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Preparation and characterisation of gelatins from the skins of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*)

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Abstract

Gelatins were prepared from the skins of the tropical fish, sin croaker (*Johnius dussumeiri*) and shortfin scad (*Decapterus macrosoma*). Visual appearance, colour, pH, bloom strength, viscoelasticity, melting point and amino acid profiles of the fish gelatins were evaluated. Shortfin scad gelatin had higher melting and gelling temperatures than those of sin croaker gelatin. The bloom strengths of gelatins from sin croaker and from shortfin scad were 125 and 177 g, respectively, compared to 240 g for commercial bovine gelatin. The pH values were significantly different between the solutions of the two fish gelatins. The elastic modulus (G') of the fish gelatin gels increased by more than 10-fold and the viscous modulus (G'') of fish gelatin solution increased sixfold after holding at 5 °C for 2 h. These viscoelastic properties of bovine gelatin only increased by less than twice.

Keywords: Fish gelatin; Bloom strength; Viscoelasticity; Melting temperature; Amino acid composition

1. Introduction

Gelatin is a protein derived from collagen, the important constituent of animal tissue (Gilsenam & Ross Murphy, 1999). The source and type of collagen will influence the properties of the resulting gelatin. New sources of gelatine, such as under-utilised fish waste, have been explored (Gilsenam & Ross Murphy, 1999). This is because there has been much interest in investigating possible means of making more effective use of under-utilised fish resources and industrial fish waste (Nagai & Suzuki, 1999). Non-mammalian gelatins, e.g., those derived from fish collagen, have obvious advantages as both minority and ethnic food products (halal, kosher) (Gilsenam & Ross Murphy, 2000).

Skins from tropical fish species, such as tilapia, have been described as an optimal raw material for gelatin production (Grossman & Bergman, 1992; Holzer, 1996). Fish gelatin is seldom used and is not mass-produced due to its dark colour and fishy odour. Some research has been devoted to the processing and functional properties of fish gelatin (Choi & Regenstein, 2000). The species of fish that were evaluated for the properties of their gelatins were: lumpfish (Osborne, Voight, & Hall, 1990), tilapia (Grossman & Bergman, 1992; Jamilah & Harvinder, 2002), conger eel and squid (Kim & Cho, 1996), cod (Gudmundsson & Hafsteinsson, 1997), shark (Yoshimura et al., 2000) and megrim (Montero & Gomez-Guillen, 2000).

The quality of gelatin depends on its physicochemical properties, which are greatly influenced, not only by the species or tissue from which it is extracted, but also by the severity of the manufacturing method (Johnston-Banks, 1990). Good rheological properties are required

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for many applications, such as thickening of sauces and gelling of pate. The aim of this work was to extract the gelatins from the skins of the tropical fish, namely sin croaker (*Johnius dussumeiri*) and shortfin scad (*Decapterus macrosoma*) and to compare their physicochemical characteristics with commercial bovine gelatin with high bloom strength.

2. Materials and methods

2.1. Raw materials

Sin croaker (*Johnius dussumeiri*), scianidae and shortfin scad (*Decapterus macrosoma*), carangidae with average sizes of 25–26 and 20–21 cm in length, respectively, were purchased fresh from a wholesaler in Port Klang, Selangor, Malaysia and transported in ice to the laboratory for beheading and gutting. The skins were removed manually after filleting and stored at −20 °C until used. The bovine gelatine, Halagel, used for comparison, was imported from Pakistan (Ahmad, 1999).

2.2. Gelatin extraction

Gelatin was prepared following the procedure described by Gudmundsson and Hafsteinsson (1997). Thawed skin was thoroughly cleaned and rinsed with excess water to remove superfluous material and treated by soaking with 0.2% (w/v) sodium hydroxide solution for 40 min. Then it was soaked with 0.2% (w/v) sulphuric acid for 40 min. This was followed by soaking with 1.0% (w/v) citric acid. After each soaking treatment, the skins were washed under running tap water until they had a pH of about 7. Each soaking and washing treatment was repeated three times with a total time of 2 h for each treatment. The ratio of skin to washing liquid used was 1 kg skin (wet weight) to 7 l of acid or alkali solution for each treatment. The skins were then subjected to a final wash with distilled water to remove any residual matter. The final extraction was carried out in distilled water at controlled temperature within the range of 40–50 °C for 12 h. The ratio used was 1 kg (weight of wet skin) to 3 l of distilled water. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (no. 4), followed by evaporation under vacuum and then freeze-drying.

2.3. Determination of colour

Colour measurement was made using a colour spectrophotometer (model spectra flash 500, Datacolor International, Berkshire, England). The samples were filled in to a clear Petri dish and readings were then taken. This procedure was performed in triplicate for each sample.

2.4. Determination of pH of raw fish skin

The pH value of raw fish skin was measured using the British Standard Institution method, BSI 757 (1975). Fish skins were chopped and blended in distilled water to form 1% (w/v) skin suspension. The pH was measured with a glass electrode (Toledo MPC 227 pH meter, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) after standardising the pH meter with pH 4.0 and 7.0 buffers.

2.5. Determination of pH of gelatin solution

The pH value of gelatin solution was measured using the British Standard Institution method, BSI 757 (1975). A 1.0% (w/v) gelatin solution was prepared in distilled water and cooled to a temperature of 25 °C in water bath. The pH was measured with a glass electrode (Toledo MPC 227 pH meter, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) after standardising the pH meter with pH 4.0 and 7.0 buffers.

2.6. Proximate compositions of gelatins

The moisture, ash, protein and fat contents of the extracted gelatins were determined by the AOAC (1995) methods.

2.7. Determination of gel strength

The bloom strength (gel strength) of gelatin gel was determined according to the method described in Winewright (1977). The gel was formed by dissolving a 6.67% (w/v) dry gelatin powder in distilled water at 60 °C. The jar was covered and allowed to cool for 15 min at room temperature. Bloom jars (Schott Duran, 55122 Mainz, Germany) (150 ml capacity) with solution were kept in a refrigerator at 7 °C (maturation temperature) for 16–18 h. Gel strength at 8–9 °C was determined on TA.XT2i Texture Analyser (Stable Micro System, Godalming, Surrey, UK) according to British Standard BS 757 (BSI, 1975), with a load cell of 5 kg, cross-head speed 1 mm/s and equipped with a 0.5 inch in diameter, flat bottomed plunger. The standard glass Bloom jar was placed centrally under the plunger and the penetration test was then performed. The maximum force (in g) was determined when the probe proceeded to penetrate into the gel to a depth of 4 mm. The reading was the average of three determinations.

2.8. Differential scanning calorimetry (DSC)

Phase transition temperature was investigated using differential scanning calorimetry, Pyris Diamond DSC, (Perkin–Elmer Instruments, Norwalk, USA). This method has been used by Bailey and Paul (2000). Sample solution at 6.67% (w/v) concentration was weighed in the order of 10 mg (±0.1 mg) in a precision balance (Sartorius, CP225D, Goettingen, Germany), and conditioned in an aluminium hermetically-sealed pan before being subjected to the DSC scan. In general, the pans were heated at 5 °C/min, between 5 and 60 °C and cooled back to 5 °C,

and in an inert atmosphere (100 ml/min of N_2). The reference was an empty pan. The equipment was calibrated with an indium sample ($T_m = 156.6$ °C, $\Delta H_m = 28.71$ J g^{-1}). All the analyses were done in triplicate. Heat absorbed or released by the gelatin solutions resulted in an endothermic or exothermic peak as a function of temperature.

2.9. Small deformation oscillatory measurement

Small deformation oscillatory measurement was performed with a controlled strain oscillatory rheometer, Physica Model No. MCR 300 (Physica Messtechnik GmbH, Darmstadt, Germany). Stainless steel concentric cylinder cup geometry (CC27, cup internal diameter, 28.925 mm) with gap size of 1 mm was used. The temperature sweep was carried out from 50 to 5 °C, holding the temperature at 5 °C for a period of 2 h and back to 50 °C at a scan rate of 1 °C/min, frequency 1 Hz and controlled strain 2%. Gelatin powder was dissolved in warm distilled water at 50 °C at 6.67% (w/v) concentration using a magnetic stirrer before the start of the test. The gelatin solution was carefully poured into the rheometer cup and covered with a thin layer of silicone oil (Sigma cat. no. M-6884). The melting temperature was taken as the point at which the phase angle peaked immediately after a sharp increase. The gelling point was taken as the point at which the first temperature of minimum phase angle occurred (Gomez, Sarabia, & Montero, 1999).

2.10. Determination of amino acid composition

Amino acid composition in gelatin was measured using waters–Pico Tag™ high performance liquid chromatography amino acid analyser, Waters Model 712 WISP (Waters, Watford, Herts, UK) according to the method of Bildlingmeyer, Cohen, and Tarvin (1987).

2.11. Statistical analyses

The SAS statistical package (SAS, 1989) was used for analyses of variance. Duncan's multiple ranges test (DMRT) was used to determine significant differences among means. All data reported are the means of at least two replicates.

3. Results and discussion

3.1. Extraction of gelatin

The yields of gelatin obtained from the skin of sin croaker and shortfin scad were 14.3% and 7.25%, respectively (see Table 1). Grossman and Bergman (1992) reported a yield of 15% for tilapia skin. Similarly, Gudmundsson and Hafsteinsson (1997) also reported a gelatin yield of about 14% for cod skins. However, Jamilah and Harvinder (2002) reported that the yields of gelatin for red and black tilapia were 7.81% and 5.39%, respectively. The lower yield recorded in this experiment could also be due to the leaching of collagen during the washing treatments. However, it was observed that sin croaker skins tended to swell more in alkaline and acidic conditions than shortfin scad skins. Therefore, sin croaker gave a better yield, possibly because the cross-links were open during swelling.

Table 1
Instrumental colour measurements of fish and bovine gelatins

	Sin croaker	Shortfin scad	Bovine
Hunter colour			
'*L*'	91.26[a]	89.33[b]	91.92[a]
'*a*'	2.24[a]	3.16[a]	1.73[a]
'*b*'	13.65[b]	18.11[a]	18.76[a]

[a–b] Means within a row with different letters are significantly different ($p < 0.05$).

Gelatin processing has two important steps: acid pretreatment and hot water extraction. The acid treatment removes non-collagen protein after the sample swells in the acid solution. The hot water extraction uses thermohydrolysis to solubilise gelatin which is then separated. Another possible reason for the lower yield of gelatin could be insufficient denaturation of soluble collagen during extraction (Jamilah & Harvinder, 2002).

3.2. Colour determination

Instrumental colour measurements of the gelatins are as shown in Table 1. The colour of gelatin depends on the raw materials extracted and whether it is the first, second or later extraction (Ockerman & Hansen, 1999). In general, colour does not influence the functional properties. The '*L*' values of bovine and sin croaker gelatin were significantly ($p < 0.05$) higher than those of shortfin scad gelatin. Bovine and sin croaker gelatins gave the brightest and whitest appearances, with '*L*' values of 91.22 and 91.26, respectively. There were no significant differences in lightness between bovine and sin croaker gelatins. However, sin croaker gelatin gave significantly ($p < 0.05$) lower '*b*' values (less yellowish) than did bovine and shortfin scad gelatin.

3.3. pH determination

There were significant ($p < 0.05$) differences among the pHs of bovine, sin croaker and shortfin scad gelatin solutions (see Table 2). The pH of sin croaker gelatin solution was the lowest and that of bovine gelatin solution was the highest. The acidic pH of the gelatin solution obtained was affected by the washing treatments. pH values of raw skin of sin croaker and shortfin scad gelatin were similar, 6.6 and 6.59, respectively.

3.4. Proximate compositions of gelatins

Table 2 shows the proximate compositions of the skins and gelatins. The protein contents of sin croaker and short-

Table 2
Proximate composition and pHs of skins and gelatins obtained from sin croaker and shortfin scad

Composition	Raw skins		Gelatins		
	Sin croaker	Shortfin scad	Sin croaker	Shortfin scad	Bovine
Moisture (%)	62.33 ± 1.49^{a}	60.43 ± 1.05^{a}	7.71 ± 0.04^{B}	11.3 ± 0.42^{A}	3.01 ± 0.02^{C}
Protein (%)	24.8 ± 0.54^{a}	24.1 ± 0.06^{a}	69.2 ± 0.13^{B}	68.7 ± 0.15^{B}	77.3 ± 0.01^{A}
Fat (%)	7.99 ± 0.42^{b}	9.63 ± 0.69^{a}	0.11 ± 0.01^{B}	0.22 ± 0.02^{A}	0.22 ± 0.01^{A}
Ash (%)	5.43 ± 1.95^{a}	5.9 ± 0.55^{a}	1.49 ± 0.15^{A}	1.15 ± 0.13^{A}	0.3 ± 0.02^{B}
pH	6.6^{a}	6.59^{a}	3.35^{C}	4.87^{B}	5.48^{A}

Values are the means ± standard deviation of triplicates.
[a–b] Means with the same superscripts within a row are not significantly different ($p < 0.05$) (raw material).
[A–C] Means with the same superscripts within a row are not significantly different ($p < 0.05$) (gelatin).

fin scad gelatins were 69.2% and 68.7%, while protein content for bovine gelatin was 77%. The protein contents of raw fish skins were 24.8% and 24.1%, respectively, for sin croaker and shortfin scad. Sin croaker skin was thicker than shortfin scad and that may be the reason for the better yield for sin croaker. Fat content was not significantly different between bovine and fish gelatins. Generally, the gelatin samples extracted were almost free (<0.5%) of fat. The values for ash contents were 0.29%, 1.36% and 1.15%, respectively, for bovine, sin croaker and shortfin scad gelatins, respectively. Ash contents were considerably higher for fish gelatins than for bovine gelatin. However, these values were considered low for fish gelatin. According to Jones (1977), the maximum ash content of gelatin was 2.6%.

3.5. Gel strength

Bloom strength is the most important physical property of a gelatin. The bloom strength of the sin croaker and shortfin scad gelatins were 124.94 and 176.92 g, respectively (Table 3). The bloom strength of bovine gelatin was 239.98 g, which was significantly ($p < 0.05$) higher than that of fish gelatins. This may be due to the higher content of hydroxyproline in bovine gelatin (see Table 5). According to Arnesen and Gildberg (2002), the low hydroxyproline content in fish skin gelatin was a major reason for the low gel strength of these gelatins. It is well established that hydrogen bonds between water molecules and free hydroxyl groups of amino acids in gelatin are essential for gel strength (Arnesen & Gildberg, 2002).

3.6. Differential scanning calorimetry (DSC)

The results from the differential scanning calorimetry studies give an indication of the thermal stability of the triple helix in gelatin (Hickman et al., 2000). The heat flow detected by DSC corresponds to the energy necessary to melt the junction zones and to achieve the helix-to-coil conformation (Michon, Cuvelier, Relkin, & Launay, 1977). The melting temperatures of the gelatin gels were 28.89, 24.57 and 18.51 °C respectively for bovine, shortfin scad and sin croaker gelatins. The melting point of bovine gelatin was significantly ($p < 0.05$) higher than that of the sin croaker and shortfin scad gelatins. These melting points were far higher than those reported for cod skin, which was in the range 8–10 °C (Gudmundsson & Hafsteinsson, 1997). Moreover, Norland (1990) reported that, generally, fish gelatin had a lower melting point than had mammalian gelatin.

Table 3
The melting point and bloom value of fish and bovine gelatins

Properties	Sin croaker	Shortfin scads	Bovine
Melting point (°C)	24.57^{b}	18.51^{c}	28.89^{a}
Bloom value, gel strength (g)	124.94^{c}	176.92^{b}	239.98^{a}

[a–c] Means within a row with same letter are not significantly different ($p < 0.05$).

3.7. Small deformation oscillatory measurement

Table 4 shows the values of storage (G') and loss (G'') moduli during both gelling (from 50 to 5 °C) and subsequent melting (from 5 to 50 °C) after holding for 2 h, at 5 °C, of the fish gelatin and bovine gelatin solutions. The graphs of storage (G') and loss (G'') moduli versus temperature are shown in Fig. 1. During gelling, the difference in gelling temperatures between bovine and shortfin scad gelatin was about 10 °C while, for sin croaker gelatin it was about 12 °C. Bovine gelatin had a higher value of storage modulus (G') than that of fish gelatins. However, after holding for 2 h at 5 °C, the storage moduli (G') of shortfin scad and sin croaker gelatins increased tremendously. This showed that, after cooling and holding for 2 h, the elastic moduli of fish gelatins increased substantially more than that of bovine gelatin. Furthermore, not only for the elastic moduli but the viscous moduli of fish gelatin solution also increased tremendously after the gelatin solution had undergone aging for 2 h. Storage modulus (G') of shortfin scad gelatin increased from 118 to 1690 Pa while that of sin

Table 4
Melting and gelling temperatures and G' and G'' values of gelatins

Gelatin	Gelling temp. (°C)	Modulus (Pa)		Melting temp. (°C)	Modulus (Pa) held at 5 °C for 2 h	
		G'	G''		G'	G''
Sin croaker	7.1	44	3.9	17.7	1270	24
Shortfin scad	9.9	118	3.1	23.8	1690	17.9
Bovine	19.6	2160	15.2	28.8	4200	20

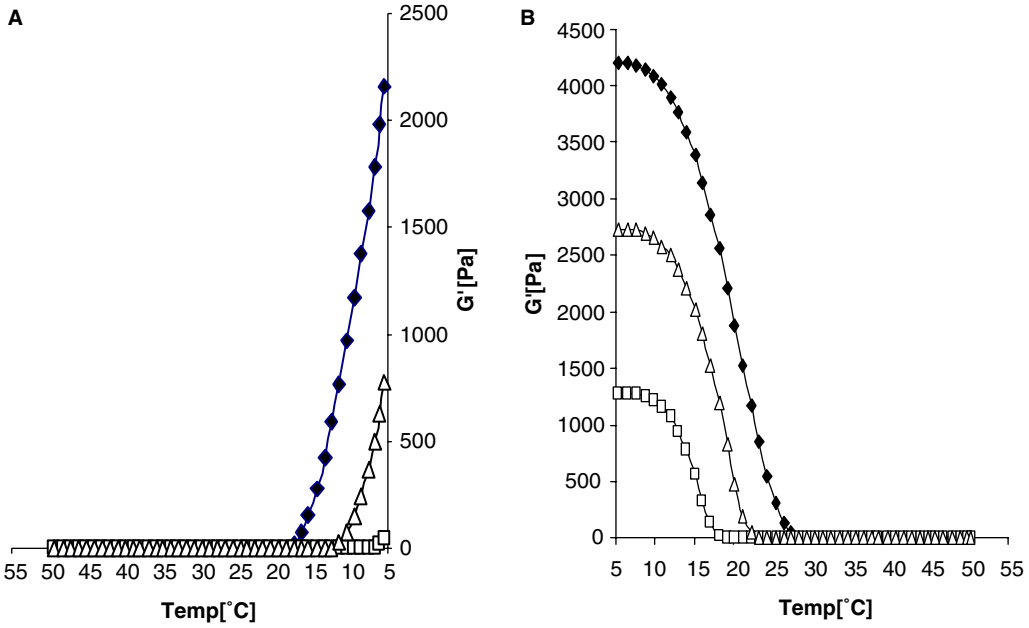

Fig. 1. Viscoelastic properties of gelatin during heating and cooling. (A) Storage modulus during cooling and (B) storage modulus during heating (–◆–◆–◆–◆–◆–, bovine; –Δ–Δ–Δ–Δ–Δ–Δ–, shortfin scad; –□–□–□–□–□–□–, sin croaker).

croaker gelatin increased from 44 to 1270 Pa but G' of bovine gelatin increased only from 2160 to 4200 Pa. In addition, it can be observed that sin croaker gelatin had a higher loss modulus (G'') than bovine gelatin after aging for 2 h.

3.8. Amino acid composition

The amino acid compositions of the two fish gelatins were similar; however, these values were different from that of bovine gelatin, as can be observed in Table 5. Glycine and imino acids (Pro + Hyp) were the most abundant amino acids in bovine gelatin, presenting 30% and 13%, respectively. Sin croaker had 29% glycine and 11% imino acid, whereas shortfin scad had 32% glycine and 10% of imino acid. Johnston-Banks (1990), reported that the imino acids (proline and hydroxyproline) impart considerable rigidity to the collagen structure and a relatively limited imino acid content should result in a less sterically hindered helix and may affect the dynamic properties of gelatin.

Table 5
Amino acid composition of fish and bovine gelatins

Amino acid content in gelatin samples (mg/100 g)			
Amino acid	Shortfin scad	Sin croaker	Bovine
Ala	20.6 ± 0.4	23.9 ± 1.0	23.7 ± 1.03
Arg	4.27 ± 0.13	4.09 ± 0.49	3.49 ± 0.37
Asp	1.81 ± 0.08	2.41 ± 0.17	1.88 ± 0.12
Cys	0.41 ± 0.11	0.69 ± 0.21	0.64 ± 0.41
Glu	3.3 ± 0.15	3.66 ± 0.17	2.86 ± 0.1
Gly	32.2 ± 0.6	29.4 ± 0.5	30.1 ± 0.58
H. Pro	8.09 ± 0.46	7.64 ± 0.51	9.76 ± 0.12
His	1.25 ± 0.04	1.06 ± 0.15	0.82 ± 0.07
Ileu	1.19 ± 0.05	1.19 ± 0.47	1.21± 0.07
Leu	2.59 ± 0.28	2.64 ± 0.77	3.53 ± 0.96
Lys	3.78 ± 0.11	5.29 ± 0.39	5.80 ± 0.2
Met	2.2 ± 0.18	1.98 ± 0.15	1.15 ± 0.1
Phe	2.2 ± 0.19	1.08 ± 0.07	1.08 ± 0.08
Pro	1.88 ± 0.16	4.14 ± 0.12	3.95 ± 0.28
Ser	2.2 ± 0.17	1.22 ± 0.19	0.80 ± 0.1
Thr	3.94 ± 0.24	2.95 ± 0.69	2.52 ± 0.63
Trp	2.48 ± 0.35	2.27 ± 0.19	2.10 ± 0.05
Tyr	2.52 ± 0.22	1.52 ± 0.54	1.46 ± 0.34
Val	3.14 ± 0.72	2.85 ± 0.42	3.11 ± 0.57

4. Conclusions

The yield of gelatin obtained from skins of sin croaker was very much higher than that of shortfin scad. The pH of sin croaker gelatin was the lowest and that of bovine gelatin was the highest. The acidic pH of the gelatin obtained was affected by the washing treatments. There were only slight differences in amino acid composition between sin croaker and shortfin scad. However, shortfin scad gelatin had better physicochemical characteristics than had sin croaker gelatin. The viscoelastic properties of shortfin scad gelatin increased greatly after holding at 5 °C for 2 h. Shortfin scad gelatin is of potential use as an alternative to mammalian gelatin due to its good viscoelastic properties.

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