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Effect of drying and freezing of Cobia (*Rachycentron canadum*) skin on its gelatin properties

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Abstract: The aim of this study is to determine the characteristics of the gelatin extracted from dried and frozen Cobia skin. The gelatin extracted from dried and frozen Cobia skin were analyzed for their proximate composition, gelatin yield, gel strength, colour, gelling properties and amino acid composition. It was found that dried Cobia skin gave higher gelatin yield compared to that of frozen Cobia skin. There were significant difference in protein, ash and fat content of both gelatin samples. Gelatin extracted from dried skin gave a snowy white, was light textured, and had bright and white appearances, while that of frozen skin was darker in color. This study also found that the gelatin extracted from dried skin gave higher gel strength. It was also found that there was no significant difference in gelling temperature and amino acid composition between both gelatin samples. This study shows that drying is a better method in preserving Cobia skin compared to freezing, prior to gelatin extraction.

Keywords: gelatin, Cobia, drying, freezing, gelatin yield, gel strength

Introduction

Gelatin has a very wide application in food industry at an estimated quantity of 200,000 metric tonnes, for pharmaceutical, biomaterial based packaging, edible film formation, and photographic industries. The common raw materials for commercial gelatin production are from pork skins, cow hides and cattle bones. Affected by the global demand for gelatin, another source of gelatin could be produced from fish skin and bones (Jamilah and Havinder, 2002). The need to provide fish gelatin as an alternative to mammalian gelatine arises from religious need, the risk of Bovine Spongiform Encephalopathy disease, the opportunity to produce value added products from fish waste as well as the need to minimize the fish waste disposal problem (Muyonga, 2003). Nagai and Suzuki (2000) reported almost 30% of fish wastes produce high collagen content, and is good source of gelatin.

Preparation of gelatin is consisted of three main stages, which are pre-treatment of the raw material, extraction of the gelatin, and purification and drying. The preservation methods of raw materials affected of the physical properties of gelatin (Gómez-Guillèn, 2002). Liu et al. (2008) has studied the properties of gelatins extracted from channel catfish skins preserved using three methods. They found that the gelatin extracted from dried channel catfish skin exhibited higher gel strength compared to those fresh and frozen skins. They have attributed this finding to the presence of large α-chain content of gelatin from the dried skins. Other that, they also observed the gelling and melting points of dried channel catfish skin gelatin solution were similar to those of fresh skin gelatin solution, but distinctly different from those of frozen skin gelatin. Giménez et al. (2005) has reported the effect of various drying methods i.e. air drying using ethanol, etahanol-glycerol mixture and marine salt followed by 160 days storage, prior to gelatin extraction on Dover sole skins. They reported that all drying methods showed similar effect on the gelatin properties. Drying operation is a cheaper preservation method compared to freezing operation in terms of weight reduction, which leads to reduced cost of transport, distribution and storage.

Cobia (*Rachycentron canadum*) is a highly potential fish species to be cultured due to its fast growth. Its weight can reach 5-6 kg within one year and 8-10kg in 16 months. The different parts of Cobia shows very unique qualities as for fat and moisture contents and has multipurpose use, such as sashimi, steamed, fried or broiled and boiled for soups. Yang et al. (2008) has reported that reported that the Cobia skin produced after processing or sashimi making was about 6%. Until now, only two studies have

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been reported on gelatin from Cobia skin. Amiza et al. (2009) has studied on optimization of gelatin extraction from Cobia skin by response surface methodology, while Yang et al. (2008) has studied the characteristics and antioxidative activity of retorted gelatin hydrolysates from Cobia skin. Thus, the aim of this study is to determine the effect of two methods of Cobia skin preservation i.e. drying and freezing on the properties of gelatin produced, in terms of gelatin yield, proximate composition, gel strength, viscosity, color and amino acid composition.

Materials and Methods

Whole Cobia fishes were purchased from a fish supplier in Langkawi Island, Malaysia. Ice storage was used during transportation of the raw materials to the laboratory to maintain the freshness. All chemicals and reagents were used of analytical grades. Fresh Cobia fish were filleted and the skin was removed from the fillet. The skin was washed to remove any contaminants. The cleaned fish skins were weighed and divided into two parts. One part was stored at frozen temperature (-20ºC) and another part was dried using a dehydrator (Model FSD-380) at 29°C for about 24 hours or until the moisture content was less than 15%.

Pre-treatment

Pre-treatment of Cobia skin prior to gelatin extraction was carried out according to the procedure of Muyonga et al. (2004). The frozen Cobia skins were thawed prior to use. After that, Cobia skin was cut into small size of about 1 cm2. Excessive water was drained manually by squeezing the skin in the cheesecloth. Then, 30 g of frozen and 30 g of dried skin separately were treated with 1.5% hydrochloric acid for 12 hours. Next, all samples were incubated at 7°C before the extraction process began. The Cobia skin samples were washed to remove excessive chemicals.

Gelatin extraction

The extraction of gelatin was carried out as described by Muyonga et al. (2004). First, the pre-treated fish skins were transferred to beakers and covered with warm water (about 60°C). The extraction process was began by placing the pretreated Cobia skin into water baths at a range between 40°C-80°C and followed by boiling for 4 hours. The volume of the extracts obtained at the different extraction time and temperature and the mass of the residue volume were recorded as well. Portions of the gelatin extracts was then filtered through muslin clothes rather than Whatman 1 paper because the extract was a little bit concentrated and then used to determine the solid concentration. The light liquor concentrations was determined by evaporating duplicate 10 ml portions to a stable weight and the concentration was then used to the calculate percentage of gelatin yield.

Yield of extracted gelatin

The light liquor concentrations was determined by evaporating duplicate 10 ml portions of filtered gelatin to a stable weight, by oven drying at 105°C for 48 hours (Muyonga et al., 2004). The concentration was then used to the calculate percentage of gelatin yield.

The following equation was adopted to calculate the yield of extracted gelatin:

$$\text{Yield (\%)} = \frac{C \times V}{M} \times 100$$

Where C = Light liquor concentration (g or ml),
V= liquor volume and
M = weight of sample (g) used for extraction

Determination of gel strength

The determination of gel strength was carried out as described by Muyonga et al. (2004). The determination of gel strength was used TAXT2 Texture Analyzer Stable Micro System. The sample preparation used a 6.67% concentration gelatin gel w/v was made up and poured into standard glass Bloom jars (150 capacities). The filled Bloom jars were chilled in a water bath at 7°C-8°C for 18hours. After conditioning, the Bloom jars were removed from the water bath just prior to testing. The Bloom jar was positioned centrally under the standard probe and the penetration test was started. After a trigger force of 5 g was attained, the probe proceeded to penetrate into the gel to a depth of 4 mm. At the depth the maximum force, reading was obtained and translated as the 'Gel Strength or Bloom Value' (g) of the gelatin gel.

Determination of viscosity

To determine the viscosity, the Brookfield DV-III Viscometer was used. In this analysis, a small sample adapter and SC4-27 spindle at 25rpm were used. The viscosity during cooling of gelatin solution from 40°C to 5°C was measured at 0.2°C /min (Kasankala et al., 2007).

Determination of color

To determine the color, the colorimeter (Minolta Cr-300 series, Japan) was used. L* (lightness), a* (redness/greenness) and b* (yellowness/blueness)

values were measured.

Determination of amino acids

The determination of amino acids was carried out as described by Liu et al. (2008). To determine the amino acid, a dedicated Amino Acid Analyzer (L-8800 model, Hitachi) was used. About 1 mg sample were weighed into hydrolysis tube and added with 1 ml 6N HCl. Sample was then hydrolyzed at 110°C for 22 hours. After hydrolyze, the sample was dried using rotary evaporator. Then, 1 ml of 0.02N HCl was added to the residue and centrifuged for 30 min. The supernatant was collected and then filtered with 0.45 µm membrane filter. Finally the sample was injected to the Amino Acid Analyzer. Amino acid content was stated as percentage.

Proximate analysis

Oven drying method (AOAC, 2000) was used to determine the moisture content of the gelatin. Ash content was determined using AOAC method (AOAC, 2000). Crude fat content was determined using Soxhlett method (AOAC, 2000). Protein content was determined using Kjedahl method (A0AC, 2000).

Statistical analysis

All data will be stated as mean ± standard deviation. The characteristics of gelatin extracted from frozen skin and dried skin were analyzed using t-test at 95% confidence level ($p<0.05$).

Results and Discussion

Gelatin extraction

Table 1 shows the gelatin yields obtained from frozen and dried Cobia skin. It was found that dried Cobia skin gave higher gelatin yield compared to that of frozen Cobia skin ($p<0.05$). The gelatin yield from Cobia skin is higher than those reported for megrim (8.3%), cod (7.4%) and hake (6.5%) (Gómez-Guillèn et al., 2002), but lower than those of salmon and cod skins (39.7% and 44.85%, respectively) (Arnesen and Gildberg, 2007).

Table 1. Gelatin yield extracted from dried and frozen Cobia skin

Method	Gelatin yield (%)	$p<0.05$
Dried skin	18.47 ± 0.727^{a}	0.01
Frozen skin	14.245 ± 0.395^{b}	0.04

[a-b] Value with different letter showed significant difference at $p<0.05$

The yield and quality of gelatin are influenced by the species and age of the fish, extraction process and pretreatment temperature (Karim and Bhat, 2009). Amiza et al. (2009) has reported similar results, whereby they found that the maximum gelatin yield from Cobia skin was around 21%. Generally, the extraction yield of gelatin from fish gelatin was approximately between 6% and 19% (Karim and Bhat, 2009). The highest yield of gelatin from Cobia skin was reported by Yang et al. (2008), whom reported a yield of 46.9% from gelatin hydrolysate of retorted Cobia skin.

Gel strength

Gel strength is one of the most important functional properties of gelatin. Gel strength is a function of complex interactions determined by amino acid composition and the ratio of α-chains content and the amount of β-components (Cho et al., 2004). The gel strengths of commercial gelatins are expressed using Bloom values and the value is the weight in grams that is required for a specified plunger to depress the surface of a standard. Johsnton-Banks (1990) reported that the average molecular weight of gelatin is largely responsible for its gelling behaviour.

After overnight maturation (18 hour, 7-8°C), gelatin extracted from dried Cobia skin showed higher gel strength (319 g) than that of frozen skin (237 g). Similar trend was reported in a study by Liu et al. (2008). They reported that the gel strength of gelatin from dried channel catfish skins (256 g) was higher than those of the fresh skin gelatin (243 g) and frozen channel catfish skins gelatin (246 g). They also found that there was a strong correlation between gel strength and the content of α-chains in catfish gelatin. High content of α-chains can increase the gel strength. However, gelatin from fresh catfish skin gave lower gel strength because of the increasing low molecular weight fragments. Fernández-Díaz et al. (2003) also reported that gelatin extracted from frozen flounder skin had lower gel strength than that of fresh flounder skin. They found that that freezing of fish skins affected the molecular composition of the resulting gelatins, leading to a decrease of the amount of extracted α-chains as well as higher molecular weight polymers. The absence of high molecular weight polymers and α-chains in gelatins from frozen skins prevented a correct annealing of protein chains during maturation period, leading to decreased gel strength and decreased ulterior renaturation ability.

The gelling strength of commercial gelatins ranges from 100 to 300, but gelatins with Bloom values of 250–260 are the most desirable and gel strength is also affected by concentration, conditions

of preparation such as temperature, pH, solids, and the presence of other hydrocolloids such as agar, pectin, or carrageenan.

Gel strength is one physical properties and most important functional of gelatin and it is governed by molecular weight, as well as by complex interactions determined by the amino acid composition and the ratio of a/b-chains present in the gelatin and mainly dependent on the proportion of fractions having a molecular weight of approximately 100,000 g mol^{-1} (Cho et al., 2004).

The gel strength of Cobis kin gelatin was higher than that of salmon skin gelatin (108 g) and cod skin gelatin (71 g) (Arnesen and Gildberg, 2007). Nevertheless, a Bloom value as high as 426 has been reported for yellowfin tuna skin gelatin (Cho et al., 2004). Some species of warm water fish gelatins have been reported to exhibit relatively high Bloom values, close to that of high Bloom pork gelatin (Gudmundsson and Hafsteinsson, 1997). Such high gel strength characterizes only those gelatins extracted from the skins of warm-water fish such as tilapia and grass carp. For example, Bloom values ranging from 128 to 273 have been reported for tilapia gelatin (Jamilah and Harvinder, 2002).

The large range of Bloom values found for the various gelatins arises from differences in proline and hydroxyproline content in collagens of different species, and is also associated with the temperature of the habitat of the animals. Badii and Howell (2006) have shown that hydrophobic amino acids (Ala, Val, Leu, Ile, Pro, Phe, and Met) could also contribute to the high Bloom value of tilapia fish gelatin. They found a lower number of hydrophobic amino acids in the commercial non-gelling cod gelatin compared to tilapia and horse mackerel gelatin. This study shows that the gel strength of Cobia skins was better than those of porcine and bovine gelatin.

Viscosity

Viscosity is the second most important commercial physical property of a gelatin. It is a property of fluids that indicates resistance to flow. Higher viscosity indicates greater thickness. Gelatin solution viscosity typically increases with Bloom strength and it's depending on temperature (Karim and Bhat, 2009). Figure 1 shows the gelling temperature of gelatin extracted from dried and frozen Cobia skin.

Figure 1 shows that both gelatin samples had the same gelling temperature (13°C), but different viscosity. Viscosity of gelatin extracted from dried Cobia skin was higher than that of frozen Cobia skin. Fish gelatin has lower gelling temperature ranging from 8-25°C, compared to mammalian gelatin (11-28°C) (Cho et al., 2004). The wide range of gelling temperature is influenced by the origin of the raw

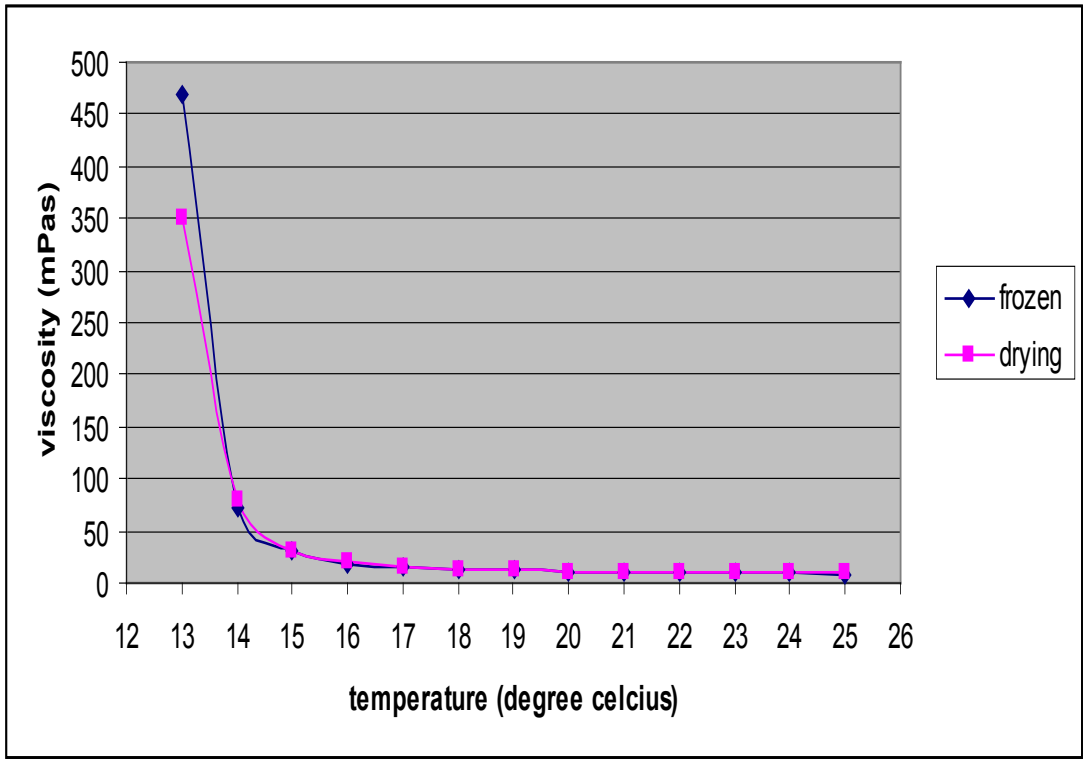

Figure 1. Effect of temperature on the gelling properties of Cobia gelatin extracted from frozen and dried skin

Table 2. Colour analysis of gelatine powder

Color Measurement	Gelatin	
	Dried skin	Frozen skin
L*	79.37 ± 0.23[a]	68.71 ± 0.13[b]
a*	-1.65 ± 0.01[a]	1.04 ± 0.02[b]
b*	10.54 ± 0.01[a]	7.91 ± 0.02[b]

[a-b] Values with different letter showed significant difference at $p<0.05$ between the two gelatin preparation.

Table 3. Amino acid composition of gelatin extracted from dried and frozen Cobia skin

Amino acid	% Amino Acid	
	Dried skin gelatin	Frozen skin gelatin
Aspartic	2.82 ± 0.38a	3.08 ± 0.0495a
Threonine	1.31 ± 0.16a	1.46 ± 0.014a
Glutamic	5.65 ± 0.95a	6.22 ± 0.16a
Glycine	**19.03 ± 3.68a**	**20.85 ± 0.64a**
Alanine	8.17 ± 0.59a	8.45 ± 0.01a
Cystine	0.00a	0.00a
Valine	1.38 ± 0.22a	1.44 ± 0.01a
Methionine	0.07 ± 0.10a	0.00a
Isoleucine	0.59 ± 0.11a	0.68 ± 0.01a
Leucine	1.20 ± 0.20a	1.43 ± 0.01a
Tyrosine	0.21 ± 0.03a	0.27 ± 0.01a
Phenylalanine	1.03 ± 0.15a	1.16 ± 0.00a
Lysine	2.03 ± 0.33a	2.25 ± 0.01a
Histidine	0.47 ± 0.03a	0.54 ± 0.05a
Arginine	4.61 ± 0.69a	4.93 ± 0.03a
Proline	**4.58 ± 0.42a**	**5.01 ± 0.27a**
Serine	1.79 ± 0.11a	1.90 ± 0.04a

[a] Values with different letter showed significant difference at $p<0.05$

Table 4. Proximate Composition of Gelatin extracted from dried and frozen skin (dry basis)

Chemical composition (%)	Freeze-dried gelatin powder extracted from	
	Dried skin	Frozen skin
Ash	2.90 ± 0.00[a]	3.15 ± 0.01[b]
Protein	93.14 ± 0.08[a]	79.12 ± 0.10[b]
Fat	2.62 ± 0.02 [a]	3.66 ± 0.07[b]

[a-b] different letter indicates a significant difference between gelatin powder extracted from dried and frozen skin

material used in process. The viscosity of gelatin solutions is partially controlled by molecular weight and polydispersity. The viscosity of gelatin solutions increases with increasing concentration and with decreasing temperature (Chiou et al., 2006).

The viscosity of gelatin plays a significant role in certain food systems. Examples of this include starch-molded confectionery applications where the high working speeds demanded by modern processing equipment require a gelatin with a low viscosity to prevent the formation of "tails" together with a rapid distribution in the molds. Gelatins viscosity also affects gel properties including setting and melting point. High-viscosity gelatin gives gels with a higher melting and setting rate than do gelatins of lower viscosity. For stabilizing the fish gelatin, certain emulsions gelatins of higher viscosity are preferred. The viscosity characteristics displayed by a given gelatin grade are primarily related to the molecular weight distribution of the gelatin molecules (Karim and Bhat, 2009).

Color analysis

Color analysis of gelatin samples was determined using the colorimeter (Minolta Cr-300 series, Japan) L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) values were measured. Table 2 shows that gelatin from dried skin were significantly ($p<0.05$) different in L*, a*, and b* values compared to those of frozen skin. Gelatin extracted from dried skin gave a snowy white, and was light textured, had bright and white appearances, while gelatin extracted from frozen skin was darker and less yellowish in color. Jamilah and Havinder (2002) reported that color of both gelatins (black tilapia and red tilapia) had a snowy white appearance and were light-textured. The color of the gelatin depends on the raw material. However, it does not influence other functional properties. Lighter color of fish hydrolysate is preferred and considered as a positive attribute, since it is easier to incorporate these light colored gelatin into any food system without imparting any strong color attribute to the product.

Amino acid composition

Table 3 shows the amino acid composition of gelatin extracted from frozen and dried Cobia skin. There t-test showed that there was no significant difference in the total and individual amino acid composition between both gelatin samples ($p<0.05$). Table 3 shows that Glycine (Gly) content in each type of gelatin is far higher than any other amino acids. Glycine was the most abundant amino acid in both of gelatin sample and it was about 19-21% of total amino acids. Glycine content in the gelatin extracted from frozen skin was slightly higher than that of dried skin. The important amino acid besides the Glycine is Proline (Pro) and Hydroxyproline (Hyp). Proline also showed frozen skin gelatin has slightly higher (5.01 ± 0.27) value from dried skin gelatin (4.58 ± 0.42). However, there was no significant difference in Glycine and Proline content in both gelatin samples ($p<0.05$). In this study, hydroxyproline could not be detected because the method used in this study could not detect hydroxyproline.

Gómez-Guillèn et al. (2002) and Cho et al. (2004) reported a higher glycine and proline content in fish gelatin. Gómez-Guillèn et al. (2002) reported that the amino acids composition of gelatin from the skin of sole, megrim, cod, hake and squid had more than 30% Gly and ~17% imino acids. However, Cho et al. (2004) reported that content of Gly was much higher about 32.1% whereas proline content was 12% in yellowfin tuna gelatin. However, Jamilah and Harvinder (2002) reported that the proline contents of the gelatins from red and black tilapia was very low and nearly not detectable

The amino acid composition plays main role in the physical properties of gelatin. However, the physical properties of the gelatin depends not only on the amino acid composition, but also on the relative content of α or ß components and higher molecular weight aggregates, as well as on the presence of lower molecular weight protein fragments. Thus, in addition to the source or species, gelatin properties will also strongly depend on the preservation of raw materials (Johnston-Banks, 1990; Giménez et al., 2005).

Importance in fish collagens has been stimulated by the suggestion that their reduced structural stability compared with mammalian collagen is related to a lower content of hydroxyproline. Proline contents also lower than those in mammalian collagens and this is compensated for by higher serine and threonine contents and gelatin usually repeated structure of Gly-X-Y with X being Proline and Y being hydroxyproline. When Proline (Pro) and Hydroxyproline (Hyp) were located in the X and Y positions, the gelatin structure ordered conformation when gelatin forms a gel network. Besides the imino acids, lysine also stabilizes gelatin structure by forming crosslinking structure between chains (Jamilah and Havinder, 2002).

The imino acids proline and hydroxyproline impart considerable rigidity to the collagen structure. Relatively, limited imino acid content should result in a less sterically hindered helix and may affect the dynamic properties of the gelatins. Although proline

is important, hydroxyproline is believed to play a singular role in the stabilization of the triple-stranded collagen helix due to its hydrogen bonding ability through its hydroxyl group. The lower content of proline and hydroxyproline probably gives low gel modulus, gelling and melting temperature (Gómez-Guillèn et al., 2002).

Proximate composition of Cobia gelatin

Table 4 shows the proximate composition of gelatin extracted from frozen and dried Cobia skin (dry basis). Table 4 shows that there were significant difference in protein, ash and fat content of gelatin extracted from both Cobia skin samples ($p<0.05$).

The results show that gelatin extracted from dried Cobia skin (93.14±0.08) gave higher protein content compared to that of frozen Cobia skin (79.12± 0.10). Gelatin extracted from frozen Cobia skin was significantly lower that of dried Cobia skin. The reduction in protein content could be due to tissue damage occurred by ice-crystal formation during the freezing process (Fernández-Díaz et al., 2003). Such freeze-induced protein aggregation was higher with increasing freezing temperature. Therefore, the more covalently cross-linked collagen makes more difficult the extraction and solubilisation of α-chain dimers and trimers and high molecular weight polymers, and on the contrary, smaller collagen fractions could be more easily extracted (Fernández-Díaz et al., 2003). The protein content of Cobia gelatin is in similar ranges reported for fish gelatin. Jongjareonrak et al. (2006) reported that the protein content of Bigeye snapper and Brownstripe red snapper gelatin was 87.9±0.8% and 88.6±0.7%, respectively. While, Cheow et al. (2007) reported a lower protein content of 69.2% and 68.7% for Sin croaker and shortfin scad gelatin, respectively.

It was found that the gelatin extracted from dried Cobia skin had lower ash and fat content compared to those of frozen skin gelatin. The ash content of both Cobia gelatin samples was in the same range as reported for Bigeye snapper, Brownstripe red snapper, sin croacker and shortfin scad (Jongjareonrak et al., 2006; Cheow et al., 2007). They reported the ash content in the range of 0.9-3.2%. High quality of gelatin should no more than 0.5% ash, while guidelines for ash content in food samples must be less than 3% (FAO guidelines). Further purification is needed to produce high quality gelatin with ash content lower than 0.5%. Ash content of gelatin may be contributed by the residual of chemicals after processing, come from raw material, or also the possibility of mixing with other ingredients (Jamilah and Havinder, 2002).

The fat content in Cobia gelatin in this study (2.62% - 3.66%), was higher compared to fish gelatin from Bigeye snapper, Brownstripe red snapper, sin croacker and shortfin scad (Jongjareonrak et al., 2006; Cheow et al., 2007), which was in the range of 0.1-0.8%. These differences in fat content might be due to the differences in fat content in Cobia skin compared to other fish species.

Conclusion

This study shows that the gelatin yield extracted from dried Cobia skin was significantly higher than that of frozen Cobia skin. The gelatin extracted from dried Cobia skin was also found to have higher gel strength, higher protein content and lighter color compared that of frozen skin. However, the gelatin extracted from dried Cobia skin gave lower viscosity, ash and fat content compared those of frozen skin gelatin. It was found that both gelatin samples gave similar gelling temperature and amino acid composition. The light texture and white color, give added advantage for gelatin extracted from dried skin to be incorporated in food product. This study showed that drying of Cobia skin is a better preservation method compared to freezing.

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