the highest viscosity, and the lowest viscosity was shown by gelatin from yellowfin tuna skin. In all fish species, drying reduced significantly ($p > 0.05$) viscosity of the gelatin. Viscosity is measuring resistance force of the solution. The value is closely related to the molecular

weight of the component that resulted in cohesion force between molecules. The resistance force is also influenced by the other components that are probably present in the system such as ash and lipid, and they lead to adhesion force. In term of gelatin molecule, the viscosity has close relation with the length of polypeptide chain of amino acids. In addition, the viscosity of all fish gelatins was considerably higher compared to bovine hide gelatin with viscosity of 3.31 cP as reported by See *et al.* (2010). Commercial gelatins set the viscosity specification around 7 cP, thus the viscosity of these gelatins were close to the commercial gelatin. British Standard Institution categorized the grade A of gelatin having viscosity of 4.5 cP (BSI, 1975).

Melting points of the gelatins extracted from fish skins ranged from 19.67 to 28.67°C, as shown in Table 3. The melting point of gelatins was closely related to the fish species. Fish gelatin of yellowfin tuna had lowest melting point, both extracted from fresh and dried skins which yielded 20.33 and 19.67°C, respectively. Sun-drying affected slightly on the melting point. Gelatins from both skin conditions of brown stingray fish exhibited the highest melting point, around 28°C. The melting point of gelatin from brown stingray fish was similar to that of extracted from fish skin of black tilapia as reported by Jamilah and Harvinder (2002). Bovine gelatin was reported to have high melting point of 33.8°C (Cho *et al.*, 2005). Thus, fish gelatins studied here had lower melting point compared to commercial bovine gelatin.

Gel strength is the most important characteristic of the gelatin properties. Similar to the viscosity and melting point characteristics, the same trend occurred on the gel strength, which is closely related to the fish species (Table 3). Sun-drying on fish skin affected slightly gel strength. The highest gel strength was shown by both gelatins from fresh and dried skins of brown stingray, resulted in 266.42 and 337.00 Bloom, respectively. The lowest gel strength was shown by those extracted from yellowfin tuna, 159.03 Bloom from fresh skin and 163.36 Bloom from dried skin. In addition, the gel strength of gelatins from red snapper and white cheek shark was in medium ranging from 218.59 to 245.21 Bloom. The gel strength of these gelatins was higher than those from the skins of red

Table 3. Viscosity, melting point, gel strength and turbidity of gelatin extracted from fresh and dried fish skins

Fish skins	Viscosity (cP)		Melting point (°C)		Gel strength (Bloom)		Turbidity (NTU)	
	Fresh skins	Dried skins	Fresh skins	Dried skins	Fresh skins	Dried skins	Fresh skins	Dried skins
Yellowfin tuna	6.64±0.26 ^{aA}	6.29±0.01 ^{aB}	20.33±1.53 ^{aA}	19.67±0.58 ^{aA}	159.03±1.72 ^{aA}	163.36±1.19 ^{aA}	2.27±0.04 ^{aA}	3.15±0.05 ^{aB}
Brown stingray	8.00±0.12 ^{aA}	7.52±0.03 ^{aB}	28.33±0.58 ^{aA}	28.67±0.58 ^{aA}	266.42±8.16 ^{aA}	337.00±5.59 ^{aB}	1.72±0.02 ^{aA}	2.75±0.11 ^{aB}
Red snapper	7.07±0.06 ^{aA}	6.76±0.03 ^{aB}	25.33±0.58 ^{aA}	22.67±0.58 ^{aB}	218.58±12.69 ^{aA}	245.21±5.94 ^{aB}	1.98±0.08 ^{aB}	2.53±0.13 ^{aB}
White cheek shark	7.44±0.54 ^{aA}	6.94±0.01 ^{aB}	24.67±0.58 ^{aA}	25.00±1.00 ^{aA}	226.54±7.39 ^{aA}	229.39±4.48 ^{aA}	1.63±0.02 ^{aA}	2.12±0.05 ^{aB}

Values were given as mean + standard deviation. Values with the same superscript letters within a column and value with the same superscript capital letters within a row of each parameter are not significantly different ($p < 0.05$)

tilapia and black tilapia, which resulted in 128.11 and 180.76 Bloom, respectively (Jamilah and Harvinder, 2002). In all fish species, sun-drying tended to increase gel strength, as similar to that of reported by Liu *et al.* (2008). Lower molecular weight fragment of gelatin from fresh skins is the reason that results in lower gel strength. Meanwhile, commercial bovine hide gelatin was reported to have 239.98 Bloom (Cheow *et al.*, 2007). Fish gelatins from brown stingray skins showed considerably higher gel strength compared to bovine hide gelatin. Gelatin from yellowfin tuna was lower and those of extracted from red snapper and white cheek shark were close to bovine hide gelatin, depending on the skin condition. According to British Standard Institution (BSI, 1975), all gelatins fell down into grade A, which has gel strength specification at 220 Bloom, except for yellowfin tuna fish skins.

Turbidity of the gelatin solutions extracted from fresh and dried skins of seawater fishes is shown in Table 3. All gelatins showed similar trend, in which the gelatin from dried fish skin had significantly ($p < 0.05$) higher turbidity. Gelatin from yellowfin tuna skin had highest turbidity, whether from fresh or dried skins. On the other hand, gelatins from white cheek shark skins showed the lowest turbidity. The drying process affected significantly ($p < 0.05$) to the turbidity of the gelatins. There is no scientific report explaining the reason of this phenomenon. It was probably also due to color change of the skins toward brown during drying. Muyonga *et al.* (2004) stated that the turbidity is closely associated with the color measurement as well. The turbidity is largely dependent on the clarification process which should eliminate the particle. Higher value indicates inadequate filtration process during gelatin manufacture. The turbidity in this study was higher than that of reported by Muyonga *et al.* (2004), who found the turbidity of the gelatins from Nile perch fish skins varied from 20-158 NTU. This large difference was not only due to the different fish species, but also due to the processing and other technical aspects during gelatin extraction. Although there is no standard or requirement for the turbidity, but it is considerably necessary as a technical assessment to meet better quality for wider scope of gelatin applications.

Color properties

The color properties of the gelatins extracted from different species of seawater fishes from fresh and dried skins are presented in Table 4. In term of lightness (**L** value), it was observed in both skin conditions that the value was closely associated with the fish species. The gelatin from brown stingray showed significantly ($p > 0.05$) the highest **L** value, indicating the bright color. On contrary, the gelatins from white cheek shark fish skins revealed the darkest, shown by their lowest **L** value. Meanwhile, the gelatin extracted from yellowfin tuna and red snapper had a close lightness level. This study suggested that there was a little effect of the sun-drying on the **L** value of gelatin, in which the drying led to a bit darker in the gelatin appearance.

Parameter **a** of the gelatin color was also strongly associated with the fish species, observed in both skin conditions. It was in order array from the highest to the lowest found in yellowfin tuna, red snapper, white cheek shark and brown stingray. It means that gelatin from yellowfin tuna fish skin tends to have red appearance, and less was observed in the gelatin from brown stingray fish skin. The drying process increased slightly **a** value of the gelatin.

The yellowish color (**b** value) of the gelatin was quite similar, only those of extracted from yellowfin tuna skin performed the highest value. It indicated that the gelatin from yellowfin tuna had more yellow appearance than others. Drying affected significantly ($p < 0.05$) **b** value, it could increase or decrease the value. Jamilah and Harvinder (2002) reported that gelatin extracted from tilapia fish skins had **L** = 92.35 to 93.32; **a** = -0.47 to -0.56; **b** = 2.30 to 3.09. It seems that the gelatin of this study is darker, red and more yellow in appearance. The gelatin color is most likely influenced by drying process and the remained natural color of the fish skins.

Amino acids composition

Amino acid composition of the gelatin extracted from fresh and dried skins of seawater fishes is shown in Table 5. The analysis detected the presence of 15 amino acids and it mostly contained the high percentage of glycine, followed by glutamic acid and alanine. On the other hand, histidine and tyrosine

Table 4. Color properties of gelatin extracted from fresh and dried fish skins

Fish skins	L		a		b	
	Fresh skins	Dried skins	Fresh skins	Dried skins	Fresh skins	Dried skins
Yellowfin tuna	63.60±1.21 ^{bA}	60.37±0.35 ^{cB}	12.67±1.45 ^{dA}	13.67±0.84 ^{dA}	37.43±1.17 ^{cA}	35.57±0.74 ^{dB}
Brown stingray	75.93±0.35 ^{cA}	71.83±1.36 ^{dB}	4.77±0.15 ^{aA}	6.70±0.10 ^{aB}	27.43±0.06 ^{aA}	30.80±0.53 ^{bB}
Red snapper	63.57±1.72 ^{bA}	50.27±5.23 ^{bB}	10.03±0.23 ^{cA}	10.60±1.57 ^{cA}	30.50±1.18 ^{bA}	25.27±1.10 ^{aB}
White cheek shark	41.87±0.81 ^{aA}	42.13±1.27 ^{aA}	8.00±0.17 ^{bA}	8.37±0.21 ^{bB}	30.87±0.12 ^{bA}	32.87±0.72 ^{cB}

Values were given as mean + standard deviation. Values with the same superscript letters within a column and value with the same superscript capital letters within a row of each parameter are not significantly different ($p < 0.05$)

Table 5. Amino acids composition of gelatin extracted from fresh and dried fish skins

Amino acids	No. residues/100 residues							
	Yellowfin tuna		Brown stingray		Red snapper		White cheek shark	
	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
Aspartic acid	5.18	5.28	4.92	4.85	5.22	5.14	4.99	5.03
Glutamic acid	10.17	10.23	10.54	10.57	9.41	9.18	10.04	10.12
Serine	2.46	2.26	2.22	2.26	2.25	2.27	2.43	2.45
Histidine	0.62	0.65	0.90	1.04	0.74	0.77	0.98	1.04
Glycine	24.51	24.67	25.72	25.85	22.88	22.93	22.86	22.93
Threonine	2.38	2.47	2.25	2.28	2.09	2.04	1.87	1.85
Arginine	8.67	8.45	8.65	8.67	7.69	7.62	8.27	8.26
Alanine	10.44	10.52	9.73	9.70	9.33	9.34	9.38	9.40
Tyrosine	0.32	0.41	0.37	0.42	0.44	0.42	0.23	0.35
Methionine	1.52	1.55	1.64	1.68	1.53	1.58	1.64	1.61
Valine	2.21	2.09	2.95	2.99	2.10	2.21	2.43	2.57
Phenylalanine	2.11	2.24	2.22	2.21	2.00	2.21	2.12	2.22
Isoleucine	1.22	1.16	1.89	1.83	1.40	1.48	2.55	2.60
Leucine	2.56	2.37	3.11	3.21	2.66	2.68	2.86	2.82
Lysine	3.50	3.57	3.07	3.09	3.83	3.87	3.48	3.55

were found to be least in the gelatins. Proline and hydroxyproline are known as the essential amino acids of the gelatin. They were not found in these fish gelatins. It occurred because this amino acid analysis used OPA (orthophaldehyde) in alkali condition, in which the proline could not react well. Hence, it did not form derivate that absorbed UV light or fluorescence. Therefore, the amino acid could not be detected by a fluorescence detector. According to Ashman and Bosserhof (1985) analysis of amino acids using HPLC method could not detect secondary amino acids like tryptophan, cysteine, proline and hydroxyproline, because this method uses OPA derivation conducted in pre-column. In general, the percentage of amino acids of gelatin in this study was higher than that of reported by Jamilah and Harvinder (2002). In addition to proline and hydroxyproline, glycine is also limiting amino acids in the gelatin, because these three amino acids are found dominantly in commercial gelatin. In term of water binding property, glycine is important. Higher percentage of glycine leads to better water binding of the gelatin that is normally indicated by high viscosity, gel strength and melting point.

Based on the amino acid composition, it showed that the gelatin extracted from brown stingray fish skins, both from fresh and dried skins showed the highest glycine. This amino acid was closely associated with the viscosity, gel strength and melting point value, as shown by their highest viscosity, melting point and gel strength compared to gelatins from other fish species. Montero and Gómez-Guillén (2000) also stated that amino acid composition of the gelatin affected the physical properties.

Conclusions

It could be concluded that sun-drying seems to be suitable method for preservation of seawater fish skins. The optimization of dry skin rehydration need to be investigated accordingly to obtain the optimum yield as close to those of extracted from fresh skins. Drying of fish skin affected slightly the viscosity, melting point and gel strength of the gelatin extracted, but it increased the turbidity. Drying affected obviously the color of the gelatin. It was observed that drying did not interfere with amino acid composition of the gelatin. Sun-drying on fish skins has advantages for preservation compared to freezing since the dried skins are able to stand at room temperature for longer time and the weight is largely decreased, which leads to reduction in transportation, distribution and energy cost.

Acknowledgment

The authors thank Decentralization Project of Gadjah Mada University through Fundamental Research Grant Contract No. LPPM-UGM/566/2007 for financial support.

References

- Ashman, K. and Bosserhof, A. 1985. Amino acid analysis by high performance liquid chromatography and precolumn derivatisation. In Tschesche, H. (ed). *Modern Methods in Protein Chemistry*, p. 155-171. Berlin: de Gruyter.
- AOAC. 1996. *Official methods of analysis*, 16th edition, Washington DC: Association of Official Analytical

Chemists.

- Baziwane, D. and He, Q. 2003. Gelatin: The paramount food additives. *Food Reviews International* 19(4): 423-435.
- BSI (British Standard Institution). 1975. Methods for sampling and testing gelatin (physical and chemical methods). London: BSI.
- Cho, S.M., Gu, Y.S. and Kim, S.B. 2005. Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. *Food Hydrocolloids* 19: 221-229.
- Cheow, C.S., Norizah, M.S., Kyaw, Z.Y. and Howell, N.K. 2007. Preparation and characterisation of gelatins from the skins of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*). *Food Chemistry* 101: 386-391.
- Fernández-Díaz, M.D., Montero, P. and Gómez-Guillén, M.C. 2003. Effect of freezing fish skins on molecular and rheological properties of extracted gelatin. *Food Hydrocolloids* 17: 281-286.
- Giménez, B., Gómez-Guillén, M.C. and Montero, P. 2005. Storage of dried fish skins on quality characteristics of extracted gelatin. *Food Hydrocolloids* 19: 958-963.
- Gómez-Guillén, M.C. and Montero, P. 2001. Extraction of gelatin from megrim (*Lepidorhombus boscii*) skins with several organic acids. *Journal of Food Science* 66(2): 213-216.
- Gómez-Guillén, M. C., Turnay, J., Fernández-Díaz, M. D., Ulmo, N., Lizarbe, M. A. and Montero, P. 2002. Structural and physical properties of gelatin extracted from different marine species: A comparative study. *Food Hydrocolloids* 16(1): 25-34.
- Gudmundsson, M. 2002. Rheological properties of fish gelatins. *Journal of Food Science* 67(6): 2172-2176.
- Jamilah, B. and Harvinder, K.G. 2002. Properties of gelatins from skins of fish-black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). *Food Chemistry* 77: 81-84.
- Liu, H., Li, D. and Guo, S. 2008. Rheological properties of channel catfish (*Ictalurus punctatus*) gelatin from fish skins preserved by different methods. *LWT Food Science and Technology* 41: 1425-1430.
- Moeljanto. 1992. Pengawetan dan pengolahan hasil perikanan. Jakarta: Penebar Swadaya.
- Montero, P. and Gómez-Guillén, M.C. 2000. Extracting conditions for megrim (*Lepidorhombus boscii*) skin collagen affect functional properties of the resultant gelatin. *Journal of Food Science* 65: 434-438.
- Muyonga, J.H., Cole, C.G.B. and Duodu, K.G. 2004. Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. *Food Hydrocolloids* 18: 581-592.
- See, S.F., Hong, P.K., Ng, K.L., Wan Aida, W.M. and Babji, A.S. 2010. Physicochemical properties of gelatins extracted from skins of different freshwater fish species. *International Food Research Journal* 17: 809-816.
- SNI 1995. Standar Nasional Indonesia 06-3735: Mutu dan cara uji gelatin. Jakarta: Badan Standarisasi Nasional.
- Suwetja, I.K. 1997. Biokimia hasil perikanan Jilid I: Komposisi kimia ikan, protein dan lipida. Manado: Fakultas Perikanan dan Ilmu Kelautan, Universitas Sam Ratulangi.
- Wahyuni, M. and Peranginangin, R. 2007. Perbaikan daya saing industri pengolahan perikanan melalui pemanfaatan limbah non ekonomis ikan menjadi gelatin. Downloaded from <http://ikanmania.wordpress.com/2008/01/page/14/> on 19th April 2007.