

Research Article

FUNCTIONAL PROPERTIES OF GELATIN EXTRACTED FROM FOUR DIFFERENT TYPES OF FISHES: A COMPARATIVE STUDY

***Koli J.M., Sagar B.V., Kamble R.S. and Sharangdhar S.T.**

*Fish Processing technology and Microbiology, College of Fisheries,
Shirgaon, Ratnagiri, Maharashtra State*

**Author for Correspondence*

ABSTRACT

The present study was carried out to determine the functional properties of extracted gelatins from four different fish skins: Catla (*Catla catla*), Black tilapia (*Oreochromis mossambicus*), Stripped catfish (*Pangasius hypophthalmus*) and Black kingfish (*Ranchycentron canadus*). The extraction yields for four types of extracted gelatins were high, ranging from 7.8% (stripped catfish gelatin) to 13.88% (Black kingfish). Stripped catfish gelatin presented the highest gel strength (238.20 g). At 45 °C, the viscosity of Black kingfish gelatin (13.15 cP) was the highest. Four extracted gelatins had higher melting point. It can be concluded from the present study that these fish skins are prospective source to produce gelatin in good yield with desirable functional properties.

Keywords: *Gelatin; Catla, Black Tilapia, Stripped Catfish, Black Kingfish, Gel Strength, Viscosity and Mating Point*

INTRODUCTION

Gelatin is the denatured collagen fraction having a molecular weight higher than 30 kDa (Boran *et al.*, 2010). In aqueous solutions, gelatin is a mixture of different polypeptide chains including α -chains, β (dimers of α -chain) and γ (trimers of α -chain) components with a molar mass of around 90, 180 and 300 $\times 10^3$ g/mol respectively (Rbii *et al.*, 2011). Gelatin is being widely used in food, drug and cosmetic industries as stabilizing, thickening and gelling agent (Boran *et al.*, 2010; Gomez-Guillen *et al.*, 2001; Kittiphattanabawon *et al.*, 2010). The quality of gelatin is largely determined by its gelling strength and thermal stability. This is dependent on the amino acid composition which is species specific and molecular weight distribution as influenced by processing conditions (Gomez-Guillen *et al.*, 2002).

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

Fish by-products are seldom used as a source of raw materials for gelatin extraction. They are mainly used for animal feed supplements due to their small size.

However, some studies have ascertained freshwater and marine water fish to have vast amounts of waste after removal of useful edible parts and high gelatin yield being expected from them. Additionally, most findings suggest that gelatin from these species has an advantage over those extracted from cold water species, providing better rheological properties nearly similar to mammalian gelatins. The quality of gelatin for a particular application largely depends on its physicochemical properties, which are greatly influenced by both the species and tissue from which it is extracted and the method of extraction. Gelatin extraction from fish skin is generally achieved by pre-treatment with acid or alkali to give the desired properties. The present study aims to utilize skins of the Catla, Black Tilapia, Stripped catfish and Black kingfish as a raw material for extraction of gelatin and to estimate the functional properties of gelatin at different temperatures.

Consequently, increasing interest has been paid to other gelatin sources, especially fish skin and bone from seafood processing waste. A number of studies have addressed properties of fish skin gelatins (Choi

Research Article

and Regenstein, 2000; Gomez-Guillen and Montero, 2001; Jamilah and Harvinder, 2002; Muyonga *et al.*, 2004) showing that their properties differ from those of mammalian gelatins and vary between species. The quality of gelatin depends on its physicochemical properties which are greatly influenced by the species or tissue from which it is extracted and also by the severity of the manufacturing method. The functional properties of gelatin such as gel strength, viscosity and melting point depend on their molecular weight distribution and the amino acid composition (Johnston-Banks, 1990).

The objective of this study was to determine the functional properties of extracted gelatins from four different fish skins including Catla (*Catla catla*), Black tilapia (*Oreochromis mossambicus*), Stripped catfish (*Pangasianodon hypophthalmus*) and Black kingfish (*Ranchycentron canadus*).

MATERIALS AND METHODS

Raw Material and Chemicals

Four types of fishes viz. catla (*Catla catla*), Black tilapia (*Oreochromis mossambicus*), Stripped catfish (*Pangasius hypophthalmus*) and Black kingfish (*Ranchycentron canadus*) were obtained from a local market in Ratnagiri and Sangali market Maharashtra. Upon arrival at the laboratory, the fishes were filleted and the skin manually removed by using a sharp knife. After filleting, these fish skins were cleaned by tap water for three times and drained. Then the fish skins were frozen at -20°C until further use.

Glass Wares, Chemicals and Packaging Material

All glass wares used for were procured from Borosil Laboratories, India. Polythene zip bags were used for packing of the dried gelatin. Sodium hydroxide (NaOH), sulphuric acid (H₂SO₄) and citric acid (C₆H₈O₇) were purchased from MERCK Chemical Company Ltd. Mumbai, India.

Equipments and Machineries

Electronic Monopan balance of 'Sartorius' make (Citizen Scale Pvt. Ltd. Mumbai, India) were used for weighing purpose. Water bath of 'Bio-Technics' make was used for extraction of gelatin. Refrigerator of 'Godrej COLD GOLD' make was used for keeping gelatin for testing gel strength of sample. Autoclave of EQUITRON brand (Medical Instruments mfg. Co, Mumbai) was used for sterilization of glassware and media. 'TEMPO' brand hot air oven was used for moisture estimation. Heating mantle was used during estimation of proximate composition. Muffle furnace (Classic Scientific make) was used for ash estimation. pH was estimated by pH meter (ECOSCAN brand, Singapore). Aluminum trays and stainless steel were used for drying the gelatin solution.

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 °C and for 12 hrs. The clear extract obtained was filtered with Whatman filter paper (no. 1) using a Buchner funnels. The filtrate was then in tray and dried in oven at 60 °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 °C) for further study. The yield of gelatin was calculated on wet weight basis of raw material and expressed as percentage yield.

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 Wainwright (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 °C. Then it was cooled for 15 min at room temperature and kept at 7 °C for 18 h for maturation. Gel strength was determined on TA-RT-KI

Research Article

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 60⁰ C for 15 min. The tubes were immediately cooled in ice-chilled water and matured at 10⁰ 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 10⁰ C and the bath was heated at rate of 0.2-0.4 ⁰C /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 ⁰C 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 ⁰C ±1 ⁰C (Cho et al., 2006).

RESULTS AND DISCUSSION

Gelatin Yield

The gelatin yield extracted from skins of Catla, Stripped cat fish, Black tilapia and Black king fish at 45 ⁰c were 10.50 %, 7.8 %, 8.49% and 13.88% respectively as shown in figure 1.

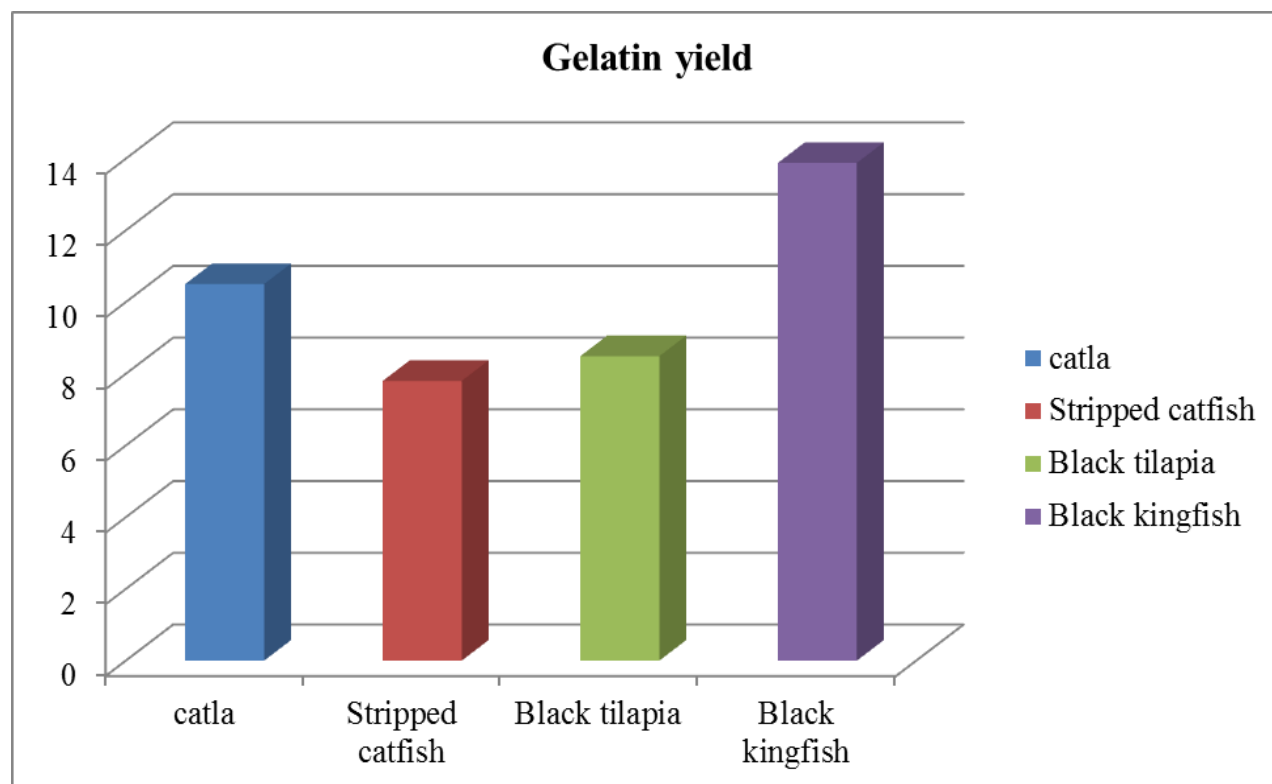


Figure 1: Yields of gelatins extracted from four fish skins

Research Article

The gelatin yield of black king fish was found high. 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). Jamilah and Harvinder (2002) reported that the yield of red tilapia gelatin and black tilapia gelatin were 7.81% and 5.39% respectively. Leaching of collagen during washing and treatments of skin could result in the lower yield of gelatin. Insufficient denaturation of soluble collagen during extraction can also result in lower yield.

Cho *et al.*, (2006) reported 9.63 % yield from patin fish. 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.

Gel Strength

In present study, Gel Strength of gelatin extracted from skins of Catla, Stripped cat fish, Black tilapia and Black king fish at 45 °C were 180.76 g, 238.20 g, 190.24 g and 222g respectively as shown in table 1.

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 perch (229 g) (Muyonga *et al.*, 2004) of yellow fin tuna (426 g) (Cho *et al.*, 2005), tilapia (263 g) (Grossman and Bergman, 1992) and grass carp (267 g) (Kasankala *et al.*, 2007). 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). However, Grossman *et al.*, (1992) reported gel strength of 263.00 g for the tilapia spp. which was higher than those reported by Jamilah and Harvinder (2002). The variation in the reported gel strength could be explained by differences in molecular weight distribution rather than in amino acid composition without disregarding the existence of additional factors that may influence these parameters. It is well established that fish gelatin has a lower melting point than mammalian gelatins (Norland, 1990) and melting point of gelatins increase with increasing in molecular weight (Ward and Courts, 1977). It is also well established that hydrogen bonds between water molecules and free hydroxyl groups of amino acid in gelatin are essential for the gelatin's gel strength (Arnesen *et al.*, 2002). The higher the hydroxyproline content, the higher the gel strength of the gelatin (Sarabia *et al.*, 2000).

Melting Point

In this study, melting point of black tilapia skin gelatin extracted from skins of Catla, Stripped cat fish, Black tilapia and Black king fish at 45 °C were found to be 24.50 °C, 26.20 °C, 27.48 °C and 22.1 °C respectively as shown in table 1. It is known that fish gelatin has lower melting point than mammalian gelatin. The amino acid composition may also contribute to the melting point characteristics (Norland, 1990). The melting point of bovine gelatin and porcine gelatin has been reported as 29.7 °C and 32.3 °C respectively (Gudmundsson, 2002). The melting points observed were 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) (Mohtar *et al.*, 2010). Gilsenan and Ross-Murphy (2000) reported There was a relationship between melting point and molecular weight of gelatin, low molecular weight gelatins melt at lower temperature than high molecular weight ones.

Table 1: Functional properties of gelatin extracted from skins of different fishes

Fish	Gel strength	Melting point	Viscosity
Catla	180.76±0.28 ^a	24.50± 0.20 ^a	9.5±0.20 ^a
Stripped catfish	238±4.64 ^b	26.20±1.35 ^b	8.21±0.84 ^a
Black tilapia	190.24±3.2401 ^c	27.48±0.0529 ^b	6.9066±0.0611 ^b
Black kingfish	222±1.15 ^d	22.13±0.12 ^c	13.5333±0.57 ^c

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

Research Article

Viscosity

In present study, viscosity of gelatin extracted from skins of Catla, Stripped cat fish, Black tilapia and Black king fish at 45 °C were found to be 9.5 cP, 8.21 cP, 6.9 cP and 13.15 cP respectively as shown in table 1. Viscosity is the second most important commercial property of gelatin after gel strength (Ward and Courts, 1997). Gudmundsson and Hafsteinsson (1997) reported 7.5 cP as the highest viscosity nom cod skin gelatin using chemical treatment. 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, whereas for channel cat fish it was 3.23 cP (Yang *et al.*, 2007). The changes in pH are known to influence the viscosity and minimum viscosity for gelatin has been in the range of 6-8 (Stainsby, 1987).

Conclusion

Gelatins were extracted from four types of fish skins. With alkaline and acidic pretreatment following with hot water extraction at 45 °C followed by overnight drying in hot air oven gave the high gelatin yield and gel strength of extracted gelatins. Gelatins extracted from these fish skins exhibited high gel strength. These four types of fish skin gelatins exhibited the functional properties close to the bovine skin gelatins and much better than cold water fish skin gelatin. The high turbidity values of extracted gelatins were due to inadequate filtration. This study showed that these extracted fish skins gelatins are potentially to be utilized as alternative sources of mammalian gelatins and may be used in various applications in the food, pharmaceutical, and photographic industries.

REFERENCES

- AOAC (2005).** *Official Methods of Analysis of the Association of Official Analytical Chemists International*, 18th edition, In: Association of Official Analytical Chemists, Washington (D. C.).
- Arnesen JA and Gildberg A (2002).** Preparation and characterisation of gelatin from the skin of harp seal (*Phoca groenlandica*). *Bioresource Technology* **82** 09191-194.
- Boran G, Mulvaney SJ and Regenstein JM (2010).** Rheological properties of gelatin from silver carp skin compared to commercially available gelatins from different sources. *Journal of Food Science* **75** E565-E571.
- Cheow CS, Norizah MS, Kyaw ZY and Howell NK (2007).** Preparation characterization of gelatin from the skin of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*) *Food Chemistry* **101** 386-391.
- Cho SM, Jahncke ML, Chin KB and Eun JB (2006).** The effect of processing conditions on the properties of gelatin from skate (*Raja kenoei*) skins. *Food Hydrocolloid* **20** 810-816.
- Cho SM, Gu YS and Kim SB (2005).** Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. *Food Hydrocolloid* **19** 221-229.
- Choi SS and Regenstein JM (2000).** Physicochemical and sensory characteristics of fish gelatin. *Journal of Food Science* **65** 194-199.
- Gilsenan PM and Ross-Murphy SB (2000).** Rheological characterisation of gelatins from mammalian and marine sources. *Food Hydrocolloids* **14** 191-195.
- Gomez Guillen MC and Montero P (2001).** Extraction of Gelatin from Megrim (*L. boscii*) Skins with several organic acids. *Journal of Food Science* **66**(2) 213 -16.
- Gomez-Guillen MC, Turnay J, Fernández-Díaz MD, Ulmo N, Lizarbe MA and Montero P (2002).** Structural and physical properties of gelatin extracted from different marine species: a comparative study. *Food Hydrocolloids* **16** 25-34.
- Grossman S and Bergman M (1992).** Process for the production of gelatin from fish skins. US Patent 5,093,474.
- Gudmundsson M and Hafsteinsson H (1997).** Gelatin from cod skins as affected by chemical treatment. *Journal of Food Science* **62**(1) 37-39, 47.

Research Article

- Jamilah B and Harvinder KG (2002).** Properties of gelatins from skins of fish-black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). *Food Chemistry* **77** 81–84.
- Johnston-Banks FA (1990).** *Gelatin Food Gels, Applied Food Science Series* (Elsevier) New York 233-289.
- Kasankala LM, Xue Y, Weilong Y, Hong SD and He Q (2007).** Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. *Bioresource Technology* **98**(17) 3338-43.
- Kittiphattanabawon P, Benjakul S, Visessanguan W and Shahidi F (2010).** Effect of extraction temperature on functional properties and antioxidative activities of gelatin from shark skin. *Food and Bioprocess Technology* 10.1007/s11947-010-0427-0.
- Koli JM, Basu S, Nayak BB, Patange SB, Pagarkar AU and Venkateshwarlu G (2011).** Functional characteristics of gelatin extracted from skin and bone Tiger Toothed croaker (*Otolithes ruber*) and Pink perch (*Nemipterus japonicus*). *Food and Bioproducts Processing*.
- Mohtar NF, Conrad P and Siew-Young Q (2010).** Optimization of gelatin extraction from hoki (*Macruronus novaezelandiae*) skins and measurements of gel strength and SDS-PAGE. *Food Chemistry* **122** 307-313.
- Muyonga JH, Cole CGB and Doudu KG (2004).** Characterization of acid soluble collagen from skin of young and adult Nile perch. *Journal of Food Chemistry* **85** 81-89.
- Norland RE (1990).** Fish Gelatin. In: *Advances in Fisheries Technology and Biotechnology for Increased Profitability*, edited by Voight MN and Botta JK (Technomic Publishing Co.) Lancaster 325-333.
- Rbii K, Surel O, Brambati N, Buchert AM and Violleau F (2011).** Study of gelatin renaturation in aqueous solution by AFIFFMALS: Influence of a thermal pre-treatment applied on gelatin. *Food Hydrocolloids* **25** 511-514.
- Sagabia AL, Gomez-Guillen MC and Montero P (2000).** The effect of 7 added salt on the viscoelastic properties of fish skin gelatin. *Food Chemistry* **70** 71-76.
- Shahidi F (1994).** Seafood processing by-products. In: *Seafoods Chemistry Processing Technology and Quality*, edited by Shahidi F and Botta JR, Blackie, Glasgow 320–334.
- Wainwright FW (1977).** Physical tests for gelatin products. In: *The Science Technology of Gelatins*, edited by Ward AG and Courts (Academic Press Inc.) London 508-531.
- Ward AG and Courts A (1977).** *The Science and Technology of Gelatin* (Academic Press Inc.) New York 420.
- Zar HJ (1999).** *Biostatistical Analysis*, 4th edition (Dorling Kindersley (India) Pvt. Ltd.) Delhi 663.