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Research Article

PHYSICO-CHEMICAL PROPERTIES OF GELATIN EXTRACTED FROM CATLA SKIN (*CATLA CATLA*) (*HAMILTON, 1822*)

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ABSTRACT
Studies on gelatins from fish processing by-products, such as the skin and bone, has increased for the replacement of mammalian sources of gelatin. So for evaluating the alternative good quality source of gelatin, the gelatin was extracted by the best method to achieve better gelatin yield and gel strength by alkali (NaOH, 0.2 %) pre-treatment followed by acid treatments ($H_2 SO_4$, 0.2 % and citric acid, 1 %) with pre-treatment time for 40 Min., from skin of Catla and its rheological and functional properties were examined at three different temperature (40, 45 and 50 ^{0}C).The proximate composition of raw material was found to be moisture74.77%, crude protein 18.87%, crude fat 3.22% and ash 3.51%. The proximate composition of extracted gelatin was found to be comparatively better at 45 ^{0}C than 40 and 50 ^{0}C. The yield of gelatin was high at 45 ^{0}C temperature. The gel strength of extracted gelatin of Catla at 45 ^{0}C temperature (180.76 g) was found to be higher than 40 and 50 ^{0}C. Similarly the viscosity, melting point, emulsifying capacity and stability extracted gelatin at 45 ^{0}C from Catla skin were in general significantly higher than those of gelatin extracted at 40 and 50 ^{0}C temperature. Hydroxiproline content in extracted gelatin of Catla was found to be in the range of 6.53-8.67 mg/g and highest content of hydroxiproline was obtained at 45 ^{0}C temperature. It can be concluded from the above results that the Catla is prospective source to produce gelatin of higher yield and quality and desirable rheological and functional properties at 45 ^{0}C temperature. These promising finding may contribute to the on-going efforts for using fish gelatin as an alternative source for mammalian gelatins and for various applications.

Keywords: *Gelatin; Catla, Black tilapia, Stripped catfish, Black Kingfish, Gel Strength, Viscosity and Meting Point*

INTRODUCTION
In India, carps contribute almost 85% of the harvest from freshwater aquaculture production. The three Indian major carps, viz. Catla (*Catla catla),*Rohu (*Labeo rohita*), and mrigal (*Cirrhinus mrigala*) contribute bulk of the production in the country, whereas the three domesticated exotic carps such as Silver carp (*Hypophthalamichthys molitrix*), Grass carp (*Ctenopharyngodon idella*) and Common carp (*Cyprinus carpio*) form the second important group. The skin constitute about 6-7% of the processing waste from these fishes which is a good source of gelatin and a gelatin yield of 10.5 -12.9% is obtained from the skin of these species (Ninan *et al*., 2009). Previous studies ascertained freshwater fish to have contained vast amounts of waste after removal of useful edible parts and high gelatin yield can be expected from them (Grossman and Bergman, 1992).
Gelatin has wide applications in the food and pharmaceutical industries. Most of the commercial gelatins are derived from mammalian sources, mainly pigskin and cowhide. Gelatin from marine sources is possible alternative to bovine gelatin (Rustad, 2003; Kim and Mendis, 2006; Wassawa *et al*., 2007).
Fish skin contains large amounts of collagen and can be considered as a potential source of gelatin. One major advantage of gelatin from aquatic sources is that it is not associated with the risk of Bovine Spongiform Encephalopathy and is acceptable to most religious groups. Further, the utilization of fish skin for the extraction of gelatin can significantly address the problem of waste disposal in the fish processing industry. Although fish gelatin will be unable to completely replace mammalian gelatin, in future it might become a niche product offering unique and competitive properties to other biopolymers, as well as meeting the demand of global halal market (Karim and Bhat, 2009).

There are two main types of gelatin. Type A, with iso-ionic point of 7 to9, pH 3.8 to 5.5,gel strength 50 to 300 g, viscosity (mps) 15 to 75,ash 0.3 to 2% is derived from collagen with exclusively acid pretreatment. Type B, with iso-ionic point of 4.7 to 5.4, pH 5 to 7.5,gel strength 50 to 300, viscosity (mps) 20 to 75, ash 0.5 to 2 %, is the result of an alkaline pre-treatment of the collagen (GMIA, 2012).

Annually, more than 100 million tons of fish are being harvested worldwide. 29.5% of the total catch is used for fishmeal due to its poor functional properties (Kristinsson and Rasco, 2000). 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).

The global demands for gelatin have been increasing over the years. Recent reports indicate the annual world output 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%). Since most commercial gelatins are obtained from pig skins or cow skins and bones (perhaps due to the relatively low cost of the final gelatin product), the issue of gelatin replacement has existed for many years for the vegetarian, halal and kosher markets, particularly within Europe with the emergence of bovine spongiform encephalopathy (Karim and Bhat, 2008).

Gelatin is the most versatile of the hydrocolloids in the modern food industry. In comparison to gelatin, carrageenan forms brittle, barely elastic gel. Pectin gels have no elastic properties at all and are not stable as a result. Alginates form clear, elastic gels, but their melting point is much higher than that of gelatin (GME, 1990). The use of starches and modified starches in food processing can lead to unpleasant textures due to the large quantities needed.

Gelatin, a protein derived from collagen is the major structural protein in connective tissue of animal skin and bone (Leuenberger, 1991; Cho *et al*., 2004). It is an important constituent in a number of food and non-food products due to its multi-functional properties, thermal stability, digestibility, solubility and its biological characteristics. In the food industry, it serves primarily as a gelling agent, but it is also used as a thickener, film former, stabilizer, emulsifier, adhesive agent, foaming agent, protective colloid and as a beverage fining agent (Johnston-Banks, 1990). The quality of gelatin for a particular application therefore depends largely on its rheological properties that are desirable for that application (Stainsby, 1987; Gomez- Guillen *et al*., 2002).

Commercially, gelatin is made from skins and skeletons of bovine and porcine. Mammalian gelatin has been intensively studied (Ward and Courts, 1977). 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 (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. Several patents (Grossman and Bergmann, 1992) as well as several published methods (Gudmundsson and Hafsteinsson, 1997) for fish gelatin production have been reported.

Gelatin consists of different amounts of 18 amino acids, where glycine, proline and hydroxyl-proline are the most abundant. Commercially, two main types of gelatin are used: Type A and Type B gelatins (Ward and Courts, 1977). Type A gelatins result from acid process and Type B gelatin results from alkaline process. Dry commercial gelatins for the food industry usually contain about 88% protein, 10% water and 1 – 2% salts (GME, 1990).

Some studies on fish gelatin extraction has been reported for cod (Gudmundsson and Hafsteinsson, 1997), megrim (Montero and Guillen, 2000), tilapia (Jamilah and Harvinder, 2002), Nile pearch (Muyonga *et al*., 2004) and tuna (Cho *et al*., 2005), croaker and pink pearch (Koli *et al*., 2011), Rohu and common carp (Ninan *et al.*, 2009, 2010, 2011).

MATERIALS AND METHODS

Raw Materials and Chemicals

Skin of Catla (Catla catla) was collected from Ratnagiri and Sangali fish market. The skin was washed and stored at – 200Cuntil further use. Sodium hydroxide (NaOH), sulphuric acid (H_2SO_4) and citric acid ($C_6H_8O_7$) were purchased from MERCK Chemical Company Ltd. Mumbai, India.

Methods for Extraction of Gelatin

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 40^0C, 45^0C and 50^0C 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 60^0C for 16 hrs. The thin film of dried matter was powdered, weighed and packed in zip pack bags, stored at ambient temperature (25± 2^0C) for further study. The yield of gelatin was calculated on wet weight basis of raw material and expressed as percentage yield. 45 ^{0}C extraction temperatures showed higher yield, rheological and functional properties.

Proximate Compositions

Proximate compositions were analyzed by AOAC method (1995).

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 60^0C for 15 min. The tubes were immediately cooled in ice-chilled water and matured at 10^0C. 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 10^0C and the bath was heated at rate of 0.2-0.4^{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 60^0C 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).

Hydroxy Proline Content

Hydroxy proline 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-dimethyl-alamine benzaldehyde in 3ml of isopropanol) was added. The mixture was mixed and heated at 60 ^{0}Cfor 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 10rom 0628). Hydroxy proline standard solutions, with concentration ranging from 10 to 60 ppm, were also run simultaneously. Hydroxy proline content was calculated and expressed as mg/g sample.

Determination of Isoionic Point (pI)

Isoionic point of fish gelatin was determined according to the method described by Zhang *et al.* (2011). The pI was determined by measuring the transparence of 2 % (w/v) gelatin solution with different pH values at 660 nm spectrophotometer (Thermospectronic, Instrument). The pH value at which the solution has the lowest transparence was the pI value of the gelatin.

RESULTS AND DISCUSSION

Table 1: Proximate composition of raw material, yield and extracted gelatin Catla skin

Sr. No	Proximate compositions (%)	Catla skin	Extracted gelatin at different temperature		
			40 ^{0}C	45^{0}C	50 ^{0}C
1	Moisture	74.77±0.40	9.53±0.23	8.13±0.07	7.45±0.05
2	Protein	18.87±0.05	88.49±0.30	89.45±0.36	88.71±0.10
3	Fat	3.22±0.04	1.02±0.21	0.86±0.03	1.75±0.05
4	Ash	3.51±0.08	1.32±0.18	1.53±0.06	1.91±0.03
5		Yield Of gelatin (%)	8.61±0.06	10.50±0.05	9.94±0.04

Table 2: Rheological/ Functional properties, pH and isoionic point of gelatin extracted from skin of Catla *(Catla catla)*

Sr. No	Functional properties and other characteristics	40 ^{0}C	45^{0}C	50 ^{0}C
1	Gel strength/ Bloom value (gm)	172.29±0.16	180.76±0.28	174.25±0.15
2	Viscosity (cP)	7.2±0.15	9.5±0.20	6.5±0.20
3	Hydroxiproline content (mg/g)	6.53±0.06	8.87±0.06	7.53±0.06
4	Melting point (^{0}C)	21.61±0.10	24.50± 0.20	23.43 ±0.31
5	pH	3.6667±0.15	4.2333±0.15	5.4333±0.21
6	Isoionic point	4.5	5	4.5

The proximate compositions of raw materials, extracted gelatin at different temperature level as shown in table no. 1, while the functional properties, yield pH and isoionic point as (Table 2). Gelatin is an important biopolymer normally derived from beef or pork. It is used to increase the viscosity of aqueous

systems and to form aqueous gels. It's useful properties include thermo-reversibility, a characteristic rheology described as melt-in-the-mouth, and an excellent release of flavour. Jones (1977) and Anonymous (1980) described the food and nonfood uses of gelatin. Gelatin's single largest use is in gel desserts. Estimated world usage is 200,000 metric tons per year with U.S. usage being about 30,000 metric tons per year for food and about 10,000 metric tons per year for pharmaceutical applications (Holzer, 1995). The traditional sources of gelatin present problems for such religions as Judaism and Islam. These communities cannot accept pork gelatin, and beef gelatin is acceptable only if it has been processed in accordance with religious requirements. Products from fish with removable scales (that is, those that can be removed without tearing the skin) are acceptable in Judaism with minimal restrictions, while all fish are acceptable in Islam. In addition to religious needs, the commercial use of fish skins, bones, and swim bladders, which are usually wasted, to yield additional income has both economic and waste management benefits for the fish industry because of the large quantities of these materials generated. Therefore, the present study was taken up to explore the possibility of preparing quality gelatin from the locally available fish i.e. Catla (*Catla catla*).

The proximate composition of raw material and extracted gelatin are shown in (Table 1) respectively. The gelatins extracted from skin of catla at different temperatures (40^0C, 45^0C and 50^0C) showed high values of proteins and low values for moisture and fat, indicating efficient removal of water and fat from the skin (Ninan *et al.,* 2011). The gelatin obtained at 45^0C extraction temperature contained higher content of protein than other temperatures i.e. 89.54%. 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 50^0C (Muyonga *et al.,* 2004). The protein content from red tilapia obtained 89.70% (See *et al*., 2010). Ninan *et al.,* (2011) reported protein content of 90.43% and 89.91% from Rohu and Common carp respectively. Koli *et al.,* (2012) 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 catla at three different temperatures (40^0 C, 45^0 C and 50^0 C) were 9.53%, 8.13% and 7.45% respectively (Table 1). Moisture content of all samples was well below the limit prescribed for edible gelatin i.e. 15% (GME, 2012). At 6-8% moisture, gelatin is very hygroscopic and it becomes difficult to determine the physiochemical attributes with the accuracy (Cole, 2000). The moisture content varied not only with the extent of drying, but also with the humidity during storage (Ockerman and Hansen, 1988). Ninan *et al.,* (2011) reported moisture content of 8.10% and 8.48% from Rohu and common carp respectively. Moisture content was found 10.98%, 11.89% and 10.01% from red tilapia, snakehead fish and *pangasious* catfish respectively (See *et al.,* 2010).

The ash content of gelatin extracted from skin of Catla at three different temperatures (40^0C, 45^0C and 50^0C) were 1.32%, 1.53% and 1.91% respectively(Table 1), 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, 2012). Low ash content suggested that the extracted gelatin was of high quality, as the ash content for a high quality gelatin should be lower than 2% (Ockerman and Hansen, 1988). Ninan *et al.,* (2011) reported ash content between 1.10% -1.18 for Rohu and common carp. See *et al.,* (2010) reported ash content 0.67% and 0.39% for red tilapia and pangasious catfish respectively.

The gelatin yield was extracted from skin of Catla at three different temperatures (40^0C, 45^0C and 50^0C) were 8.60%, 10.49% and 9.94% respectively (Table 1). The gelatin yield was found high at 45^0C. Decrease in the yield of gelatin could be due to the loss of extracted collagen due to incomplete hydrolysis of the collagen (Jamilah and Harvinder, 2002). 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). 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. The maximum swelling of skins of Catla was observed during pre-treatment with alkali and acid correlating

with better yield due to opening of cross links during swelling. A high degree of cross linking via covalent bonds can cause decrease in solubility of collagen and leading to lower content of extractable gelatin (Foegeding *et al.,* 1996).

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%,young and adult Nile perch 12.5% and 16% respectively (Muyonga *et al.,* 2004). Ninan *et al.,* (2011) reported that the gelatin yield of Rohu and common carp were 12.93% and 12%.

The gel strength or bloom strength is a measure of the hardness, stiffness, strength, firmness and compressibility of the gel at a particular temperature and is influenced by concentration and molecular weight (Ockerman and Hansen, 1988).

In present study, gel strength of Catla skin gelatin extracted at different temperatures (40^0C, 45^0C and 50^0C) was found to be 172.29 g, 180.76 g and 174.25 g respectively (Table 4.4). The gel strength for the samples was in the range of 172.29-180.76 g. The gel strength was significantly ($p<0.05$) higher at 45^0C of Catla gelatin compared to 40^0C and 50^0C (Table 4.4b).

The gel strength obtained in this study were in the range of tilapia (180.76 g) (Jamilah and Harvinder, 2002), short fin scad (176.92 g) (Cheow *et al*., 2007), common carp and rohu (181.31g, 188.63g respectively) (Ninan *et al*., 2011), silver carp (176 g) (Tavakolipour, 2011) and lower than that of Nile pearch (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).

The gel strength of fish gelatin has been reported in a wide range 124-426 g, compared to 200-300 g for bovine or porcine gelatin (Karim and Bhat, 2009). The difference in gel strength among the various species could be explained by differences in extraction process used and the intrinsic properties of collagen which varies among fish species. Gudmundsson and Hafsteinsson (1997) suggested that the gel strength may depend on isoelectric point and may be controlled, to certain extent, by adjusting the ph. The low gel strength was due to low concentrations of imino acids (proline and hydroxyproline). The proline and hydroxyproline contents are approximately 30% for mammalian gelatins, 22% to25% for warm-water fish gelatins, and 17% for coldwater fish gelatins (Muyonga *et al.,* 2004).

In present study, viscosity of Catla skin gelatin extracted at different temperatures (40^0C, 45^0C and 50^0C) was found to be 7.2 cP, 9.5 cP and 6.5 cP respectively (Table 2). Viscosity is the second most important commercial property of gelatin after gel strength (Ward and Courts, 1977). The viscosity for the samples was in the range of 6.5-9.5 cp. The viscosity was significantly ($p<0.05$) higher at 45^0C of Catla gelatin compared to 40^0C and 50^0C (Table 4.4d). 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, for rohu, common carp and grass carp it was 6.06 cP, 5.96cP and 7.07 cP respectively (Ninan *et al.,* 2011) and whereas for channel cat fish it was 3.23 cP (Yang *et al*., 2007), 5.24 cP, 3.40 cP for catfish and snakehead respectively (See *et al*., 2010). 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).

The pH of extracted gelatin varied between 3.66 and 5.43 (Table 2) indicating their category as Type B. This is because the pre-treatment method employed during the extraction process involves both alkaline and acid treatments. It has been reported that alkali pre-treatment results in Type B gelatin with pH in the range of 4.7 to 5.7 (Baziwane and He, 2003) and in the present study an alkali pre-treatment 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). Ninan *et al*., (2011) reported the pH range of 4.05-4.42 for rohu and Common carp and Grass carp respectively. pH range for snakehead and red tilapia were 5.39 and 5.50 reported (see *et al.*, 2010).

In the present study, isoionic points of gelatin extracted from catla gelatin were found to be in the range of 4.5-5 pI (Table 2). Generally, raw materials used to extract gelatin are pre-treated with either dilute acid or alkali solution. These two pre- treated methods produce two types of gelatins Type A and Type B. The Type A gelatin, produced by the acid pre-treatment is reported to exhibit pI range from 7 to 9; whereas Type B gelatin produced by the alkali processing, has an pI range of 4.8–5.1 (Cole, 2000).

In another study, Stainsby (1987) has reported that isoionic point of fish gelatin depending on the method in which the collagens are pre-treated, two different types of gelatin (each with differing characteristics) can be produced. The lower pI for bone gelatins may be attributed to the prolonged exposure of bones to acid treatment during demineralization, de-amidation of asparagines and glutamine occur during prolonged exposure of collagenous material to alkali, leading to decrease in pI value (Eastoe and Leach, 1977). In this experiment, the skins of Catla fish were pre-treated with alkali solution resulting the gelatin having pI value of about 4.5 to 5 which is in accordance with the value reported for Type B gelatin (Cole, 2000) However, Gudmundsson (2002) reported isoionic point (pI) values for megrim (9.5), tilapia (9.1), and cod (8.9). These values of pI were very high as compared to Catla fish gelatin.

In present study, hydroxyproline content of Catla skin gelatin extracted at different temperatures (40^0C, 45^0C and 50^0C) were found to be 6.53 mg/g, 8.87 mg/g and 7.53 mg/g respectively (Table 2). Hydroxyproline content was significantly ($p<0.05$) higher at 45 ^{0}C of Catla gelatin compared to 40 ^{0}C and 50 ^{0}C (Table 4.6b) 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 (Haug *et al.,* 2004; 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. Ninan *et al.,* (2011) reported that the hydroxyproline content in Rohu, common carp and grass carp skin gelatin were 7.90 mg/g ,7.78 mg/g and 11.66 mg/g respectively. While in Pink pearch skin and bone gelatin were 7.63 mg/g and 7.41 mg/g. In adult nile perch the hydroxyproline content were 9.76 mg/g (Muyonga *et al.,* 2004).

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). Cho *et al.,* (2006) stated that, the stability of collagen and gelatin is proportional to their total amino acid (Proline and Hydroxyproline) and glycine content. Ultimate gel strength is related to its imino acid and glycine content.

In present study, melting point of Catla skin gelatin extracted at different temperatures (40^0C, 45^0C and 50^0C) were found to be 21.7^0C, 24.50^0C and 23.43^0C respectively (Table 4.6). The melting point of Catla gelatin was significantly higher ($p<0.05$) at 45^0C compared to 40^0C, 50 ^{0}C (Table 2). 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) and hoki (16.6^0C) (Mohtar *et al*., 2010). However, these melting points were lower than that of black tilapia (28.9^0C) (Jamilah and Harvinder, 2002) which was warm water fish. Ninan *et al.,* (2011) reported the melting point in Grass carp, Rohu and Common carp were 29.1 ^{0}C,28.13 ^{0}C and 28.27 ^{0}C respectively, Melting point of grass carp 24.7 ^{0}C (Duan *et al*., 2010). Fish gelatin with lower melting temperature had a better release of aroma and offered stronger flavour and useful in the product development to control the texture and flavour release during mastication.

Choi and Regenstein (2000) reported that melting point increase with the maturation time and it has been observed that the levels of imino acids (proline and hydroxyl proline) contributed to the melting point characteristics (Gudmundsson, 2002).

Conclusion

On the basis of present study it was concluded that, extraction of Catla skin be could utilize for the extraction of gelatin. The skin was subjected for extraction of gelatin at three different extraction temperatures (40^0C, 45^0C and 50^0C). Among these three temperatures were tried the best temperature for extraction was identified on the yield and gel strength of the gelatin. The 45^0C temperature was found better results of functional properties of extracted gelatin from Catla. Though the gel strength of fish gelatin did not match that of mammalian gelatin, the optimized parameters gave reasonably good gel strength and yield. By changing the parameters, the gel strength of the gelatin could be controlled to yield the desired properties of fish gelatin made suitable for various applications.

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