Isolation and characterization of gelatin from the skins of skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*)

K. Shyni*, G.S. Hema, G. Ninan, S. Mathew, C.G. Joshy, P.T. Lakshmanan

Biochemistry & Nutrition Division, Central Institute of Fisheries Technology, Cochin, India

**Article Info**

**Article history:**
Received 27 January 2013
Accepted 4 December 2013

**Keywords:**
Gelatin
Skipjack tuna
Dog shark
Rohu
Gel strength
Fish gelatin

**Abstract**

Gelatin was extracted from the skins of dog shark (*Scoliodon sorrakowah*), skipjack tuna (*Katsuwonus pelamis*) and rohu (*Labeo rohita*) and their physico-chemical properties were measured. The skins of shark, tuna and rohu yielded 19.7, 17.2 and 11.3% gelatin, respectively. The gel strength of dog shark gelatin (6.67%, 10°C) was found to be higher (206 g) than tuna and rohu skin gelatins (177 g and 124 g, respectively). Similarly, molecular weight, viscosity, melting point, foaming properties, water holding capacity, odour, colour and clarity of dog shark gelatin were in general better than the tuna and rohu skin gelatins. The amino acid analysis showed that hydroxyproline content in dog shark skin gelatin was the highest when compared to tuna and rohu skin gelatins.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Gelatin is a denatured fibrous protein derived from collagen by partial thermal hydrolysis. It is an important functional biopolymer that has broad applications in the food, pharmacy and photography industries (Hao et al., 2009). The source, type of collagen and the processing conditions will influence the properties of the resulting gelatin. Different types of gelatins have varying thermal and rheological properties such as Bloom strength, melting and gelling temperatures. These properties are governed by factors such as chain length or molecular weight distribution, amino acid composition and hydrophobicity, etc. (Gomez-Guillen et al., 2002; Norziah, Al-Hassan, Khairulnizam, Mordi, & Norita, 2009).

The global demand for gelatin has shown an increasing trend in recent years. Recent reports indicate that the annual world production of gelatin is nearly 326,000 tonnes, with pig skin derived gelatin accounting for the highest (44%) output, followed by bovine hides (28%), bovine bones (27%), and other sources (1%) (Ahmad & Benjakul, 2011). Other sources, which include fish gelatin, accounted for around 1.5% of total gelatin production in 2007, but this percentage was double that in 2002, indicating that gelatin production from alternative non-mammalian species had grown in importance (Gomez-Guillen et al., 2009). This may be due to the shortage of the primary raw materials mostly cattle hides, bones and pigskins (Spend Matters, 2012).

Gelatins from land animal sources are preferred over marine sources due to their superior gel strength, melting point and viscosity (Cho, Gu, & Kim, 2005). However, fish gelatin received increasing attention as an alternative to land animal gelatin due to religious constraints and health issues associated with the latter. Both Judaism and Islam forbid the consumption of any pork-related products and non-religiously slaughtered beef, while Hindus refrain from consuming cow-related products (Karim & Bhat, 2009). In addition, gelatin from aquatic sources has been shown to be free of infectious materials such as bovine spongiform encephalopathy (Sadowska, Kolodziejka, & Niecikowska, 2003).

The fish skins and bones contribute almost 30% of the total weight of the fish (Gomez-Guillen et al., 2002). Fish skins are a major by-product of the fisheries and aquaculture industry. The skin yield is highly variable according to species, fish size and processing styles. Conversion of these wastes into value-added products such as gelatin to yield additional income has both economic and waste management benefits for the fish industry (Choi & Regenstein, 2000).

A number of studies have addressed properties of fish skin gelatins (Arnesen & Gildberg, 2007; Choi & Regenstein, 2000; Fernandez-Diaz, Montero, & Gomez-Guillen, 2001; Gomez-Guillen & Montero, 2001; Grossman & Bergman, 1992;...
Dry wt

Studies have ascertained that freshwater gelatin from the skins of skipjack tuna, the marine sh species (Gilsenan & Ross-Murphy, 2000; Gudmundsson & Bergman, 1992; Leuenberger, 1991).

Dog shark and skipjack tuna are available along the south west coast of India. Rohu is one of the major carp species, a natural inhabitant of the freshwater sections of the rivers of India and contributes to the inland catch. At present, the fishery is sustainable for all the above species.

The present study was undertaken to extract and characterize gelatin from the skins of skipjack tuna (Katsuwnous pelamis) from the family scombridae, dog shark (Sciliodon sorrakowah) from the family of carcharhinidae, a cartilaginous fish, and rohu (Labeo rohita) from the family of cyprinidae, a freshwater fish.

2. Materials and methods

2.1. Raw material

The species used for the study were skipjack tuna (K. pelamis), dog shark (S. sorrakowah), and rohu (L. rohita). The marine fishes were landed from long-line day boats that iced the fish on board, at the local fish landing centre in Cochin during May 2011, from the Arabian sea. The length of the fishes were measured and recorded, i.e., tuna (92 ± 2.4 cm, 17 in number) shark (73 ± 2.9 cm, 23 in number). The skinning of the fish was carried out by the fisherman at the market under supervision. The iced skin in prime quality was subjected to a preliminary extraction trial, and then skinning was carried out at laboratory in the iced condition.

The skins were cut into 2–3 cm² pieces using a scalpel and washed with ice cold tap water. They were then placed in polyethylene bag with added glaze water (10%) and stored at −20 °C until use. The storage time was less than 2 months. All chemicals used unless otherwise noted were of analytical grade (Merck KGa Chemical & Pharmaceutical Company, Darmstadt, Germany; Sigma–Aldrich Corporation, MO, USA).

2.2. Gelatin extraction

Based on preliminary extraction trials, it was decided to follow the gelatin extraction method of Gudmundsson and Hafsteinsson (1997). Thawed skins were thoroughly washed and treated with warm water (38–40 °C) for 10 min to remove superfluous material and reduce the fat content. Before gelatin extraction, skins were soaked in 0.1 M NaOH at ambient temperature (∼27 °C) with a skin/solution ratio of 1:10 (w/v) for 2 h. The alkaline solution was changed every 1 h to remove non-collagenous proteins and pigments. Alkaline-treated skins were washed with tap water until the wash water was neutral or faintly basic. The pH of wash water was monitored using Cyberscan 510 pH meter (Eutech Instruments Pte Ltd, Singapore). The skins were then soaked in 0.2 M acetic acid with a skin/solution ratio of 1:10 (w/v) for 24 h with gentle stirring at 4 °C. The acidic solution was changed every 12 h to swell the collagenous material in the fish skin matrix. Acid-pretreated skins were washed thoroughly with tap water until the wash water became neutral. 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 45 °C for 12 h with a skin/water ratio of 1:10 (w/v). The clear extract obtained was filtered through a Buchner funnel with Whatman filter paper No. 4 (Sunshine Instruments, Coimbatore, Tamil Nadu, India). Fat separation was done using a simple fat separating funnel (India-MART, Maharashtra, India). The extract was poured in to the funnel and was allowed to settle for few seconds. Within few seconds, suspended fat formed a layer over the gelatin extract. Then the gelatin was allowed to flow slowly through the outlet valve of the funnel. The valve was carefully closed on reaching the level of suspended fat layer. The clear extract obtained was concentrated by evaporation under vacuum at 5 °C, with a flash evaporator (Buchi rotavapor R215, Buchi Labortechnik, Flawil, Switzerland). The concentrated viscous solution was frozen in an air blast freezer (Icematic T10, Castelman SpA, Castelfranco Veneto (TV), Italy) at −40 °C and then freeze-dried with freeze drier (Gamma 10, Osterode am Harz, Germany) in two steps. The solution was subjected to main drying for 8 h at set shelf temperature of 20 °C and set pressure of 0.01 mbar. The final drying was done for 2 h at set shelf temperature of 25 °C and set pressure of 0.01 mbar at a condenser temperature of −55 °C.

2.3. Yield of gelatin

The yields of the gelatins obtained were calculated as:

\[
\text{% Yield (wet wt. basis)} = \frac{\text{Dry wt. of gelatin}}{\text{Wet wt. of skins}} \times 100
\]

2.4. Determination of proximate composition

Moisture, lipid, ash and protein were determined by AOAC (1995) methods 950.46, 960.39, 900.2A and 928.08, respectively. Protein digestion was done as described by Eastoe and Eastoe (1952) to ensure complete hydrolysis of collagen. A conversion factor of 5.4 was used for calculating the protein content from the Kjeldahl nitrogen content since collagen, the main protein in skin, contains approximately 18.7% nitrogen (Eastoe & Eastoe, 1952). This is an estimate and is based on mammalian gelatin.

2.5. Determination of pH

The pH values of raw fish skins and gelatin solutions were measured using the British Standard Institution method (BSI, 1975). For determining the pH of the skins, samples were chopped and blended for 5 min at ambient temperature (37 °C) by vigorous shaking (ICS-BLENDER, Hyderabad, Andhra Pradesh, India) in distilled water to form a 1% (w/v) skin suspension. For the gelatin solution, a 1% (w/v) gelatin solution was prepared by adding 1 g of gelatin in 99 ml of distilled water. The mixture was heated to 45 °C
for 5 min, for dissolving gelatin. The solution was allowed cool down to room temperature before pH measurement. The pH was measured using a Cyberscan 510 pH meter (Eutech Instruments Pte Ltd, Singapore).

2.6. Gelatin colour and gel clarity

Instrumental colour measurements of the dry gelatin samples were made using a HunterLab MiniScan XE Plus Spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) based on three colour co-ordinates, namely L* (lightness), a* (redness/greenness) and b* (yellowness/blueness). The equipment was standardized using a white tile and black glass. The gelatin sample was filled into the glass sample cup and the cup was fed into the instrument. Instrumental colour measurement readings were taken from the instrument monitor. Visual observations for colour were also noted.

Clarity was determined by measuring transmittance (%T) at 620 nm in spectrophotometer (Spectronic Instruments Inc., Rochester, N.Y., USA) through 6.67% (w/v) gelatin solutions which were heated at 60 °C (Julabo TW20 water bath, Allentown, PA, USA) for 1 h (Avena-Bustillos et al., 2006).

2.7. Determination of odour

Sensory evaluation was conducted using a seven member expert panel according to the method of Muyonga et al. (2004a). The samples were prepared in test tubes with screw caps. by dissolving 0.5 g of gelatin in 7 ml of distilled water, thus obtaining a solution containing approximately 6.67% gelatin. The tubes were then held in a water bath at 50 °C containing approximately 6.67% gelatin. The tubes were then held and left to stand at room temperature for 15 min at room temperature and then placed in a water bath (Julabo TW20, Allentown, PA, USA) for 1 h. (Avena-Bustillos et al., 2006).

2.8. Determination of viscosity

The viscosity of the gelatin (6.67% concentration at 60 °C) was measured using a Brookfield digital viscometer (model DV-E, Brookfield Engineering, Middleboro, MA, USA) equipped with a No. 1 spindle at 30 ± 0.5 °C. The measured values were obtained directly in centipoises (cp) from the instrument.

2.9. Determination of gel strength

The gel strength (Bloom) was determined according to British Standard 757:1975 method (BSI, 1975), by using a texture analyzer (Lloyd Instruments, Model LRX Plus, Sussex, U.K.). A solution containing 6.67% (w/v) gelatin was prepared by mixing 7.50 g of gelatin and 105 ml of distilled water in a Bloom bottle (Schott Duran, Mainz, Rhineland-Palatinate, Germany). The mixture was swirled and left to stand at room temperature for 1 h, for allowing the gelatin to absorb water and swell. The Bloom bottles were then transferred to a water bath (Julabo TW20, Allentown, PA, USA) maintained at 65 °C and held for 25 min with occasional swirling to dissolve the gelatin. The bottles were taken out of the water bath, allowed to cool for 15 min at room temperature and then placed in a cold-water bath (circulating bath, Haake D3 Model, Berlin, Germany) maintained at 10 ± 0.1 °C and held at this temperature for 16–18 h before the determination of the gel strength. The Bloom bottle was placed centrally under the plunger (Delrin probe, which is an acetal homopolymer, 12.7 mm diameter, black in colour) of the instrument. The Bloom strength was determined with a load cell of 5 kg and crosshead speed of 0.5 mm/s. The maximum force (g) was determined when the probe penetrated to a depth of 4 mm into the gel.

The test determines the weight (in grams) needed by a probe (normally with a diameter of 0.5 inches) to deflect the surface of the gel 4 mm without breaking it. The result is expressed as Bloom (grams). Gelatin, if it gels, is usually between 30 and 300 Bloom.

2.10. Determination of amino acid composition

A dry sample (100 mg) of gelatin was weighed and taken in a test tube. 10 ml 6 N HCl was added to in to the test tube. Then the tubes were filled with nitrogen, sealed and hydrolyzed at 110 °C for 24 h. After the hydrolysis, the contents were quantitatively transferred into a round bottom flask through Whatman filter paper. No. 42. The contents were flash evaporated to dryness. Distilled water was added and flash evaporated again to remove traces of HCl. The process was repeated thrice. The residue was then dissolved and made up to 5–10 ml (known volume) with 0.05 M HCl. The sample was then filtered through a membrane filter (Thermo Scientific, MK, Buckinghamshire, England) with a nominal pore size of 0.45 μm. The sample (20 μl) was injected into the amino acid analyzer (HPLC–LC 10 AS, Hichichi L-2130 Elite La Chrome, Kyoto, Japan) and the amino acid composition was determined as per the method of Ishida, Pugita, and Asai (1981). The system consists of a binary pump (L-7100), autosampler (L-2200), column oven (L-2350), fluorescent (FL) detector (L-2485) and a post-column derivatization unit fitted with a peristaltic pump. The column used is a cation exchange column (Shodex, CX Pak, 4.6 × 15 mm) connected with a guard column. The eluents used were buffer A (13.3 g tri-sodium citrate, 70 ml ethanol, 12.8 ml citric acid monohydrate, 3.74 g NaCl, 4 ml Brij (Sigma–Aldrich Corporation, MO, USA), pH 3.2 made up to 1 l with distilled water) and buffer B (117.6 g trisodium citrate and 24.8 g boric acid, 500 ml distilled water, 45 ml 4 N NaOH, pH to 10 and make up to 2 l with distilled water). The separation is effected using a gradient programme (0 min–100% A, 30 min–50% B). The eluted amino acids are subjected to post-column derivatization with ortho-phthaldialdehyde (OPA) reagent delivered using a peristaltic pump at the rate of 0.5 ml/min at 30 °C. Detection was done using a FL detector with absorption wave length of 340 nm and emission wave length of 450 nm. Amino acid standards for collagen hydrolyzate (Sigma A-9531) were run to calculate the concentration of amino acids in the sample. Base line auto-zero was made through software D-2000 Elite, Hitachi. The method showed a linear response (R² = 1.00) in this range. The identification of amino acids in the sample was done using D-2000 Chromatography Data Station Software. Trypotphan analysis was carried out according to colorimetric method (Sastry & Tammuru, 1985).

2.11. Determination of molecular weight distribution

Electrophoretic patterns of the different gelatins were analyzed according to the method of Laemmli (1970). The samples were dissolved in 50 g/l SDS solution. The mixtures were then heated at 85 °C for 1 h, followed by centrifugation at 8500 × g for 5 min at room temperature using a microcentrifuge (MIK-RO20, Hettich Zentrifugen, Germany) to remove undissolved debris if any. Solubilized samples were mixed (1:1 v/v) with the sample buffer (0.5 mol/l Tris–HCl, pH 6.8 containing 40 g/l SDS, 200 ml/l glycerol in the presence or absence of 100 ml/l β mercaptoethanol). The mixtures were loaded onto a polyacrylamide gel having a 7.5% resolving gel (10% SDS: 100 μl, acrylamide: 2.5 ml, ammonium persulphate (APS) 10%: 50 μl, distilled water: 4.85 ml, TEMED: 5 μl, Tris–HCl, 1:5 M: 2.5 ml) and a 4% stacking gel (10% SDS: 100 μl,
acrylamide: 1.33 ml, APS 10%: 50 μl, distilled water: 6.1 ml, tetramethylethylenediamine (TEMED): 10 μl, Tris–HCl, 1.5 M: 2.5 ml). The electrophoresis was done till the base line reached bottom of the gel, at a constant current of 20 mA per gel using a Bio-Rad Tetra Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA) with gel documentation system. After electrophoresis, gels were fixed with a mixture of 500 ml/l methanol and 100 ml/l acetic acid for 30 min, followed by staining with 0.5 ml/l Coomassie blue R-250 (ultra-pure grade) in 150 ml/l methanol and 50 ml/l acetic acid for 1 h. Finally, they were destained with a mixture of 300 ml/l methanol and 100 ml/l acetic acid for 1 h and destained again with the same solution for 30 min. High molecular weight protein marker (Sigma Chemical Co., St. Louis, MO, USA) with a molecular weight range of 36,000–205,000 Da was used to estimate the molecular weight of proteins. The molecular weight distribution of sigma marker (Da) used in the study is

- Myosin, rabbit muscle — 205,000
- β-Galactosidase, Escherichia coli — 116,000
- Phosphorylase b, rabbit muscle — 97,000
- Fructose-6-phosphate Kinase, rabbit muscle — 84,000
- Albumin, bovine serum — 66,000
- Glutamic dehydrogenase, bovine liver — 55,000
- Ovalbumin, chicken egg — 45,000
- Glyceraldehyde-3-phosphate dehydrogenase, rabbit muscle — 36,000

2.12. Determination of melting point of gelatin

The melting point measurement was done according to the method of Wainewright (1977). Gelatin solutions, 10% (w/w), were prepared and 5 ml aliquot of each sample was transferred to a small culture tube (12 × 75 mm). The samples were degassed in vacuum desiccators (Heraeus vacuum, Hohenpriesnitz, Sachsen, Germany) for 5 min. The tubes were then covered with Parafilm (Pechiney Plastic Packaging, Inc., Chicago, IL, USA) and heated in a water bath at 60°C for 15 min. The tubes were immediately cooled in ice-chilled water and matured at 0°C for 18 h. Five drops of a mixture of 75% chloroform and 25% red food colour (Magnil Dye Chem, Mumbai, Maharashtra, India) was placed on the surface of the gel. The gels were put in a water bath (Haake D3, Lab Instruments, Maharashtra, India) for 5 min. The tubes were then covered with Parafilm and vacuum desiccators (Heraeus vacuum, Hohenpriesnitz, Sachsen, Germany) for 5 min. The gelatin sample was taken as the melting point.

2.13. Determination of setting point and setting time

The method used for the determination of setting point (SP) and setting time (ST) of gelatin was that described by Muyonga et al. (2004a). Gelatin solutions of 10% (w/v) dissolved in thin wall culture tube (12 × 75 mm) test tubes were prepared in the same way as described for the Bloom samples. For setting point determination, the dissolved samples from the warm-water bath were transferred to a circulating water bath held at 40°C (Haake D3). The bath was then cooled at the rate of 2°C/min. A thermometer was inserted into the sample and lifted out at 15 s intervals. The temperature of the mixture at which the gelatin solution no longer dripped from the tip of the thermometer was recorded as the setting temperature.

Setting time was determined on samples prepared in the same way as those for the determination of the setting temperature. Samples were transferred to the water bath maintained at 10°C (Haake D3). A rod was inserted into the gelatin solution and raised at intervals of 15 s. The time at which the rod could not detach from the gelatin sample was recorded as the setting time.

2.14. Determination of foaming properties

Foam formation ability (FA) and foam stability (FS) of gelatin were determined by the procedure of Cho et al. (2004). Gelatin solution, 1 g/100 ml was put in a beaker and swelled at 60°C. The foam was prepared by homogenizing at 10,000 rpm for 5 min in a homogenizer (Euro Turrax T20b, IKA Labortechnik, Staufen, Germany). The homogenized solution was then poured into a 250 ml measuring cylinder. The FA was calculated as the ratio of volume of foam to the initial volume of liquid. The foam stability was calculated as the ratio of the initial volume of foam to the final volume of foam after 30 min.

2.15. Determination of water holding capacity and fat binding capacity

Fat binding capacity (FBC) and water holding capacity (WHC) of gelatin were determined as per the procedure of Cho et al. (2004). For measuring FBC, 1 g of gelatin powder was placed in a centrifuge tube and weighed (tube with gelatin). Then, 10 ml sunflower oil was added, and held at room temperature for 1 h. During this period, the gelatin solutions were mixed with a Vortex mixer (CM-101 Plus, REMI Instruments, Maharashtra, India) for 5 s every 15 min. The gelatin solutions were then centrifuged at 450 × g (Model CPR 24, REMI Instruments, Maharashtra, India) for 20 min with cylinder bottom centrifuge of 20 ml capacity (REMI Instruments, Maharashtra, India). The upper phase was removed by tilting the centrifuge tube to 45° angle and draining on to a filter paper for 30 min. The FBC was calculated as the weight of the contents of the tube after draining divided by the weight of the dried gelatin, and expressed as the weight % of dried gelatin.

For measuring WHC, 1 g of gelatin powder was placed in a centrifuge tube and weighed (tube with gelatin). Distilled water (50 ml) was added, and held at room temperature for 1 h. During this period, the gelatin solutions were mixed with a Vortex mixer (CM-101 Plus, REMI Instruments, Maharashtra, India) for 5 s every 15 min. The gelatin solutions were then centrifuged at 450 × g (Herieux Multifuge 3SR Plus, Thermo Scientific, MK, Buckinghamshire, England) for 20 min with 600 ml bucket centrifuge (Thermo Scientific, MK, Buckinghamshire, England). The upper phase was removed and the centrifuge tube was drained for 30 min on a filter paper after tilting to 45° angle. WHC was calculated as the weight of the contents of the tube after draining divided by the weight of the dried gelatin, and expressed as the weight % of dried gelatin.

2.16. Statistical data analysis

One way analysis of variance was used to find the effect of different species on yield and functional properties of gelatin. Once ANOVA was found significant, Tukey’s test was used as a post-hoc test to compare the means of different species. The statistical analysis was carried out using SAS 9.3 software (SAS Institute Inc, Cary, NC, USA). The mean value of three replications and its standard deviations are shown in the tables. Letters are placed as a superscript along with the mean to indicate significant difference between the means in all the tables except the amino acid table, i.e., means with different superscripts are statistically significant at 1% level of significance (p < 0.01).
3. Result and discussion

3.1. Proximate composition of fish skins

The protein, moisture and ash content values of three selected fish skins after cleaning, are tabulated in Table 2. All three fish skins had considerably higher ash contents (2.03–4.39%) compared to that of the Red tilapia (0.51%), Walking catfish (0.52%) and Striped catfish (0.46%) (Jamilah, Tan, Umi Hartina, & Azizah, 2011). Compared to the other two species, ash content in rohu skins was lower, probably due to perfect de-scaling. De-scaling was not carried out for shark and tuna skins since the scales were too tiny for tuna and was deeply embedded in the shark skin. Shark skins contained comparatively higher amount of protein (27.7%) than other two species. The skins of rohu had highest moisture content, followed by shark and tuna skins. Subcutaneous fat was highest (18.3%) for rohu skins when compared with shark and rohu skins. This can be explained as the site of lipid storage in shark is liver, whereas it is skin and viscera in tuna. The results of lipid can be correlated with the moisture content in reverse order in the above species.

3.2. Yield of gelatin

Gelatin yield is calculated as g of gelatin per 100 g of clean skins. The yield of shark skin gelatin (SSG), tuna skin gelatin (TSG) and rohu skin gelatin (RSG) were significantly different (p < 0.01) and were 19.7 ± 0.04%, 11.3 ± 0.03% and 17.2 ± 0.03%, respectively. It was observed that shark skins tend to swell more in the alkaline and acidic solutions compared to the skins of rohu and tuna. Therefore, shark skins gave a better yield: possibly due to increased opening of cross-links during swelling. As a cartilaginous fish, shark has comparatively more connective tissue and hence more collagenous material than bony fishes. This can be correlated with the high yield of gelatin from shark skins.

Gelatin processing has three steps, alkali pretreatment, acid pretreatment and hot water extraction. The alkali & acid treatment removes non-collagenous protein and the sample swells in the acid solution. The hot water extraction uses thermohydrolysis to solubilize gelatin which is then separated. The lower yield observed in rohu could be due to the leaching of collagen during the washing treatment or insufficient denaturation of collagen during extraction (Jamilah & Harvinder, 2002). The crude protein content and hydroxyproline content were also lower in rohu skins.

3.3. Proximate composition of gelatin

The proximate compositions of extracted gelatins are tabulated in Table 1. The gelatin extracted from skins of the three species showed protein as the major component and protein content varied between 88.4 and 90.1%. Jongjareonrak, Benjakul, Visessanguan, Prodpran, et al. (2006) reported a protein content of 87.9% and 88.6% in gelatin extracted from the skins of bigeye snapper and brown eye snapper, respectively. The gelatin from the skins of adult Nile perch also contained 88% protein when extracted at 50 °C (Muyonga et al., 2004a). Moisture content in all the samples was well below the prescribed limit of 15% (GME, 2005) for edible gelatin. At 6–8% moisture, gelatin is very hygroscopic and it becomes difficult to determine the physico-chemical attributes with accuracy (Cole, 2000). The gelatin samples extracted were almost free of fat. This showed that the simple de-fattening method followed here had eliminated the fat content as desired. The gelatins were found to be low in ash content, well below the recommended maximum of 2.6% (Jones, 1977).

3.4. The pH values of gelatin samples

The pH of extracted gelatins varied between 4.17 and 4.34 as shown in Table 1, indicating their category as Type B. It has been reported that alkali pretreatment results in Type B gelatin with pH in the range of 4–5 (Baziwane & He, 2003) and in the present study an alkali pretreatment was employed during the extraction of gelatin. Wide variations in the pH of skins gelatin of cod (2.7–3.9) (Gudmundsson & Hafsteinsson, 1997), red tilapia (3.05) and black tilapia (3.91) (Jamilah & Harvinder, 2002), freshwater carps (4.01–4.88) (Ninan, Abubacker, & Jose, 2011; Ninan, Jose, & Abubacker, 2011) and bigeye snapper (6.44) (Binsi, Shamasundar, Dilee, Badii, & Howell, 2009) have been reported. This was, most likely due to the different pretreatments employed during the extraction involving both alkaline and acid treatments.

It has also been reported that for Type B gelatin, the viscosity is minimum and gel strength is maximum at pH 5.0 (Cole, 2000) signifying the importance of pH for gelatin’s rheological properties. Hence from the manufacturer’s point of view it is advantageous to manufacture gelatin with a pH of 5.0. However, due to the strong buffering capacity of gelatin, this pH may not be the most advantageous for the customer.

3.5. Gelatin colour and gel clarity

Both colour and clarity of a gelatin gel are important aesthetic properties, depending on the application for which the gelatin is intended. Instrumental colour measurements of gelatins are tabulated in Table 2. The colour of gelatin depends on the raw materials used and method of extraction (Ockerman & Hansen, 1999). In general, colour does not influence the functional properties. However light colour is preferred because it is easier to incorporate gelatins into any food systems without imparting any strong colour attribute to the product. According to the instrumental colour measurement, shark skins gelatin was significantly lighter/whiter than the other two samples, Rohu skins gelatin was the greenest and it was significantly (p < 0.01) less yellowish when compared to shark and tuna gelatins. By visual observation, shark and tuna skins have a small yellowish hue at the upper surface whereas the skins extracted from tuna and shark are deep red-brown. Gelatins from rohu have a reddish-brown hue and it is intermediate in colour between the two extremes. Gelatins from shark and tuna have a slightly sheen on the surface, whereas the gelatins from rohu are dull in appearance.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Components and pH</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shark</td>
<td>68.4 ± 0.43</td>
<td>27.7 ± 0.36</td>
<td>0.16 ± 0.02</td>
<td>4.19 ± 0.03</td>
<td>6.23 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Rohu</td>
<td>76.5 ± 0.45</td>
<td>18.8 ± 0.06</td>
<td>2.93 ± 0.05</td>
<td>2.03 ± 0.04</td>
<td>6.37 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Tuna</td>
<td>56.5 ± 0.09</td>
<td>20.5 ± 0.26</td>
<td>18.3 ± 0.11</td>
<td>4.39 ± 0.03</td>
<td>6.31 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shark</td>
<td>8.7 ± 0.25b</td>
<td>90.1 ± 0.30b</td>
<td>0.42 ± 0.05b</td>
<td>0.72 ± 0.06b</td>
<td>4.34 ± 0.01b</td>
<td></td>
</tr>
<tr>
<td>Rohu</td>
<td>9.3 ± 0.36b</td>
<td>89.2 ± 0.49b</td>
<td>0.48 ± 0.08b</td>
<td>0.71 ± 0.07b</td>
<td>4.17 ± 0.01b</td>
<td></td>
</tr>
<tr>
<td>Tuna</td>
<td>10.9 ± 0.24b</td>
<td>88.4 ± 0.12b</td>
<td>0.82 ± 0.05a</td>
<td>0.68 ± 0.06b</td>
<td>4.29 ± 0.01b</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation of triplicate. Values with the same superscript letters within a column are not significantly different (p < 0.01).
gelatin has been observed in the pH range of 6–8 (Stainsby, 1987). The above results thus indicate that natural variations in the viscosity can be expected from different fish species, although the extraction methods do play a role.

3.8. Gel strength values of gelatin samples

Gel strength (Bloom) is the most important physical property of a gelatin. The gel strengths of shark, rohu and tuna gelatins were 206,124 and 177 g, respectively. All three samples were significantly (p < 0.01) different (Table 3). The gel strength of fish gelatin has been reported in a wide range of 124–426 Bloom, compared to 200–300 Bloom for bovine or porcine gelatin (Karim & Bhat, 2009). This may be due to many factors such as pH, molecular weight distribution and amino acid content. Hydroxyproline contents of shark, rohu and tuna gelatins were about 9.85%, 6.78% and 7.86%, of the total amino acids (Table 4), while that of bovine gelatin was 14% (Nalinanon, Benjakul, Vissessanguan, & Kishimura, 2008). The gel-like properties of gelatin are influenced by the source of raw materials, which vary in protein and hydroxyproline contents (Jongjareonrak, Benjakul, Vissessanguan, & Prodpran, et al., 2006). The main difference between fish and mammalian gelatins is the imino acid content, where the mammalian gelatins have higher amounts (Gudmundsson, 2002). Imino acids played a role in gel formation. The hydroxyl groups of hydroxyproline play a part in the stability of the helix by inter-chain hydrogen bonding via a bridging water molecule as well as direct hydrogen bonding to the carbonyl group (Wong, 1989). Gelatin, with the shorter chain lengths cannot form as strong a gel due to the lower inter-junction zones (Intarasirisawat et al., 2007). The quality of gelatin is generally determined by the gel strength or Bloom value, including low (<150), medium (150–220) and high Bloom (220–300) (Johnston-Bank, 1983). Gudmundsson and Hafsteinsson (1997) suggested that the gel strength may depend on the isoelectric point and may be controlled, to certain extent, by adjusting the pH.

3.9. Amino acid composition

The amino acid composition of gelatin extracted from the skins of shark, rohu and tuna are shown in Table 4. It is expressed as g of gelatin.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Instrumental colour, visual observation and transmittance of shark skin gelatin (SSG), rohu skin gelatin (RSG) and tuna skin gelatin (TSG).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>L'</td>
</tr>
<tr>
<td>---------</td>
<td>--------------</td>
</tr>
<tr>
<td>SSG</td>
<td>82.8 ± 0.40a</td>
</tr>
<tr>
<td>RSG</td>
<td>78.1 ± 0.62b</td>
</tr>
<tr>
<td>TSG</td>
<td>75.3 ± 0.60b</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation of triplicate. Values with the same superscript letters within a column are not significantly different (p > 0.01).

gelatins appeared pearly white while rohu gelatin was shiny whitish.

While shark and rohu gelatins showed good transmittance (%T), tuna gelatin showed significantly (p < 0.01) poor %T values (Table 3). The turbidity and dark colour of gelatin is commonly caused by inorganic, proteinaceous and mucosubstance contaminants introduced or not removed during its extraction (Eastoe & Leach, 1977). Muonyga et al. (2004a) stated that the efficiency of the filtration process during gelatin extraction affected the degree of clarity of gelatin solution.

3.6. Odour of gelatin samples

Sensory evaluation showed a difference in odour between gelatins. The shark and rohu gelatins were found to be free of fishy odour and had a mild putrid odour (hedonic score of 1.83 ± 0.29 and had a mild putrid odour (hedonic score of 1.83 ± 0.29 and 2.17 ± 0.29, respectively), whereas the tuna gelatin had a detectable fishy odour with a hedonic score of 3 which was significantly (p < 0.01) higher than shark and rohu gelatins. Muonyga et al. (2004a) reported that the gelatins prepared from the skins and bones of Nile perch by activated carbon treatment were found to have a mild putrid odour. Strong fishy odour was reported for freeze-dried gelatin prepared from the skins of black tilapia (Jamilah & Harvinder, 2002), Choi and Regenstein (2000) observed that activated carbon treatment at the final stage of extraction can further reduce the odour and improve the acceptability of gelatin.

3.7. Viscosity values of gelatin samples

Viscosity is the second most important commercial property of gelatin after gel strength (Ward & Courts, 1977). The viscosity for the samples was in the range of 2.5–5.6 cP and all the samples were significantly (p < 0.01) different. The viscosity was lowest in rohu gelatin compared to shark and tuna gelatins (Table 3). Viscosity is partially controlled by molecular weight and molecular size distribution (Sperring, 1985). The viscosities of most of the commercial gelatins have been reported to be up to 13.0 cP (Johnston-Banks, 1990). Jamilah and Harvinder (2002) reported the viscosity values of 3.2 cP and 7.12 cP for red and black tilapia, respectively, whereas for channel catfish, it was 3.23 cP (Yang et al., 2007). The changes in pH are known to influence the viscosity and minimum viscosity for

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Viscosity and Bloom values of shark skin gelatin (SSG), rohu skin gelatin (RSG) and tuna skin gelatin (TSG).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Viscosity</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>SSG</td>
<td>5.60 ± 0.10a</td>
</tr>
<tr>
<td>RSG</td>
<td>2.50 ± 0.00b</td>
</tr>
<tr>
<td>TSG</td>
<td>4.37 ± 0.06b</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation of triplicate. Values with the same superscript letters within a column are not significantly different (p < 0.01).

Table 4 Amino acid composition of shark skin gelatin (SSG), rohu skin gelatin (RSG) and tuna skin gelatin (TSG).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues/100 residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSG</td>
<td>RSG</td>
</tr>
<tr>
<td>Aspartic</td>
<td>3.64 ± 0.09</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.08 ± 0.19</td>
</tr>
<tr>
<td>Glutamic</td>
<td>7.69 ± 0.27</td>
</tr>
<tr>
<td>Glycine</td>
<td>32.8 ± 0.62</td>
</tr>
<tr>
<td>Alanine</td>
<td>10.9 ± 0.30</td>
</tr>
<tr>
<td>Cystine</td>
<td>0</td>
</tr>
<tr>
<td>Valine</td>
<td>2.52 ± 0.24</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.07 ± 0.15</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.58 ± 0.05</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.17 ± 0.17</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.58 ± 0.10</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.77 ± 0.13</td>
</tr>
<tr>
<td>Hydroxylysine</td>
<td>0.73 ± 0.10</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.86 ± 0.07</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.29 ± 0.09</td>
</tr>
<tr>
<td>Proline</td>
<td>9.90 ± 0.11</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>9.85 ± 0.33</td>
</tr>
<tr>
<td>Serine</td>
<td>3.61 ± 0.11</td>
</tr>
<tr>
<td>Total</td>
<td>99.1 ± 0.74</td>
</tr>
</tbody>
</table>
aminos acids per 100 g of solids. All gelatins had glycine as their major amino acid (32.8–33.4) and had relatively high content of alanine (10.9–12.2). For imino acids, all samples had proline and hydroxyproline content of 9.90–11.6 and 6.78–9.85, respectively. It was observed that the level of tryptophan was below the detectable limit in all three samples. The preparation of amino acid monomers from gelatin through the acid hydrolysis process leads to the destruction of indole ring of tryptophan. Hence the detection of tryptophan is not possible in HPLC. The absence can also be resulted from acid hydrolysis during the gelatin extraction process (Chapman & Hall, 1997; Jamilah & Harvinder, 2002). Cysteine and tryptophan are not commonly present in collagen and gelatin (Foegeding, Lanier, & Hultin, 1996; Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006; Muyonga, Cole, & Duodu, 2004b; Yata, Yoshida, Fujisawa, Mizuta, & Yoshinaka, 2001). Regenstein and Zhou (2007) reported that glycine, alanine, proline and hydroxyproline are four of the most abundant amino acids in gelatin.

The samples had an imino acid content of 17.96–19.75% of protein. Kasanka, Xue, Weilong, Hong, and He (2007) reported an imino acid content of 19.47% for gelatin prepared from the skin of carp. Imino acid content of Nile perch gelatin was 21.5% (Muyonga, 2004a). Grossman and Bergman (1992) reported an imino acid content of 17% and 5%, for gelatin from cod and tilapia, respectively. Mammalian gelatins generally contain 30% imino acids (Poppe, 1992). Johnston-Banks (1990), reported that the imino acids, especially hydroxyproline, impart considerable rigidity to the collagen structure and a relatively limited imino acid content might affect the dynamic properties of gelatin. Maximum gel strength was observed for shark skin gelatin which shows that hydroxyproline is the major determinant of stability due to its hydrogen bonding ability through its hydroxyl group, although proline is also important (Burjandze, 1979; Ledward, 1986).

3.10. Molecular weight distribution

The gelatin preparations from the skins of three species were analyzed using SDS-PAGE, to characterize differences in molecular weight distribution (Plate I). For gelatin gels α1 and α2 chains were found as the major components for all the samples. However, β component (covalently linked α chain dimer) with molecular weight ~ 200 kDa was observed in shark gelatin and it was absent in the other two samples. The presences of peptides with molecular weight below 100 kDa were slightly noticeable in all three samples. That may be the results of heat induced cleavages of protein chains that occurred during the extraction process (Muyonga et al., 2004a). The result suggested that β chains of shark skin gelatin were more tolerant to thermal hydrolysis than those of tuna and rohu skin gelatin.

3.11. Melting point of gelatin

The melting point of shark and tuna gelatins was significantly higher (p < 0.01) than that of rohu gelatin (Table 5). This can be correlated with the higher hydroxyproline content in these gelatins. Melting point increases with the maturation time and it has been observed that the levels of hydroxyproline contribute to the melting point characteristics (Choi & Regenstein, 2000; Gudmundsson, 2002). It is known that fish gelatins have lower melting points than mammalian gelatins. Norland (1990) and Gudmundsson (2002) had observed melting points of 29.7 °C and 32.3 °C, for bovine and porcine gelatin, respectively. The melting points observed in the present study are far higher than those reported for cold-water fishes such as cod (13.8 °C), hake (14 °C) (Gomez-Guillen et al., 2002) and hoki (16.6 °C) (Mohr, Perera, & Quek, 2010). However, these melting points were lower than that of black tilapia (28.9 °C) (Jamilah & Harvinder, 2002) which is a warm-water fish. Fish gelatins with lower melting temperatures had a better release of aroma and offered stronger flavour and useful in product development to control the texture and flavour release during mastication (Zhou, Steven, & Regenstein, 2006).

3.12. Setting point and setting time of gelatin

Setting point denotes the temperature at which setting/gelling process begins, which involves the transition from random coil to triple helical structure of gelatin (Haug, Draget, & Smidsrod, 2004). Setting points of shark and tuna gelatins were significantly higher (p < 0.01) than that of rohu gelatin (Table 5). Bovine and porcine gelatins have considerably higher setting and melting points than most fish gelatins (Choi & Regenstein, 2000; Gilsen & Ross-Murphy, 2000; Gudmundsson, 2002; Leuenberger, 1991). Setting points of cold-water fish gelatin was in general lower than warm-water fish/mammalian gelatins. This difference is related to the imino acid composition. Setting and melting temperatures are also influenced by the change in ionic strength and pH of gelatin. They decreased with the increase in ionic strength of >0.5 mol/L, which

<table>
<thead>
<tr>
<th>Sample</th>
<th>MP (°C)</th>
<th>SP (°C)</th>
<th>ST (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSG</td>
<td>25.8 ± 0.29°C</td>
<td>20.8 ± 0.29°C</td>
<td>93.3 ± 1.15 s</td>
</tr>
<tr>
<td>RSG</td>
<td>18.2 ± 0.29°C</td>
<td>13.8 ± 0.29°C</td>
<td>14.7 ± 1.53 s</td>
</tr>
<tr>
<td>TSG</td>
<td>24.2 ± 0.29°C</td>
<td>18.7 ± 0.29°C</td>
<td>103 ± 1.15 s</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation of triplicate. Values with the same superscript letters within a column are not significantly different (p > 0.01).
was probably due to the reduced electrostatic interaction preventing ionic inter-chain bridging and gelation of fish gelatin (Haug et al., 2004).

Setting time required for shark skin gelatin was significantly (p < 0.01) lower than rohu and tuna gelatins (Table 5). Gelling temperatures reported for Nile perch bone gelatin were 18.5–19.0 °C and gelling time of 90 s (Muyonga et al., 2004a). Similarly, Ninan, Abubacker, et al. (2011) and Ninan, Jose, et al. (2011) observed gelling temperatures of 18.5 °C and 18.0 °C and gelling times of 106 and 103 s in rohu and common carp skin gelatin which differed from the result of the present study. That may be attributed to the difference in age and size of the fish or because of the difference in extraction conditions.

3.13. Foaming properties

Foaming ability (FA) and foam stability (FS) are another important property of gelatin for commonly used foods such as marshmallows (Zuniga & Aguilera, 2009). Foaming ability and foam stability of the gelatins are shown in Table 6. The foaming capacity of shark gelatin was found to be significantly higher (p < 0.01) than both tuna and rohu gelatins. Foam formation is generally controlled by transportation, penetration and reorganization of protein molecules at the air–water interface. A protein must be capable of migrating rapidly to the air–water interface, unfolding and rearranging at the interface to show good foaming properties (Halling, 1981). Foam stability depends on the nature of the film and indicates the extent of protein interaction within the matrix (Mutilangi, Panyam, & Kilara, 1996). Foam stability was significantly lower (p < 0.01) in rohu gelatin when compared to shark and tuna gelatins. Foams with higher concentration of proteins were denser and more stable because of an increase in the thickness of interfacial films (Zayas, 1997). It has been suggested that reduced foam formation and stability may be due to aggregation of proteins which interfere with interactions between the protein and water needed for foam formation (Kinsella, 1977).

3.14. Water holding capacity and fat binding capacity

Water holding and fat binding capacities are functional properties that are closely related to texture by the interaction between components such as water, oil and other components (Cho et al., 2004). Water holding capacity and fat binding capacity of gelatins are shown in Table 6. Water holding capacity is significantly higher (p < 0.01) in shark gelatin. Higher water holding capacity is mainly related to the higher amounts of hydrophilic amino acids and higher hydroxyproline content (Ninan, Abubacker, et al., 2011; Ninan, Jose, et al., 2011). Fat binding capacity is significantly higher (p < 0.01) in tuna gelatin. The degree of exposure of the hydrophobic residues and the high amount of tyrosine were found responsible for the high fat binding capacity (Ninan, Abubacker, et al., 2011; Ninan, Jose, et al., 2011).

4. Conclusion

It may be concluded that there are considerable differences between yield and functional properties of gelatin from the skins of dog shark, skipjack tuna and rohu. The characteristics of the gelatins extracted through this process indicate that they have good yield and functional properties. There is, therefore, a potential for exploitation of processing waste for gelatin extraction from these species. The potential is higher for dog shark skins than the other two species because dog shark skins give higher gelatin yield and the skin gelatin showed better functional properties.

The ability to form weak gels may find new applications for fish gelatin and it could possibly be used in refrigerated products and in products where low gelling temperatures are required (Gudmundsson, 2002).

Acknowledgements

The authors are thankful to the Director, Central Institute of Fisheries Technology (Kochi), Kerala, India for according permission to publish the paper. The authors also wish to express their gratitude to the Department of Biotechnology, Ministry of Science and Technology, Government of India, for the financial support given for this research work.

References


