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Characterization of fish gelatin from Blackspotted Croaker (*Protonibea diacanthus*)

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ABSTRACT

The global demand for gelatin has been increasing over the years, because it has broad applications in the food, pharmaceutical, photographic, cosmetic and packaging industries. In the food industry, gelatin is used as an ingredient to enhance the elasticity, consistency and stability of food products. It is also used in some medical and biotechnological applications. So, the present study aims to extract fish gelatin from the fish processing waste, i. e skin of Black spotted Croaker (Protonibea diacanthus). The qualitative and quantitative parameter includes proximate analysis (viz. moisture, fat, protein, ash and pH), viscosity, color, Hydroxyproline content and electrophoresis studies were done on the extracted fish gelatin from skin of Black spotted Croaker. It was found that, the extracted fish gelatin is well suited to exploit gelatin, which can be used in the food industry. Ghol fish skin has been recognized as a good source of high quality collagen that can be employed to manufacture functional food, medicine and cosmetic products.

Key words: Characterization, fish gelatin, *Protonibea diacanthus*

INTRODUCTION

The global demand for gelatin has been increasing over the years. The major source of collagen for the manufacture of gelatin is porcine skins, cattle hides and bones. Recent reports suggest the annual world production of gelatin is nearly 326,000 tons, with pig skin-derived gelatin accounting for the highest (46%) output, followed by bovine hides (29.4%), bones (23.1%), and other sources (1.5%). Unfortunately, these sources of gelatin present religious and safety oriented concerns for various consumer communities (Both Judaism and Islam forbid to consume any pork related products, while Hindus do not consume cow related products) [1]. However, the outbreak of bovine spongiform encephalopathy (BSE) and the foot-and-mouth disease (FMD) crisis have also resulted in anxiety among users of collagen and gelatin products from land-based animals [2]. Therefore, alternative sources, especially fish processing wastes including skin, bone or scale, have been paid increasing attention for gelatin extraction. These sources are good substitute for mammalian gelatin. The waste from fish processing after filleting can account for as much as 75% of the total catch weight [3]. About 30% of such waste consists of skin and bones with high collagen

content. This waste is excellent raw material for the preparations of collagen and gelatin. Thus, preparations of gelatin from marine by-products not only satisfy the kosher and halal requirements and consumers concern for BSE, but also increase economic returns for the fishing industry.

Gelatin has broad applications in the food, pharmaceutical, photographic, cosmetic and packaging industries. In the food industry, gelatin is used as an ingredient to enhance the elasticity, consistency and stability of food products. It is also used in some medical and biotechnological applications. In general, there are two methods to obtain gelatin from skins and bones, an acid process (gelatin A with isoelectric point at pH 6-9) and an alkaline process (gelatin B with isoelectric points at pH 5) [4]. Type of methods is applied depends on the collagen source, the number of covalent cross-linkages together with the age of the animal as well as the desired quality of the final gelatin. The age of the animal is a significant factor for those gelatins yield and quality. Chemicals used for pre-treatment as well as extraction condition such as temperature and time can influence the length of polypeptide chains and the functional properties of the gelatin [5]. The gelling properties of gelatin are also influenced by the source of raw material, which vary in proline and hydroxyproline contents.

Ghol fish *(Protonibea dicanthus)* is a popular seawater fish well-accepted by consumer all over the world due to the tasty mouth feel and abundant nutrients, like unsaturated fatty acids and proteins. So far, there is no studies have been conducted on gelatin extraction from this fish and this species is harvested in sufficient quantity which may has a commercial potential for gelatin production. Against this background the present study was aimed to extract and characterize the gelatin from the Ghol fish.

MATERIALS AND METHODS

Raw material

Blackspotted croaker *(Protonibea diacanthus)* fish skin with average size of 70-100 cm was procured fresh from Shivaji fish market located in Mumbai, India and transported in ice in the ratio of 1:1 to the laboratory in an insulated container. The skins were washed thoroughly and were cut into small pieces (1×1 cm^2). The prepared skin was stored at -20 °C until used for further study.

Gelatin extraction

Gelatin was extracted following the procedure described by Gudmundsson and Hafsteinsson [6] with some modification. Thawed Blackspotted croaker fish skin was thoroughly cleaned and rinsed with excess water to remove superfluous material and then treated with alkali (NaOH) solution at varying concentrations and soaking time according to the experimental design. Then, it was soaked with 0.2% sulphuric acid for 40 min. and followed by soaking with 0.7 % citric acid for 40 min. After each soaking treatment, the skin was washed under running tap water until they had a pH of about 7.0. 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 varying temperature and time. The ratio of skin: water used was 1:3 (w/v). The clear extract obtained was filtered with Whatman filter paper (No.1), using a Buchner funnel. The filtrate was then kept in a tray and dried in oven at 60 °C for 16 h. The thin film of dried matter was powdered, weighed and packed in Zip pack bags, stored at ambient temperature for further study. The yield of gelatin was calculated on wet weight basis of raw material and expressed as percentage yield. Percentage yield of extracted gelatin was calculated by the following formula.

$$\% \text{ Yield of gelatin} = (\text{Weight of gelatin}/\text{Weight of fish skin}) \times 100$$

Proximate Composition Analysis

Moisture

Moisture content in the sample was determined by using automatic moisture analyzer (IR 120, Denver, moisture analyzer). 1 g of the sample (Fish skin or gelatin) was taken and cut into small pieces and spread on the clean plate. The sample was heated at an initial temperature of 100^0C and a final temperature of 170^0C until a stable weight was achieved. Moisture percentage was obtained from the weight loss due to heating.

Protein

Crude protein content was determined by using Automatic Microkjeldahl unit (Kel Plus - Classic DX (VA), Pelican Equipments) was followed by AOAC [7]. Briefly, 0.5 gm of sample (Fish skin or Gelatin) was taken and digested with 1.6 g of digestion mixture (K_2SO_4 & $CuSO_4$ in 5:1 ratio) in 20 ml conc. H_2SO_4. The digestion was carried out in the digestion unit till the solution become clear. Digested sample was diluted to 250 ml & 5 ml of the digested solution was taken for automatic distillation in the Microkjeldahl unit. The total programme time was 9 min and the liberated NH_3 was collected in a conical flask containing Boric acid and mixed indicator (Bromo cresol green and methyl red). The amount of NH_3 liberated was determined by titrating with 0.1 N H_2SO_4. Crude protein content was calculated by multiplying the total Nitrogen content with conversion factor of 6.25 and expressed as percentage.

Crude fat

The crude fat content of gelatin was determined by Soxhlet extraction method [7]. About 1g of moisture free gelatin sample was taken in a whatman thimble. The thimble was plugged with cotton loosely and placed in a Soxhlet extraction unit. Petroleum ether AR grade was used as solvent. Extraction was continued for 16 hours. After the extraction, the pre-weighed receiver flask containing the extracted fat was dried initially on a water bath at 98°C±5°C. After complete drying, the receiver flask was cooled in desiccators and the weight was obtained. The difference in the initial and final weight of receiver flask was determined as fat content of gelatin calculated on wet weight basis.

Ash

The ash content of the samples was determined by using Muffle furnace (Phoenix CEM Corporation, USA) was followed by AOAC [7]. Briefly, 5 g of the sample (fish skin or gelatin) was taken in a previously ignited and weighed silica crucible. It was then transferred to muffle furnace and the temperature was raised to 600 °C and kept for 6 hours until white ash was obtained. Weight was taken after cooling and the percentage of ash was calculated from the weight difference.

Determination of Hydroxyproline

Determination of Hydroxyproline content was carried out according to Muralidharan et al. [8] method. Briefly, 0.1 gm gelatin sample was introduced into a round bottom flask and added 100 ml 6N HCl solution and boiled at 110°C under reflux for 16 hours. The cooled hydrolysate was transferred to a 250 ml volumetric flask through a funnel containing a filter paper and diluted to volume with distilled water. Hydroxyproline standard solution was prepared by dissolving 100 mg standard Hydroxyproline in distilled water. One drop 6N HCl was added and diluted to 100 ml. For use, 5 ml of the standard solution was diluted to 500 ml. Five standards were prepared by diluting 10, 20, 30, 40 and 50 ml of this solution to 100 ml with distilled water.

Four ml of the final dilution was taken in test tube; 2 ml oxidant solution (chloramines-T) was added and left to stand for 20 minute to the mixture. 2 ml of color reagent (4-dimethylaminobenzaldehyde solution) was added, mixed and covered with the aluminum foil. This resulting mixture was placed in a water bath at 60 °C for 15 minutes. The tubes were cooled under running tap water. The absorbance of the solution was read at 558 nm (Thermo scientific spectrophotometer) against a blank. A calibration curve was performed using five standard solutions of Hydroxyproline. Hydroxyproline content in the sample was calculated from the standard curve.

Determination of Colour

Colour measurement was made by using a Lab Scan XE spectrophotometer (Hunter Lab scan XE, USA) and it was calibrated to white and black standard sites of sample. 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 in red and -a* intensity in green) and the b* scale represents the yellow/blue (+b* intensity in yellow and -b* intensity in blue). The samples were filled into clear Petri dish and readings were then taken. This procedure was performed in triplicate for each sample.

Determination of viscosity

Viscosity of gelatin sample was determined according to the method of Cho *et al*. [9]. Gelatin solutions (10 g/100 ml) were prepared by dissolving the dry powder in distilled water and heating at 60^0C. Viscosity was determined using a Brookfield digital viscometer (Model LV-DV-II, Brookfield Engineering; MA, USA) equipped with No.1 spindle (Model LV) at 60 rpm at 40 $\pm1^0$C. The viscosity was read and reported in term of centipoises (cP). This procedure was performed in triplicate for each sample.

Electrophoretic analysis

Protein patterns of gelatin and gelatin gel samples were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli [10]. The samples (1 g) were dissolved in 10 ml of 5% (w/v) SDS solution. The mixture was heated at 95°C for 1 h in a water bath. Samples were centrifuged at 3000 *g* for 3 min and the supernatants were collected and mixed with sample buffer (0.5 M Tris-HCl, pH 6.8) containing 5% (w/v) SDS, 20% (v/v) glycerol and 10% (v/v) b-mercaptoethanol at the ratio of 1:1 (v/v) using 4% stacking gel and 7.5% resolving gel. The samples were run at 20 mA in a Mini- PROTEAN Tetra Cell unit (Bio-Rad Laboratories, Inc. CA). The gel was stained with Biosafe Coomassie G250 Stain. The load volume was 40 µl in all lines. SDS-PAGE Standards (BIO-RAD), Pre-stained SDS-PAGE Standards Broad Range was used to identify the protein fractions with molecular masses ranging from 6 to 198 kDa.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of Ghol fish skin was found in this study for moisture, crude protein, crude fat and ash were 75.80 ± 1.249 %, 20.63 ± 0.55 %, 2.48 ± 0.29 % and 1.06 ± 0.05 % respectively. The proximate composition of Ghol fish gelatin samples of 25, 26, and 27 (centre samples) is for moisture, crude protein and crude fat was 8.43 ± 0.86 %, 90.36 ± 0.47 % and 0.27 ± 0.067 % respectively. These results are in line with the findings of other fish available in the literature. Cheow *et al*. [11] reported the proximate composition from sin croaker fish as moisture (62.33%), crude protein (24.8%), crude fat (7.99%) and ash (5.4 %). Muyonga *et al*. [12] also reported 20 - 22% protein from Nile perch skin. The protein content of Ghol skin gelatin obtained was 90.36%. Jamilah and Harvinder [13] reported the protein content of tilapia gelatin as 89.30. Rahman *et al*. [14] reported the protein content of bovine and porcine gelatin as 88.7% and 90.65% respectively. It was observed that the protein content of Ghol skin gelatin was more or equal to commercial tilapia gelatin, bovine gelatin and porcine gelatin. The ash content of Ghol skin gelatin was observed as 0.93%. Jamilah and Harvinder [13] reported the ash content of commercial tilapia gelatin, bovine gelatin and porcine gelatin as 1.56, 1.65 and 1.43 % respectively. Ash content was more in bovine gelatin than other gelatin samples. The difference in ash content may be due to mineral contents in the raw material and variation in extraction method. The ash content of Ghol skin gelatin (0.93 %) was within the standard limit. Generally the gelatin samples are fat free [11]. The observed fat content of Ghol skin gelatin (0.27%) was low compared to the reported values of gelatin from tilapia, bovine and porcine. The presence of very low fat and ash content showed that the acid extraction process followed in the present study was appropriate in producing good quality gelatin. In this study, the moisture content of Ghol skin gelatin was observed as 8.43 %. Haug *et al*. [15] recorded the moisture and ash content as 12.9% and 0.82 % respectively in cod skin gelatin. The difference in moisture content in gelatin may be due to the variation in drying process. The moisture content in edible gelatin should be less than 15%. The observed moisture content of Ghol gelatin (8.43%) was within the prescribed limits of GME.

Viscosity of gelatin extracted from Ghol fish skin

The viscosity of gelatin was obtained from centre sample is found to be 8.41±0.43 cP. The average viscosity of centre was 8.41 cP (Centipoise). Viscosity is the second most commercially important physical property of a gelatin. Viscosity of Ghol skin gelatin is measured for only centre samples. This result was similar to the values previously reported by Zhou and Regenstein [16] for skin gelatin extracted from Alaska Pollock, which were between 1.56 and 6.62 cP. Boran and Regenstein [17] also reported the viscosity for the skin gelatin extracted from silver carp which was between 2.5 to 13.5 cP. The viscosity obtained in this study was higher or similar to pork skin gelatin suggesting that Ghol skin gelatin might successfully be used as an alternative raw material in place of pork skin where high viscosity is needed. Viscosity values for most gelatins are reported to be 1.5 to 7.5 cP but specialized gelatin may have the viscosity up to 13.0 cP [18]. The viscosity of Ghol skin gelatin was relatively high when compared with other kinds of gelatin like 3.2 cP for red tilapia [13]. The viscosity of Ghol gelatin was relatively low as compared to other kinds of gelatin samples which were 6.2 to 12.4 cP for the cod [6], and 22.5 cP for skate [9]. Low viscosity might be due to low cross linking degree of collagen molecules. Gomez-Guille´n *et al*. [19] reported that the difference in gel strength, viscosity and melting point was explained based on the amino acid composition, the α_1/α_2 collagen-chain ratio, and the molecular weight distribution. Cho *et al*. [20] reported that Viscosity is partially controlled by molecular weight and molecular size distribution. Presence of amino acid hydroxyproline has strong effect on viscosity of gelatin sample [21, 22].

Hydroxyproline content

The Hydroxyproline content of gelatin was obtained from centre sample is found to be 8.73±0.25%. The average hydroxyproline were expressed in g/100g content of centre sample of gelatin. The observed value of Hydroxyproline in Ghol fish gelatin for centre sample was 8.73 (g/100g). Jamilah and Harvinder [13] reported the hydroxyproline content of commercial tilapia fish gelatin and Bovine and porcine gelatin as 8.83 (g/100g), 10.50 (g/100g) and 6.50 (g/100g) respectively. The hydroxyproline of Ghol fish skin gelatin was lower than bovine gelatin but has similar value to commercial tilapia gelatin.

pH

The pH of gelatin was obtained from centre sample is found to be 5.5 (average). The pH values of 6.67 % gelatin solution of Ghol fish gelatin samples (centre) measured at a temperature of 60°C. The average value of pH of centre sample was observed 5.5. In this study, the acidic pH of the gelatin solution obtained was affected by the washing treatment. The pH of bovine, porcine and tilapia gelatin were 7.3, 5.4.and 5.5 respectively [11]. The pH of Ghol fish skin gelatin was similar to porcine gelatin and commercial tilapia gelatin while lower than bovine gelatin.

Color of Ghol fish skin gelatin

Instrumental colour measurements of the gelatins of Ghol skin from centre sample was found to be average L*, a* and b* values of Ghol skin gelatin was 75.86, 2.75 and 19.03 respectively. Colour of Ghol skin gelatin is measured only for centre samples. In the comparison between colour of Ghol skin gelatin and bovine, there is significant difference between L* value (91) of bovine gelatin [11] and Ghol skin gelatin. Low L* value of Ghol fish skin gelatin showed less whiteness, and high value of a* showed more redness than bovine gelatin. The b* value of Ghol skin gelatin was also somewhat higher than bovine gelatin which showed more yellowness. The difference in the value of L*, a* and b* between Ghol skin gelatin and bovine gelatin may be due to the difference in manufacturing process of gelatin. Ockerman and Hansen [23] reported that colour of gelatin depends on the raw materials extracted and whether it is the first, second or later extraction. In general, colour does not influence the functional properties.

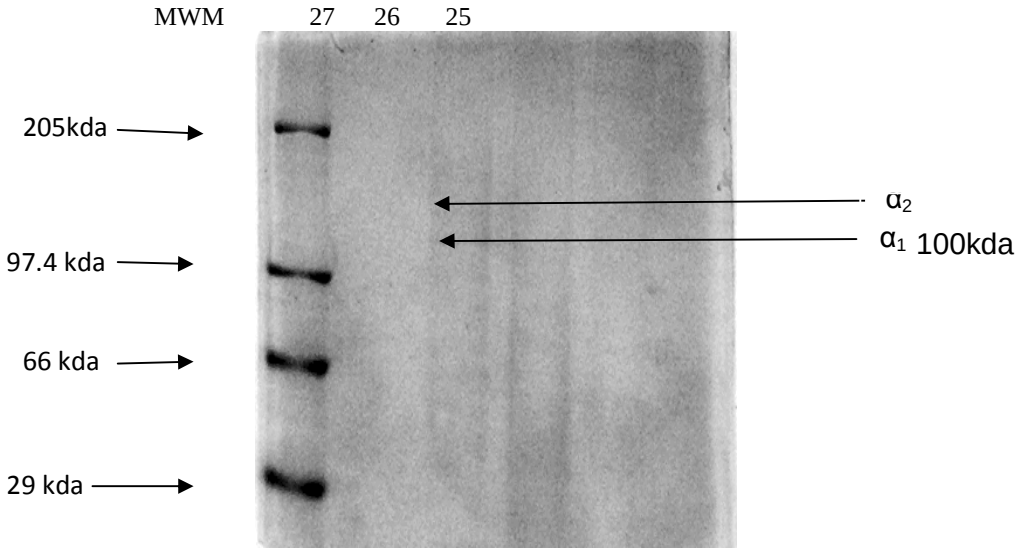

Fig.1: The protein pattern of gelatin on SDS-PAGE MWM – Molecular Weight Marker; 25, 26 and 27 are the gelatin samples.

Molecular weight distribution

The protein pattern of gelatin from Ghol fish skin (samples 25, 26 and 27) are shown in Fig.1. For gelatin of sample 27, α_1 and α_2 chains were found. The protein with molecular weight 29 kda, 66 kda and 97.4 kda were not found in any samples. β-component was not found in all the gelatin samples. The absence of β component in fish gelatin has been reported by many workers [24]. The absence of low molecular weight fraction showed that gelatin extracted in this study did not clear to release peptides and other low molecular weight (LMW) compounds. There have been reports suggesting that temperature plays major role in cleavage of high molecular weight (HMW) gelatin into LMW peptides [12, 25]. The formation of degradation fragments are associated with the low viscosity, low melting point, low setting point, high setting time, as well as decreased bloom strength of gelatin [12, 26].The high bloom

strength, high melting point and high viscosity of gelatin extracted in this study compared to the studies by above authors, corroborate the fact that the gelatin in this study is not degraded due to the low extraction temperature.

CONCLUSION

The results clearly shows that the extraction procedure of Ghol fish skin gelatin was found to be very efficient and also for the production of good quality gelatin. Since, it got a good thermal denaturation values, color and proximate analysis. So, it can be used in the food and pharmaceutical industries.

REFERENCES

[1] P. Zhou, J. M. Regenstein, *J. Food Sci*., 72 **(2007)**, C196-C201.
[2] T. Helcke, *Int. Food Ingredients*, 1 **(2000)**, 6-8.
[3] F. Shahidi, Seafood processing by-products. *In*: *Seafoods Chemistry Processing Technology and Quality.*, Shahidi, F., Botta, J. R. (Eds.)., Blackie, Glasgow, **(1994)** 320-334.
[4] G. Stainsby, Gelatin gels. *In*: *Advances in meat research* ., Pearson, A. M., Dutson, T. R., and Bailey ,A. J., (Eds.), Vol. 4. New York: Van Nostrand Reinhold Company Inc., **(1987)** 209-222.
[5] I. Kolodziejska, K. Kaczorowski, B. Piotrowska, M. Sadowska, *Food Chem.*, 86 **(2004)**, 203-209.
[6] M. Gudmundsson, H. Hafsteinsson, *J. Food Sci.*, 62 **(1997)**, 37-39
[7] AOAC., *Official methods of analysis* 18th edition. Association of official analytical Chemists, Washington, Arlington, Virginia, USA. (**2005**).
[8] N. Muralidharan, R.J. Shakila, D. Sukumar, G. Jeyasekaran, *J Food Sci Technol*. (**2011**). 1-8. (DOI 10.1007/s13197-011-0440-y)
[9] S. M. Cho, Y. S. Gu, S. B. Kim, *Food Hydrocoll*., 19 **(2005),** 221-229.
[10] U. K. Laemmli, *Nature*, 227 **(1970),** 680-685.
[11] C. S. Cheow, M. S. Norizah, Z. Y. Kyaw, N. K. Howell, *Food chem*., 101 **(2007)**, 386-391.
[12] J. H. Muyonga, C. G. B.Colec, K. G. Duodub, *Food Hydrocoll*. 8 **(2004)**, 581-592.
[13] B. Jamilah, K. G. Harvinder, *Food Chem*., 77 **(2002)**, 81-84.
[14] M. S. Rahman, G. S. Al-Saidi, M. Guizani, *Food Chem.*, 108 **(2008),** 472-481.
[15] I. J. Haug, K. I. Draget, O. Smidsrod, *Food Hydrocoll*., 18 **(2004)**, 203-213.
[16] P. Zhou, J. M. Regenstein, *J. Food Chem. Toxicol*. 69 **(2004)**, 393-398.
[17] G. Boran, J. M. Regenstein, *J. Food Sci*., 74 **(2009)**, E432-41.
[18] Gelatin Manufacturers institute of America (GMIA), Gelatin, New York, GMIA **(1993).**
[19] M. C. Gomez-Guille´n, J. Turnay, M. D. Ferna´ndez-Dı´az, N. Olmo, M. A. Lizarbe, P. Montero, *Food Hydrocolloids.*, 16 **(2002)**, 25-34.
[20] S. M. Cho, K. S. Kwak, D. C. Park, Y. S. Gu, C. I. Ji, D. H. Jang, *Food Hydrocolloids*, 18 **(2004)**, 573-579.
[21] A. I. Sarabia, M. C. Go´mez-Guille´n, P. Montero, *Food Chem*., 70 **(2000)**, 71-76.
[22] M. C. Gomez-Guille´n, A. I. Sarabia, P. Montero, *J. Food Science*, 66 **(2001)** 213-216.
[23] H. W. Ockerman, C. L. Hansen, Glue and gelatine. *In*: *Animal by-product processing and utilization.*, Baca, R.,(Etd)., FL:CRC Press., **(1999)** 183-216.
[24] A. Jongjareonrak, S. Benjakul, W. Visessanguan, M. Tanaka, *Euro. Food Res. Techn*., 222 **(2006a),** 229-230.
[25] A. Jongjareonrak, S. Benjakul, W. Visessanguan, T. Prodpran, M. Tanaka, *Food Hydrocolloids.*, 20 **(2006b),** 492-501.
[26] D. A. Ledward, Gelation of gelatin. *In*: *Functional properties of food macromolecules* ., Mitchell, J. R., and Ledward, D. A., (Eds.), London: Elsevier., **(1986),** 171-201.