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Physicochemical properties of gelatins extracted from skins of different freshwater fish species

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Abstract: The aims of this study were to determine the physicochemical properties of extracted gelatins from four freshwater fish skins: snakehead (*Channa striatus*), catfish (*Clarias batrachus*), pangasius catfish (*Pangasius sutchi*) and red tilapia (*Oreochromis niloticus*) and compare with those of commercial gelatins from cold water fish skin and bovine skin. The extraction yields for four types of extracted gelatins were high, ranging from 10.78% (w/w) (pangasius catfish gelatin) to 27.79% (w/w) (catfish gelatin). Four extracted gelatins showed lower protein content and higher lipid, moisture and ash content compared to both commercial gelatins. Red tilapia gelatin presented the highest gel strength (487.61 g). At 60°C, the shear viscosity of catfish gelatin (5.24mPa.s) was the highest. Four extracted gelatins had higher pH, isoionic point and turbidity compared to the commercial gelatins. These extracted gelatins were white in appearance and had higher L* value and lower a* value than both commercial gelatins.

Keywords: Gelatin, fish skin, physicochemical properties, freshwater fish

Introduction

Annually, more than 100 million tons of fish are being harvested worldwide. 29.5% of the total catch is used for fishmeal due to its poor functional properties (Kristinsson and Rasco, 2000). Processing discards from fisheries account for as much as 70–85% of the total weight of catch and 30% of the waste is in the form of bones and skins with high collagen content (Shahidi, 1994). These wastes are excellent raw materials for the preparation of high protein food especially gelatin. Conversion of these wastes into value-added products to yield additional income has both economic and waste management benefits for the fish industry (Choi and Regenstein, 2000).

The term "gelatin" is applied to a series of food protein products derived by partial hydrolysis of animal collagen (Gómez-Guillén and Montero, 2001). During the thermal hydrolysis of collagen with the acid or alkali pretreatment, the cross-linkages between polypeptide chains bonds of the collagen along with some amount of polypeptide chain bonds are broken down. This may cause the breakdown of fibrous structure of collagen irreversibly yielding gelatin (Yang *et al.,* 2008). Gelatin is a unique protein due to its ability to form thermo-reversible gel with a melting temperature close to body temperature and its solubility in water (Norziah *et al.,* 2009). Gelatin has a very broad application in the food, pharmaceutical and photographic industries due to its unique properties. In food industry, it can be used as an ingredient to improve the elasticity, consistency and stability of foods (Zhou and Regenstein, 2005).

The global demand for gelatin has been increasing over the years. Recent reports indicate the annual world output of gelatin is nearly 326,000 tons, with pig skin-derived gelatin accounting for the highest (46%) output, followed by bovine hides (29.4%), bones (23.1%) and other sources (1.5%). Since most commercial gelatins are obtained from pig skins or cow skins and bones (perhaps due to the relatively low cost of the final gelatin product), the issue of gelatin replacement has existed for many years for the vegetarian, halal and kosher markets, particularly within Europe with the emergence of bovine spongiform encephalopathy (Karim and Bhat, 2008). Consequently, increasing interest has been paid to other gelatin sources, especially fish skin and bone from seafood processing waste. A number of studies have addressed properties of fish skin gelatins (Choi

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and Regenstein, 2000; Gómez-Guillén and Montero, 2001; Jamilah and Harvinder, 2002; Muyonga *et al.,* 2004) showing that their properties differ from those of mammalian gelatins and vary between species.

The quality of gelatin depends on its physicochemical properties which are greatly influenced by the species or tissue from which it is extracted and also by the severity of the manufacturing method. The functional properties of gelatin such as gel strength, viscosity, setting behavior and melting point depend on their molecular weight distribution and the amino acid composition (Johnston-Banks, 1990). The amino acid composition of gelatin is mainly dependent on the source species (Muyonga *et al.,* 2004). Gelatin with high levels of imino acids proline and hydroxyproline tends to have higher gel strength and melting point. The molecular weight distribution of gelatin depends to a large extent on the extraction process. During conversion of collagen to gelatin, the inter- and intra-molecular bonds linking collagen chains and some peptide bonds are broken. The more severe the extraction process, the greater the extent of hydrolysis of peptide bonds and therefore the higher the proportion of peptides with molecular weight less than α-chain. There is a strong correlation between gel strength and the α-chain content in gelatin. Gelatin containing more α-chains would thus show higher gel strength (Karim and Bhat, 2008). Fish gelatins have low gelling and melting temperatures and also lower gel strength compared to mammalian gelatin due to its low content of imino acids proline and hydroxyproline (Norland, 1990).

In Malaysia, freshwater fish has become a significant fish resource. According to Department of Fisheries Malaysia (2007), freshwater aquaculture contributed 29.1% of the total aquaculture production in 2007, increasing by 12% from 61,652.48 tons to 70,064.27 tons in 2007. The major freshwater species cultured were red tilapia (26,175.33 tons), catfish (21,891.55 tons), black tilapia (5,848.98 tons) and pangasius catfish (5,784.44 tons). The fish-based industry in Malaysia such as surimi and fillet processing industry is developing progressively due to the high demands of fish-based products in the market. Most of these industries utilized the fish flesh only and discard the skins, bones, and fins. Therefore freshwater fish skin, comprising about 5% of the whole fish, has become an interesting raw material for gelatin production.

The objective of this study was to determine the physicochemical properties of extracted gelatins from four freshwater fish skins including snakehead (*Channa striatus*), catfish (*Clarias batrachus*), pangasius catfish (*Pangasius sutchi*) and red tilapia (*Oreochromis niloticus*). Besides, this study also compared the physicochemical properties of extracted gelatins with the commercial gelatins from cold water fish skin and bovine skin.

Materials and Methods

Chemicals and raw materials

Four types of freshwater fishes which include snakehead (*Channa striatus*), catfish (*Clarias batrachus*), pangasius catfish (*Pangasius sutchi*) and red tilapia (*Oreochromis niloticus*) were obtained from a local market in Kajang, Selangor. Upon arrival at the laboratory, the fishes were killed, filleted and the skin manually removed by using a sharp knife. After filleting, these freshwater fish skins were cleaned by tap water for three times and drained. Then the fishes were frozen at -20ºC until use.

Commercial gelatins from cold water fish skin and bovine skin were bought from Sigma Aldrich. All the chemicals used were of analytical grade.

Extraction of gelatin

Gelatin extraction procedure was carried out according to Montero and Gómez-Guillén (2000) with slightly modification. After thawing overnight at 4°C, thawed skins were first cut into small pieces (about 2 to 3 cm) and then washed with running tap water for 3 times. Skins were further cleaned with 0.8 N sodium chloride (NaCl) (1:6 w/v) at 5ºC for 10 min and rinsed with abundant running tap water. Excess water was removed by draining the cleaned skins and manual squeezing. The cleaned skins were treated with 0.2 N sodium hydroxide (NaOH) (1:6 w/v) at room temperature for 30 min with constant stirring at 120 rpm and again rinsed with tap water (repeated 3 times). Skins were caused to swell with 0.05 N acetic acid (1:6 w/v) at room temperature for three hours, rinsed with tap water (repeated 3 times) and then extracted with distilled water at 45ºC for 18 hours. The extracted gelatin solutions were concentrated by rotary evaporator until moisture was less than 15% and then the concentrated samples were freeze dried and kept for analysis. The freeze dried samples were kept for maximum two and half months. The gelatin yield was calculated as the ratio of weight of dried gelatin to the total weight of fish skin on wet basis.

Proximate composition of fish skins and gelatins

The moisture, ash and fat content of the raw fish skins and extracted gelatins were determined according to AOAC (1990). Protein content was determined by Kjeldahl method (AOAC, 1990) and a nitrogen conversion factor of 5.4 was used for

calculation of crude protein content of extracted gelatin (Muyonga *et al.,* 2004).

Determination of gel strength

A 6.67% (w/v) gelatin solution was prepared according to British Standard (BS 757:1975) by mixing 7.5 g of the extracted gelatin and 105mL of distilled water. The mixture was left at room temperature for 30 min to allow gelatin to absorb water and swell. The mixture was later heated at 65°C for 20 min to completely dissolve gelatin and the obtained gelatin solution was then kept in a refrigerator at 4ºC for 16–18 hours. The gel strength was determined by using the TAXT2 Texture Analyzer Stable Micro System equipped with a plunger (1.27 cm in diameter). The maximum force (in g) at the penetration depth of 4 mm was recorded at a rate of 0.5 mm/s and the measurements were performed in triplicate.

Determination of shear viscosity

Samples used for gel strength determination were melted in a water bath maintained at 45ºC. The samples were analyzed for shear viscosity, by employing Rheometer Physica MCR 301(Model Anton Paar) attached with 5 cm cone plate geometry with cone angle 2º and a gap set at 0.05 mm. Approximately 0.5 ml of the sample solutions were loaded onto the rheometer platform using a micropipette attached with a tapered tip. Flow curves for each sample were obtained by shearing the samples at an increasing shear rate up to 1400 s^{-1} within 240 s. The temperature of the sample was maintained at 60ºC during the measurements. The shear rate-stress data were fitted to a Newtonian model, using the inbuilt software provided with the instrument.

Determination of pH and isoionic point

The pH value of 6.67% (w/v) gelatin solution was determined by using pH meter (Cyberscan 1000, Model RS 232 Meter) at 25ºC. The isoionic point was determined by passing a 1.0% (w/v) solution of gelatin through a column of mixed bed resin (Amberlite IR 120 & IRA 400, Rohm and Hass Co.) until constant pH of deionised solution was obtained.

Determination of colour and turbidity

The colour of 6.67% (w/v) gelatin solution was determined by measuring the lightness, redness and yellowness values (L*, a* and b*) using a Hunter Colorimeter (CR 300, Minolta Co., Japan). For turbidity measurement, a standard curve with Kaolin (100 mg/L) (USP Ke-500, Fisher Sci., USA) was prepared at concentrations of 0, 100, 200, 300, 400, 500 and 600 ppm. Turbidity was determined by measuring absorbance at 660 nm using a spectrophotometer (UV-2450/2550 Spectrophotometer, Shimadzu, Japan).

Statistical analysis

All data collected were analyzed using the analysis of variance (ANOVA) and Duncan's multiple range test to determine the significant differences between means. The level of significance was 95% (P= 0.05).

Results and Discussion

Gelatin yield

Gelatin extraction was carried out following the same protocol for the skin of the different freshwater fish species. The yield of the extracted gelatins was shown in Fig. 1. The yield of the extractions, expressed as grams of dry gelatin per 100 g of clean skin, varied among the freshwater fish species. The yield of catfish gelatin was the highest (27.79%) (w/w) and followed by snakehead gelatin (16.57%) (w/w) and red tilapia gelatin (11.75%) (w/w). The yield of pangasius catfish gelatin was the lowest, which was only 10.78% (w/w). The lower yield could be due to the loss of extracted collagen due to incomplete hydrolysis of the collagen (Jamilah and Harvinder, 2002).

Fish skin represents an important source of highly soluble collagen, containing a low concentration of intra- and inter- chain non-reducible crosslinks. Therefore, a mild acid pretreatment is usually used for fish skins (Norland, 1990). The collagen rod is extracted in acid and solubilized without altering its original triple-helix configuration. Subsequent thermal treatment cleaves hydrogen and covalent bonds; this destabilizes the triple helix by means of a helix-to-coil transition, leading to conversion into gelatin (Djabourov *et al.,* 1993).

Different yield values for the gelatins extracted from other fish skins were reported in the open literature: some of these were for black tilapia (5.4%), red tilapia (7.8%) (Jamilah and Harvinder, 2002), megrim (7.4%), Dover sole (8.3%), cod (7.2%), hake (6.5%) (Gómez-Guillén *et al.,* 2002), shortfin scad (7.3%) (Cheow *et al.,* 2007), bigeye snapper (6.5%) and brownstripe red snapper (9.4%) skins (Jongjareonrak *et al.,* 2006). It was found that the yield values of gelatins extracted from four freshwater fish skins including catfish, snakehead, red tilapia and pangasius catfish were greater than those of the gelatins extracted from other fish skins. The variation in such values depends on the differences in proximate composition of skins, the collagen content and amount of soluble components in the skins, as these properties vary with the species and the age

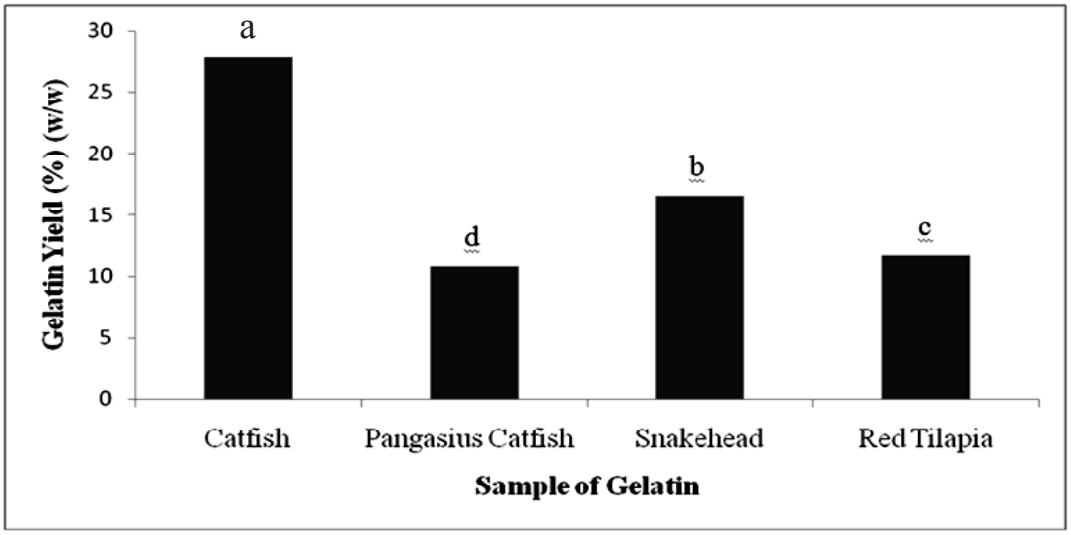

Figure 1. Yields of gelatins extracted from four freshwater fish skins
[a-d]: Means with different superscripts are significantly different (P<0.05).

of the fish, as well as the variation in the extraction method (Songchotikunpan *et al.,* 2008).

In this study, a combination of the two pretreatments was used. The alkaline and acidic pretreatments showed effects on removing noncollagenous proteins with minimum collagen loss, excluding the effect of endogenous proteases on collagen, causing a significant amount of swelling of fish skin and securing a high gelatin yield and gel strength by destroying certain chemical cross-linkages present in the collagen with less breakage of peptide bonds (Zhou and Regenstein, 2005).

Proximate composition

Proximate composition of four types of raw freshwater fish skins and gelatin extracted was shown in Table 1. The moisture content of four raw freshwater fish skins ranged from 26.93% to 39.24%. After drying, gelatins extracted from the four freshwater fish skins varied from 10.01% to 11.89%. The moisture content varied not only with the extent of drying, but also with the humidity during storage and the permeability to moisture of the packaging material (Ockerman and Hansen, 1988).

The crude protein content of four freshwater fish skins was found to be approximately 18.96–26.43%. The crude protein content of the collagenous material represented the maximum possible yield of gelatin expected from them (Muyonga et al., 2004). Gelatins extracted from four freshwater fish skins contained crude protein as the major component (75.63-89.70%). The protein content of the extracted gelatins was much greater than those for sin croaker (69.2%) and shortfin scad (68.7%) skins (Cheow et al., 2007), while it was comparable to those for young Nile perch (87.4–88.8%), adult Nile perch (87.9–88.7%) (Muyonga et al., 2004.), bigeye snapper (87.9%) and brownstripe red snapper (88.6%) (Jongjareonrak et al., 2006).

The lipid content of raw skin of pangasius catfish (10.65%) was higher than that of catfish (7.29%), snakehead (4.21%) and red tilapia (2.35%). This caused the lipid content of gelatin extracted from pangasius catfish was much higher (2.63%) than others (0.47- 0.74%). A process of degreasing could be done before gelatin extraction to reduce the fat content of the extracted gelatin. Lastly, four freshwater fish skins contained low ash content (0.55% to 0.73%). The ash content of the gelatins extracted from the four freshwater fish skins varied from 0.24 to 0.67%. Low ash content suggested that the extracted gelatin was of high quality, as the ash content for a high quality gelatin should be lower than 0.5%. To obtain the gelatin with the lower ash content, the appropriate demineralisation of the fish skins could be accomplished prior to gelatin extraction (Ockerman and Hansen, 1988).

In a comparison with commercial gelatin from cold water fish skin and bovine skin, gelatins extracted from four freshwater fish skins contained lower crude protein content and higher moisture, lipid and ash content. Commercial gelatin from bovine skin recorded the highest value of ash content (1.16%).

Gel strength

Gel strength is the most important physical property of a gelatin (Cheow *et al.,* 2007). The gel strength of gelatins extracted from four freshwater

Table 1. Proximate compositions of raw freshwater fish skins, extracted gelatins and commercial gelatins

	Proximate Composition (%)			
	Moisture	Crude Protein	Lipid	Ash
Catfish				
Fish Skin	$35.11^{AB} \pm 2.29$	$19.94^{B} \pm 0.81$	$7.29^{B} \pm 0.61$	$0.65^{A} \pm 0.04$
Gelatin	$11.04^{a} \pm 0.44$	$87.81^{bc} \pm 2.84$	$0.74^{b} \pm 0.06$	$0.62^{b} \pm 0.05$
Pangasius Catfish				
Fish Skin	$39.24^{A} \pm 2.76$	$18.96^{B} \pm 1.29$	$10.65^{A} \pm 1.58$	$0.73^{A} \pm 0.04$
Gelatin	$10.01^{b} \pm 0.11$	$81.61^{cd} \pm 1.07$	$2.63^{a} \pm 0.39$	$0.39^{c} \pm 0.02$
Snakehead				
Fish Skin	$36.79^{AB} \pm 7.16$	$19.26^{B} \pm 0.34$	$4.21^{C} \pm 0.78$	$0.55^{B} \pm 0.03$
Gelatin	$11.89^{a} \pm 0.51$	$75.63^{d} \pm 1.05$	$0.68^{b} \pm 0.01$	$0.24^{d} \pm 0.04$
Red Tilapia				
Fish Skin	$26.93^{B} \pm 0.03$	$25.43^{A} \pm 0.39$	$2.35^{C} \pm 0.98$	$0.67^{A} \pm 0.02$
Gelatin	$10.98^{a} \pm 0.38$	$89.70^{ab} \pm 3.13$	$0.47^{bc} \pm 0.01$	$0.67^{b} \pm 0.07$
Cold Water Fish				
Gelatin	$10.19^{b} \pm 0.06$	$92.07^{ab} \pm 1.26$	$0.18^{c} \pm 0.01$	$0.17^{d} \pm 0.01$
Bovine				
Gelatin	$7.44^{c} \pm 0.47$	$95.86^{a} \pm 4.38$	$0.24^{c} \pm 0.06$	$1.16^{a} \pm 0.03$

A–C Means with different superscripts within a column are significantly different ($P<0.05$) (fish skin).
a–d Means with different superscripts within a column are significantly different ($P<0.05$) (gelatin)

fish skins ranged from 278.72 g to 487.61 g (Table 2). Four extracted gelatins exhibited relatively high gel strength. Commercial gelatin from cold water fish skin showed extremely low gel strength (3.91g) compared with extracted gelatins. This gelatin solution may remain in a liquid state under the conditions of 10 °C. This was probably associated with the lower gel forming ability of this gelatin caused by the shorter chain length gelatin molecules. As a result, the weaker gel network was presumably formed (Nalinanon *et al.,* 2008).

The gel strength of the extracted gelatins was much greater than those for cold water fish gelatin including cod (90g), hake (110g), (Gómez-Guillén *et al.,* 2002), Alaska pollock (98g) (Zhou *et al.,* 2006) and salmon (108g) (Arnesen and Gildberg, 2007), while it was comparable to those for warm water fish gelatin such as yellofin tuna skin (426g) (Cho *et al.,* 2005), catfish (252g) (Yang *et al.,* 2007), Nile tilapia (328g) (Songchotikunpan *et al.,* 2008) and Nile perch (222g-229g) (Muyonga *et al.*, 2004).

Four types of freshwater fishes used are warm water fish species. Gelatins extracted from these freshwater fish skins exhibited gel strength, which were more similar to mammalian gelatins than cold water fish gelatins. This may due to higher concentrations of imino acids (proline and hydroxyproline) in warm-water fish gelatins and mammalian gelatins compared with cold-water fish gelatins. The proline and hydroxyproline contents are approximately 30% for mammalian gelatins, 22% to 25% for warm-water fish gelatins, and 17% for cold-water fish gelatins (Muyonga *et al.,* 2004).

The variation in the gel strength depends on many factors including amino acid compositions, size of protein chains (Muyonga, *et al.,* 2004), gelatin concentration and molecular weight distribution of gelatin (Ockerman and Hansen, 1988). It also associated with the temperature of the habitat of the animals (Karim and Bhat, 2008). Gudmunsson and Hafsteinsson (1997) also suggested that gel strength may depend on pH. More compact and stiffer gels are formed by adjusting the pH of the gelatin close to its isoelectric point, where the protein chains will be more neutral and thus the gelatin polymers are closer to each other. The wide range of gel strength values found for the various gelatins arises from differences in proline and hydroxyproline content in collagens of different species.

Viscosity

Viscosity is the second most important commercial physical property of a gelatin (Ockerman and Hansen, 1988). The standard temperature to measure the viscosity of gelatin is 60ºC. The shear viscosity of the gelatins extracted from four freshwater fish skins varied from 1.73 mPa.s to 5.24 mPa.s (Table 2). Gelatins extracted from catfish and pangasius catfish showed the higher shear viscosity than commercial gelatins from bovine skin and cold water fish skin. There was no significant difference ($P<0.05$) between the snakehead gelatin with commercial bovine skin gelatin. Red tilapia gelatin exhibited the lowest shear viscosity among the extracted gelatins and had no significant difference ($p>0.05$) with commercial cold water fish skin gelatin. The shear viscosity of the extracted gelatins (expect red tilapia gelatin) was relatively high as compared with the values for commercial gelatins that range from 2.0 to 7.0 mPa.s for most gelatins and up to 13.0 mPa.s for specialized ones (Johnston-Banks, 1990).

In a comparison between shear viscosity values with gel strength of extracted gelatins, it was noted that extracted gelatins which had the higher gel strength showed the lower shear viscosity and vice versa. The viscosity of gelatin solutions is partially controlled by molecular weight and polydispersity. Minimum viscosity of gelatin has been noted to be in the range of pH 6–8 for many gelatins (Ockerman and Hansen, 1988). The pH effect on viscosity is minimum at the isoionic point and maximum at pH 3 and 10.5 (Jamilah and Harvinder, 2002).

Isoionic point and pH

The isoionic points of gelatins extracted from four freshwater fish skin were higher than commercial gelatin from cold water fish skin and bovine skin (Table 2). Extracted gelatins and commercial cold water fish skin gelatin had higher isoionic points (9.01-9.64) which were close to the isoionic point of collagen (9.0-9.4). This was due to shorter period of acidic pretreatment (normally 10-72 hours) in which of deamidation of asparagines and glutamine less occurs. Commercial bovine skin gelatin is a type B gelatin which normally has lower isoionic point. This might be due to the prolonged alkaline pretreatment (7 days to 3 months). According to Muyonga *et al.* (2004), deamidation of asparagines and glutamine occur during prolonged exposure of collagenous material to acid or alkali, leading to decrease in isoionic point values.

The extracted freshwater fish gelatins showed higher pH value compared to the commercial gelatin from cold water fish skin and bovine skin (Table 2). Different pH values were reported for gelatins from different sources which include those for black tilapia (3.90), red tilapia (3.10) (Jamilah and Harvinder, 2002), sin croaker (3.30), shortfin scad (4.90) (Cheow *et al.,* 2007), Nile tilapia (5.00) (Songchotikunpan *et al.,* 2008) and Chinese Herring (4.50) (Norziah *et al.,* 2009). The difference in the pH value of gelatins may due to the type and strength of chemical(s) used during the pretreatment process (Songchotikunpan *et al.,* 2008).

Colour and turbidity

Table 3 showed the colour and turbidity of the gelatins extracted from four freshwater fish skins and commercial gelatins. The extracted gelatins had significantly ($p < 0.05$) higher L* values and lower a* values than those of commercial gelatins from cold water fish skin and bovine skin. This indicated that the colours of extracted gelatins were more brightness but less redness compared to commercial gelatins. The b* values (yellowness) of extracted gelatins (except catfish gelatin) were lower than cold water fish skin gelatin but higher than that of bovine skin gelatin. Extracted gelatins had a snowy white appearance. Cold water fish skin gelatin was visually in pale yellow colour whereas commercial bovine skin gelatins appeared in yellow brown color. The colour of gelatins depends on the raw materials and, in general, the colour does not influence the functional properties of the gelatins (Ockerman and Hansen, 1988).

The turbidity values of gelatins extracted from freshwater fish skins (except red tilapia gelatin) were much higher compared with both commercial gelatins. The higher value of turbidity in extracted gelatins reflects its poorer quality compared with commercial gelatins. High turbidity values interfere with colour measurements (Ockerman and Hansen, 1988). Higher values of turbidity may have resulted from inadequate filtration. Turbidity values are largely dependent on efficiency of the clarification (filtration) process. In this study, filtration was only done on the light liquor. In the commercial process, however, filtration is done on both the light and the heavy (concentrated) liquors. The 'heavy liquor' filtration eliminates particles that precipitate as a result of concentration. This may lead to further improvement in gelatin clarity (Muyonga *et al.,* 2004).

Conclusion

Gelatins were extracted from four types of freshwater fish skins. Combination of alkaline and acidic pretreatment following with hot water

Table 2. Gel strength, viscosity, isoionic point and pH of extracted gelatins from four freshwater fish skins and commercial gelatins

Gelatins	Gel Strength(g)	Viscosity (mPa.s)	Isoionic Point	pH
Catfish	$278.72^{c} \pm 9.43$	$5.24^{a} \pm 0.09$	$9.48^{e} \pm 0.01$	$5.22^{d} \pm 0.02$
Pangasius Catfish	$324.53^{b} \pm 7.98$	$3.82^{b} \pm 0.27$	$9.47^{c} \pm 0.01$	$5.27^{c} \pm 0.03$
Snakehead	$311.18^{b} \pm 9.62$	$3.40^{c} \pm 0.16$	$9.64^{a} \pm 0.01$	$5.39^{b} \pm 0.02$
Red Tilapia	$487.61^{a} \pm 7.52$	$1.73^{d} \pm 0.09$	$9.54^{b} \pm 0.01$	$5.50^{a} \pm 0.02$
Cold Water Fish	$3.91^{d} \pm 0.39$	$1.55^{d} \pm 0.09$	$9.01^{d} \pm 0.03$	$5.01^{f} \pm 0.01$
Bovine	$323.40^{b} \pm 9.37$	$3.32^{c} \pm 0.26$	$5.50^{e} \pm 0.01$	$5.13^{e} \pm 0.01$

a-f: Means with different superscripts within a column are significantly different (P<0.05).

Table 3. Colour and turbidity of extracted gelatins from four freshwater fish skins and commercial gelatins

Sample Gelatins	Colour			Turbidity (ppm)
	L*	a*	b*	
Catfish	$44.36^{c} \pm 0.11$	$0.56^{d} \pm 0.02$	-3,65 ± 0.06^{d}	$291.94^{c} \pm 3.76$
Pangasius Catfish	$51.84^{b} \pm 0.16$	$-0.46^{f} \pm 0.03$	-2.60 ± 0.10^{e}	$443.06^{b} \pm 1.73$
Snakehead	$61.59^{a} \pm 0.04$	$-0.24^{e} \pm 0.01$	-2.85 ± 0.02^{c}	$525.00^{a} \pm 0.83$
Red Tilapia	$40.40^{d} \pm 0.04$	$0.71^{c} \pm 0.02$	-2.86 ± 0.06^{c}	$158.05^{e} \pm 0.48$
Cold Water Fish	$37.32^{e} \pm 0.22$	$2.74^{b} \pm 0.08$	-1.83 ± 0.02^{a}	$141.67^{f} \pm 0.84$
Bovine	$36.87^{f} \pm 0.23$	$3.24^{a} \pm 0.08$	-3.00 ± 0.05^{b}	$164.45^{d} \pm 0.48$

a-f: Means with different superscripts within a column are significantly different (P<0.05)

extraction and freeze drying gave the high gelatin yield and gel strength of extracted gelatins. Gelatins extracted from four types of freshwater fish skins (warm water fishes) exhibited high gel strength. These four types of freshwater fish skin gelatins exhibited the physicochemical properties close to the bovine skin gelatins and much better than cold water fish skin gelatin. The high turbidity values of extracted gelatins were due to inadequate filtration. This study showed that these extracted freshwater fish skins gelatins are potentially to be utilized as alternative sources of mammalian gelatins and may be used in various applications in the food, pharmaceutical, and photographic industries.

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References

AOAC 1990. Official methods of analysis. 15th edn. Washington, DC: Association of Official Analytical Chemists.

Arnesen, J. A. and Gildberg, A. 2002. Preparation and characterisation of gelatin from the skin of harp seal (*Phoca groendlandica*). Bioresource Technology 82: 191-194.

BSI (British Standards Institution) 1975. Methods for sampling and testing gelatin (physical and chemical methods). London: BSI.

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.

Cho, S. H., Jahncke, M. L., Chin, K. B. and Eun, J. B. 2006. The effect of processing conditions on the properties of gelatin from skate (*Raja kenojei*) skins.

Food Hydrocolloid 20: 810–816.

Choi, S. S. and Regenstein, J. M. 2000. Physico-chemical and sensory characteristics of fish gelatin. Journal of Food Science 65: 194-199.

Department of Fisheries Malaysia 2007. Estimated aquaculture production from freshwater culture system by species, 2007. Downloaded from http://www.dof.gov.my/c/document_library/get_file?uuid=07ab7a5d-908b-4c15-9927-874ff729490e&groupId=10131.

Djabourov, M., Lechaire, J. P. and Gaill, F. 1993. Structure and rheology of gelatin and collagen gels. Biorheology 30: 191-205.

Glicksman, M. 1969. Gum technology in the food industry. New York: Academic Press.

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-Diaz, 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: 25-34.

Gudmundsson, M. and Hafsteinsson, H. 1997. Gelatin from cod skin as affected by chemical treatments. Journal of Food Science 62(1): 37–39.

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.

Johnston-Banks, F. A. 1990. Gelatin. In Harris, P. (Eds.). Food Gel, p. 233-289. New York: Elsevier Applied Sciences.

Jongjareonrak, A., Benjakul, S., Visessanguan,W., Prodpran, T. And Tanaka, M. 2000. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloids 20: 492–501.

Karim, A. A. and Bhat, R. 2008. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids, doi: 10.1016/j.foodhyd.2008.07.002

Kristinsson, H. G. and Rasco, B. A. 2000. Biochemical and functional properties of Atlantic salmon (*Salmo salar*) muscle hydrolyzed with various alkaline proteases. Journal of Agriculture and Food Chemistry 48: 657-666.

Montero, P. and Gómez-Guillén, M. C. 2000. Extracting conditions for megrim (*Lepidorhombus boscii*) skin collagen affect functional properties of the resulting gelatin. Journal of Food Science 65: 434–438.

Muyonga, J H., Colec, C. G. B. and Duodub, K. G. 2004. Extraction and physicochemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatine. Food Hydrocolloids 8: 581–592.

Nalinanon, S., Benjakul, S., Visessanguan, W. and Kishimura, H. 2008. Improvement of gelatin extraction from bigeye snapper skin using pepsin-aided process in combination with protease inhibitor. Food Hydrocolloids 22(4): 615–622.

Norland, R. E. 1990. Fish gelatin. In Voight, M. N. and Botta, J. K. Advances in Fisheries Technology and Biotechnology for Increased Profitability, p. 325–333. Lancaster: Technomic Publishing Co.

Norziah, M. H., Al-Hassan, A., Khairulnizam, A. B., Mordi, M. N. and Norita, M. 2009. Characterization of fish gelatin from surimi processing wastes: Thermal analysis and effect of transglutaminase on gel properties. Food Hydrocolloids 23: 1610-1616.

Ockerman, H. W. and Hansen, C. L. 1988. Animal by-product processing. England: Ellis Horwood Ltd.

Shahidi, F. 1994. Proteins from seafood processing discards. In Shahidi, F., Sikorski, Z. E. and Sun Pan, B. Seafood Proteins, p. 171-193. New York: Chapman and Hall.

Songchotikunpan, P., Tattiyakul, J. and Supaphol, P. 2008. Extraction and electrospinning of gelatin from fish skin. International Journal of Biological Macromolecules 42: 247–255.

Yang, H., Wang, Y., Jiang, M., Oh, J. H., Herring, J. and Zhou, P. 2007. 2-step optimization of the extraction and subsequent physical properties of Channel Catfish (*Ictalurus punctatus*) skin gelatin. Journal of Food Science 72(4): C188-C195.

Yang, H., Wang, Y., Zhou, P. and Regensteind, J. M. 2008. Effects of alkaline and acid pretreatment on the physical properties and nanostructures of the gelatin from channel catfish skins. Food Hydrocolloids 22: 1541-1550.

Zhou, P., Mulvaney, S. J. and Regenstein, J. M. 2006. Properties of Alaska Pollock skin gelatin: A comparison with Tilapia and pork skin gelatins. Journal of Food Science 71: C313-C321.

Zhou, P. and Regenstein, J. M. 2005. Effects of alkaline and acid pretreatments on Alaska Pollock skin gelatin extraction. Journal of Food Science 70: C392-C396.