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Research Article

FUNCTIONAL PROPERTIES OF GELATIN EXTRACTED FROM SKIN OF BLACK KINGFISH (*RANCHYCENTRON CANADUS*)

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ABSTRACT
The utilization of waste skin from fish for the production of value added by-products has attracted substantial attention. Black Kingfish (*Ranchycentron canadus*) was used for a culinary purpose but their skin was waste part. And convert in a value added product like gelatin was a good practice of post harvest management. In order to evaluate the waste from Black kingfish as a source of gelatin, the gelatin was extracted from skin and its rheological and functional properties were examined. The skin of Black kingfish yielded 13.88% indicating skin as an important source for gelatin production. The gel strength of gelatin from skin (222 gm), viscosity (13.53 cP), melting point (22.13° C) pH (4.81), water holding capacity 4.43 ml/g, emulsifying capacity and stability (55.66 %) and (32.5 %) respectively obtained from extracted gelatin. The hydroxyproline content in extracted gelatin was about (8.34 mg/g). It can be concluded from the present study that Black kingfish skin is prospective source to produce gelatin in good yield with desirable functional properties comparable to commercially available mammalian gelatin.

Key Words: *Gelatin; Black Kingfish, Gel Strength; Viscosity and Meting Point*

INTRODUCTION
Gelatin is heterogeneous mixture of high molecular weight water soluble protein derived from collagen by heat denaturation. It is extensively used as an ingredient to increase the viscosity of aqueous system and form aqueous gels. Traditional sources of gelatin are mainly pig skin and cow hide. In the food industry, gelatin is utilized in confections mainly for providing chewiness, texture, and foam stabilization; in low fat spreads to provide creaminess, fat reduction, and mouth feel; in dairy to provide stabilization and texturization; in baked goods to provide emulsification, gelling and stabilization and meat products to provide water-binding (Johnston-Banks, 1990; Schrieber and Gareies, 2007). Gelatin is normally recommended to enhance protein levels in foodstuffs, and especially in body- building foods. In addition, gelatin is also used to reduce carbohydrate levels in food formulated foe diabetic patients (Gans, 2007).
In the pharmaceutical industry, gelatin is used as a matrix for implants, in injectable drug delivery micro spheres, and in intravenous infusions (Pollack, 1990; Rao, 1995). There are also reports i which live attenuated viral vaccines used for immunization against measles, mumps, rubella, Japanese encephalitis, rabies, diphtheria and tetanus contain gelatin as a stabilizer (Burke *et al.*, 1999). Gelatin is also widely used for the manufacture of hard and soft capsules, plasma expanders, and in wound care.
The global demand for gelatin has been increasing over years. Recent report indicate the annual world out-put of gelatin is nearly 3, 26,000 tons, with pig skin derived gelatin accounting for the highest (46%), followed by bovine hides (29.4%), bovine bones (23.1%) and other sources (1.5%) (Karim and Bhatt, 2009).

Commercially, gelatin is made from skins and skeletons of bovine and porcine. Mammalian gelatin has been intensively studied (Ward and Courts, 1977; Gilsenan and Ross-Murphy, 2000; Cho *et al*., 2004). For many socio-cultural reasons, alternative sources are increasingly demanded. Among such reasons are religious proscription of Judaism and Islam. Diseases such as bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) crisis have also caused restrictions on collagen trade (Fernandez-Diaz, 2003; Cho *et al*., 2004). Interest in investigating possible means of making more effective use of underutilized resources and industrial wastes is not a new ambition in the food industry. The quantity of industrial waste produced is increasing by year for example the waste from fish processing after filleting can account for as much as 75% of the total catch weight (Shahidi, 1995). About 30% of such waste consists of skin and bone with high collagen content. This waste is an excellent raw material for the preparation of high protein foods, besides helping to eliminate harmful environmental aspects. Therefore, gelatin from marine sources has been looked upon as possible alternatives to mammalian gelatins.

MATERIALS AND METHODS

Proximate Composition

Proximate composition of raw materials and extracted gelatin were analyzed by measuring moisture, ash, protein and fat contents according to AOAC official methods (AOAC, 2005). The ph of raw material and extracted gelatin were measured using the British Standard Institution methods, BSI 757 (1975). Fish skins were chopped and blended in distilled water to form 1 % (w/v skin) suspension.

Gelatin Extraction

Gelatin was extracted following the procedure described by Koli *et al.,* (2011). Thawed skin was cleaned thoroughly cleaned with excess water to remove superfluous material. The cleaned materials were then sequentially soaked with 0.2% (w/v) sodium hydroxide, 0.2% sulphuric acid and 1.0% citric acid for 40 min. After each soaking treatment, the skins were washed under running tap water until had a pH of about 7 before transferring to new solution. This cycle was repeated three times with a total time of 2 hrs 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 3 volumes of distilled water at 45^0 C for 12 hrs. The clear extract obtained was filtered with Whatman filter paper (no. 1) by using a Buchner funnel. The filtrate was then in tray and dried in oven at 60^0 C for 16 hrs. The thin film of dried matter was powdered, weighed and packed in zip pack bags, stored at ambient temperature (25± 2^0 C) for further study. The yield of gelatin was calculated on wet weight basis of raw material and expressed as percentage yield.

Hydroxyproline Content

Hydroxyproline content of gelatin was determined according to the method of Bergman and Loxley (1963) with a slight modification. The samples were hydrolyzed with 6 M HCL at 110^0C for 24 hrs in reflex condenser and filtrate through Whatman no.1 filter paper. The filtrate was neutralized with 1M NaOH to pH 6.0-6.5. The neutralized sample (0.1) ml was transferred into a test tube and isopropanol (0.2ml) was added and mixed well. To the mixture, 0.1 ml of an oxidant solution (a mixture of 7% (w/v) chloroamine T and acetate/citrate buffer, pH 6, at a ratio of 1:4 (v/v) was added and mixed thoroughly. Then 1.3ml of Ehrlich's reagent solution (a

mixture of solution 2g of p-dimethylalamine benzaldehyde in 3ml of isopropanol) was added. The mixture was mixed and heated at 60^0 C for 25 min in water bath and then cooled for 2-3 min in running water. The solution was diluted to 5 ml with isopropanol. Absorbance was measured against water at 558nm using a spectrophotometer (Thermo spectronic, UV 10 rom 0628). Hydroxyproline standard solution, with concentration ranging from 10 to 60 ppm, was also run simultaneously. Hydroxyproline content was calculated and expressed as mg/g sample.

Determination of Gel Strength

The gelatin gel was prepared and the bloom value (gel strength) of gelatin gel was determined according to the method described by Wainewright (1997). The gel was prepared in bloom jar (150 ml capacity) by dissolving a 6.67% (w/v) dry gelatin powder in distilled water at 60^0 C. Then it was cooled for 15 min at room temperature and kept at 7^0 C for 18 h for maturation. Gel strength was determined on TA-RT-KI Texture Analyzer (Brookfield Engineering Labs. Inc) according to British standard BS 757 (BSI, 1975), with a load cell of 5 Kg cross-head speed 1 mm/s and using a 0.5 in. diameter bottomed plunger. The standard glass bloom jar was placed centrally under the plunger and the penetration test was then performed. The maximum force (g) was determined till the probe penetrated into the gel to a depth of 4mm.

Determination of Melting Point of Gelatin

The melting point measurement was done by a method modified from Wainwright (1977). Gelatin solutions, 6.67% (w/v) were prepared and a 5 ml aliquot of each sample was transferred to a small glass tube (borosilicate tube, 12mm × 75mm). The samples were degassed in vacuum desiccators for 5 min. The tubes were then covered with Para film and heated in a water bath at 60^0 C for 15 min. The tubes were immediately cooled in ice-chilled water and matured at 10^0 C. For 18h Five drops of a mixture of 75% chloroform and 25% reddish brown dye (food colour) was placed on the surface of the gel. The gels were put in a water bath at 10^0 C and the bath was heated at rate of $0.2\text{-}0.4^0$ C /min. The temperature of the bath was read using an electronic digital thermometer (Fisher Scientific). The temperature at which the dye drops began to move freely down the gel was taken as the melting point.

Determination of Viscosity

Gelatin solutions at the concentration of 6.67% (w/v) were prepared by dissolving the dry powder in distilled water and heating at 60^0 C for the determination of viscosity. The viscosity (cP) of 10 ml of the solution was determined using Brookfield digital viscometer (Model DV –E Brookfield Engineering, USA) equipped with a No.1 spindle at 40^0 C $\pm 1^0$ C (Cho *et al.*, 2006).

Emulsifying Capacity and Stability

The method of Yasumatsu *et al.*, (1972) was used to determine emulsifying capacity and stability. Emulsion was prepared with 1 g of each sample, 50 ml of cold distilled water (4^0 C) and 50 ml of sunflower oil. The gelatin samples were dispersed with a homogenizer/blender. Each blended samples was equally into 50 ml centrifuge tubes. One centrifuge tube was directly centrifuge at 4000 × g for 10 min while the other was centrifuged under the same conditions after heating in a water bath at 80^0 C for 30 min and cooling to room temperature (25^0 C). The height of emulsified layer, as a percentage of the total height of material in the unheated tubes, was used to calculate the emulsifying capacity and stability, using following formulae:

$$\textbf{Emulsifying Capacity} = \frac{\text{Height of emulsion layer}}{\text{Height of whole layer}} \times 100$$

$$\textbf{Emulsifying stability} = \frac{\text{Height of emulsion layer after heating}}{\text{Height of whole layer}} \times 100$$

Water holding capacity

Water holding capacity (WHC) was determined using the centrifugation method (Diniz and Martin, 1997). Duplicate samples (0.5 g) of gelatin were dissolved in 20 ml of water in centrifuge tubes and dispersed, with a vortex mixer for 30s. The dispersion was allowed to stand at room temperature for 6 h, and then centrifuge at 2800 × g for 30 min. The supernatant was filtered with Whatman No.1 filter paper and the volume recovered was measured. The difference between the initial volume of distilled water added to the protein sample and the volume of the supernatant was determined, and the result were reported as ml of water absorbed per gram of gelatin sample.

Determination of gelatin colour and gel clarity

Colour measurement was made by using a Hunter LabScan XE colorimeter (Hunter Association Laboratory, Inc., VA, USA). The tristimulus L*a*b* measurement mode was used as it relates to the human eye response to colour. The L* variable represents lightness (L*=0 for black, L*=100 for white), the a* scale represents the red/green (+a* intensity of red and -a* intensity of green) and the b* scale represents the yellow/blue (+b* intensity of yellow and -b* intensity in blue). The samples were filled into clear Petri dish and readings were taken. Clarity was determined by measuring transmittance (%T) at 620 nm in spectrophotometer (Thermospectronic, Cambridge, U. K) through 6.67% (w/v) gelatin solution which were heated at 60^0 C for 1 h (Avena-Bustillos *et al.,* 2006).

Statistical Method

The data of percentage yield, viscosity, bloom value, water holding capacity, pH, colour, clarity, emulsifying capacity and stability of gelatin extracted from black kingfish at temperature 45^0 C were analysed using appropriate statistical methods (Snedecor and Cochran, 1967., Zar 1999). Using ANOVA techniques significant difference between the treatments was determined. The significance of difference between means of treatments was further subjected to SNK test.

RESULT AND DISCUSSION

Proximate composition

The proximate composition of raw material and extracted gelatin were given in Table 1 and 2. The extracted gelatin from skin showed high value of proteins and low value for moisture, ash and fat content. Black kingfish skin gelatin contained higher content of protein i.e. 88.72%. Jongiareonrak *et al.,* (2006) reported a protein content of 87.9% and 88.6% in gelatin extracted from the skin of big eye snapper and brown eye snapper respectively. The gelatin from skin of adult Nile pearch also obtained 88% protein when extracted at 50^0 C (Muyonga *et al.,* 2004). Koli *et al.,* (2011) reported a protein content 86.45% in gelatin extracted from the skin of Tiger toothed croaker, when extracted at 45^0 C.

Moisture content of gelatin extracted from black kingfish at temperature (45^0 C) was 6.04%. Moisture content of all samples was well below the limit prescribed for edible gelatin i.e. 15% (GME, 2005). At 6-8% moisture, gelatin is very hygroscopic and it becomes difficult to determine the physiochemical attributes with the accuracy (Cole, 2000).

The ash content of gelatin extracted from skin of black kingfish at temperature (45^0 C) was 2.24%. And these values are less than the recommended maximum limit of 2.6% (Jones, 1977) and the limit given for edible gelatin i.e. 2% (GME, 2005).

Table 1: Proximate composition of black kingfish skin

Source of raw materials	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Skin of black kingfish	70±0.98	12.94±0.44	12±0.28	4.5±0.015

Values are given as ±SD from triplicate determinations; values in the same column with different superscript differed significantly ($p<0.05$).

Table 2: Proximate composition of extracted gelatin

Sr. No	Proximate composition	Percentage (%)
1	**Moisture**	6.04±0.07
2	**Protein**	88.72±0.41
3	**Fat**	0.4766±0.02
4	**Ash**	2.24±0.14

Values are given as ±SD from triplicate determinations; values in the same column with different superscript differed significantly ($p<0.05$).

Gelatin yield

The gelatin yield was extracted from skin of black kingfish at temperatures (45^0 C) was 13.88% showed in Table 3. The yield of gelatin have been reported to vary among the fish species mainly due to differences in the collagen content, the compositions of skin as well as the skin matrix. Variations in the yield have also been reported due to differences in the diverse extraction methods followed (Gomez-Guillen *et al.*, 2002; Jamilah and Harvinder, 2002; Muyonga *et al.*, 2004).

Gudmundsson and Hafsteinsson (1997) recorded the 14% yield of gelatin of cod fish. Gomez-Guillen *et al.*, (2002) recorded the percentage yield of sole fish, megrim, cod, squid and hake gelatin were 8.3%, 7.4%, 7.2%, 2.6% and 6.5% respectively. Jamilah and Harvinder (2002) reported that the yield of red tilapia gelatin and black tilapia gelatin were 7.81% and 5.39% respectively. Koli *et al.*, (2011) reported the yield of skin gelatin of Tiger-toothed croaker and Pink pearch were 7.56% and 5.57% gelatin, while their bones yielded 4.57% and 3.55% respectively.

pH of extracted gelatin

The pH of extracted gelatin 4.81 indicating their category as Type B and showed in table 3. It has been reported that alkali pretreatment results in Type B gelatin with pH in the range of 4 to 5 (Baziwane and He, 2003) and in the present study an alkali pretreatment was employed during the extraction of gelatin, the viscosity is minimum and gel strength is maximum at pH 5.0 (Cole, 2000) signifying the importance of pH for its rheological properties. The pH reported for gelatin from the skin of red tilapia was 3.05 and for black tilapia it was 3.91 (Jamilah and Harvinder, 2002).

Hydroxyproline content

In present study, hydroxyproline content of black kingfish skin gelatin extracted at different temperatures (45^0 C) was found to be 8.34 mg/g. Hydroxyproline content was significant ($p<0.05$) at 45^0 C of black kingfish gelatin showed in Table 3 which were lesser than the gelatin extracted from tilapia skin, 8.44 mg/g (Cho *et al*., 2006) and similar to cod skin, 8.30 mg/g (Gomez-Guillen *et al.*, 2002). Gelatin with high levels of amino acids tends to high gel strength and melting point (Muyonga *et al.*, 2004), as imino acids are important in the denaturation of gelatin subunits during gelling (Johnston-Banks, 1990).

Koli *et al.*, (2011) reported that hydroxyproline content in Tiger-toothed croaker skin and bone gelatins were 7.77 mg/g and 7.51 mg/g. While in Pink pearch skin and bone gelatin were 7.63 mg/g and 7.41 mg/g. Strength of gelatin gel is influenced by amino acids composition and molecular weight distribution of the gelatin itself, the strength of gelatin also varies with gelatin concentration, thermal history (gel maturation temperature and time), pH and presence of any additives (Choi and Regenstein, 2000).

Table 3: Gelatin Yield and its hydroxyproline content and pH of extracted gelatin from skin of black kingfish.

Source of raw Material	Yield (%)	Hydroxyproline content (mg/g)	pH of 1 % solution
Black Kingfish	13.88±0.31	8.34±0.04	4.81±0.02

Values are given as ±SD from triplicate determinations; values in the same column with different superscript differed significantly ($p<0.05$)

Gel strength (Bloom value)

Gelatin is highly capable of forming hydrogen bonds with water molecules to form a stable three-dimensional gel. The need to evaluate the characteristics of the gel has resulted in the concept of gel strength which is known as bloom value.

In present study, bloom values of black kingfish skin gelatin extracted at temperature (45^0 C) was found to be 222 gm. The bloom value was significant ($p<0.05$) at 45^0 C of black kingfish gelatin showed in Table 4.

The bloom value obtained in this study were higher to that of tilapia (180.76 g) (Jamilah and Harvinder, 2002), sin croaker (124.94 g) and short fin scad (176.92 g) (Cheow *et al*., 2007) and lower than that of Nile pearch (229 g) (Muyonga *et a*l., 2004) of yellow fin tuna (426 g) (Cho *et al*., 2005). The ability to form weak gels may find new application for fish gelatin as a non-gelling gelatins and it could possibly be used in refrigerated products and in products where low gelling temperature are required (Gudmundsson, 2002).

Viscosity

In present study, viscosity of black kingfish skin gelatin extracted at temperatures (45^0 C) was found to be 13.53 cP .Viscosity is the second most important commercial property of gelatin after gel strength (Ward and Courts, 1997).. The viscosity was significant ($p<0.05$) at 45^0 C of black kingfish gelatin showed in Table 4. Viscosity is partially controlled by molecular weight a molecular size distribution (Sperling, 1985). The viscosities of most of the commercial gelatins have been reported up to 13.0 cP (Johnston-Banks, 1990). Jamilah and Harvinder (2002)

reported that the viscosity of red tilapia gelatin and black tilapia gelatin were found to be 3.20 cP and 7.12 cP respectively.

Melting point

In present study, melting point of black kingfish skin gelatin extracted at temperatures (45^0 C) was found to be 22.1^0 C. The melting point of black kingfish gelatin was significant ($p<0.05$) at 45^0 C showed in Table 4. It is known that fish gelatin has lower melting point than mammalian gelatin (Norland, 1990). The melting point of bovine gelatin and porcine gelatin has been reported as 29.7 ^{0} C and 32.3 ^{0} C respectively (Gudmundsson, 2002). The melting points observed in the present study are far higher than those reported for cold water fishes such as cod (13.8^0 C), hake (14^0 C) (Gomez-Guillen *et al*., 2002). However, these melting points were lower than that of black tilapia (28.9^0 C) (Jamilah and Harvinder, 2002) which was warm water fish.

Table 4: Rheological properties of gelatin extracted from skin of black kingfish

Source of raw Material	Bloom value (g)	Viscosity (cP)	Melting point (^{0} C)
Black Kingfish	222±2	13.53±1.00	22.1±0.20

Values are given as ±SD from triplicate determinations; values in the same column with different superscript differed significantly ($p<0.05$)

Emulsifying capacity and stability

In present study, emulsifying capacity and stability of black kingfish skin gelatin extracted at temperature (45^0 C) was shown in Table 5 and significant difference at ($p<0.05$). Emulsifying capacity of black kingfish gelatin extracted at different temperature (45^0 C) was found to be 55.66% and emulsifying stability was found to be 32.5% respectively.

Emulsifiers are surface active materials that absorb to interface and facilitate the production of small droplets by lowering the interfacial during homogenization (Walstra, 2003). The amphoteric nature with hydrophobic zones on the peptode chain make gelatin to behave as an emulsifier and it is being use in the manufacture of toffees and water-in-oil emulsion such as low fat margarine, salad dressing, and whipped cream (Baziwane and He, 2003).

Water holding capacity

The functional properties of proteins in a food system depend in part on the water holding capacity (WHC) which refers to the ability of proteins to imbibe water and retain it against a gravitational force within protein matrix. The water holding of black kingfish gelatin which was extracted at 45^0 C obtained 4.43ml/g showed in Table 5. The water binding capacity of solubilised gelatin makes it suitable material for reducing drip loss and impairing juiciness in frozen fish or meat products when thawed or cooked, and where denatured protein has suffered a partial loss of its water holding capacity. Koli *et al*., (2011) reported that water holding capacity of Tiger-toothed croaker skin and bone gelatins were 4.50 ml/g and 3.00 ml/g, while Pink pearch skin and bone gelatin were 2.36 ml/g and 1.50 ml/g.

Table 5 Rheological properties of gelatin extracted from skin of black kingfish

Source of raw Material	Emulsifying capacity (%)	Emulsifying stability (%)	Water holding capacity (ml/g)
Black Kingfish	55.66±0.48	32.5±0.73	4.43±0.11

Values are given as ±SD from triplicate determinations; values in the same column with different superscript differed significantly (p<0.05).

Gelatin colour and gel clarity

Colour of gelatin extracted from black kingfish skin at was expressed in terms of L*, a* and b* and there were significant differences in colour characteristics The skin gelatin was extracted at 45^0 C showed the greatest lightness value (L*). Similar results were found to redness (a*) and there was no significant difference with respect to yellowness (b*). It can be concluded that factors such as fish species and raw material influence the colour characteristics of extracted gelatin. Both colour and clarity of a gelatin gel are important aesthetic properties, depending on the application for which the gelatin is intended. While the skin gelatin was extracted at 45^0 C showed the highest transmittance (%T) showed in Table 6. The turbidity and dark colour of gelatin is commonly caused by inorganic, protein and mucosubstance contaminants, introduced or not removed during its extraction (Eastoe and Leach, 1997).

Koli *et al.,* (2011) reported that Tiger-toothed croaker skin gelatin colour i.e. 75.41 (L*), 2.79 (a*), and 19.25 (b*) for lightness, redness and yellowness respectively, while clarity in transmittance (49.43 %T). While for Pink pearch skin gelatin colour 71.74 (L*), 2.74 (a*) and 22.07 (b*) for lightness, redness and yellowness respectively, while clarity in transmittance (44.30 %T).

Koli *et al.,* (2011) reported that Tiger-toothed croaker bone gelatin colour i.e. 65.44 (L*), 1.65 (a*), and 22.50 (b*) for lightness, redness and yellowness respectively, while clarity in transmittance (40.50 %T). While for Pink pearch bone gelatin colour 62.50 (L*), 1.97 (a*) and 22.60 (b*) for lightness, redness and yellowness respectively, while clarity in transmittance (40.13 %T). See *et al.,* (2010) reported that gelatin colour of four different fish species i.e. Catfish (44.36 L*, 0.56 a* and -3.65 b*), red tilapia (40.40 L*, 0.71 a* and -2.86 b*).

Table 6: Gelatin colour and clarity

Gelatin colour and gel clarity

Lightness (L*)	86.76±0.07
Redness (a*)	2.33±0.02
Yellowness (b*)	5.16±00.20
Transmittance (%)	41±0.11

Values are given as ±SD from triplicate determinations; values in the same column with different superscript differed significantly (p<0.05)

Conclusions

It can be concluded that black king fish skin waste may be utilized to produce gelatin. Results from the present study clearly demonstrate that the skin of black king fish is a prospective source to produce gelatin in good yield with the desirable characteristics comparable to commercially available fish gelatins.

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