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Functional Properties of Gelatin Extracted from Skin of Sole fish *(Cynoglossus macrostomus)* (Norman, 1928)

R. R. Sawant[1]*, S. Y. Metar[2], J. M. Koli[1], V. H. Nirmale[1], V. V. Vishwasrao[1] and G. S. Temkar[1]
[1] *College of fisheries, Shirgaon, Ratnagiri - 415 639, India*
[2] *Marine Biological Research Station, Ratnagiri - 415 612, India*

Abstract

Sole fish *(Cynoglossus macrostomus,* Norman, 1928*)* is a major by-catch of shrimp trawl and is a potential source of gelatin. Therefore a study on the extraction and determination of functional characteristics of gelatin obtained from the skin of sole fish was undertaken. Maximum yield obtained from skin of sole fish was 8.32% at 45ºC. The proximate composition of extracted gelatin was found to be comparatively better at 45ºC. The gelatin extracted from the skin had high gel strength at 45ºC than others. Hydroxyproline content was found to be in the range of 5.98-6.57 mg g^{-1} and highest content of hydroxyproline was obtained at 45°C temperature. Sole fish skin gelatin was found to have lower melting point than mammalian gelatin e.g. bovine and porcine gelatin. These promising finding may contribute to the on-going efforts for using fish gelatin as an alternative source for mammalian gelatins for various applications.

Keywords: Gelatin, sole fish, functional properties

Introduction

Annually, more than 100 million tons of fish are being harvested worldwide, 29.5% of the total catch is used for fishmeal (Kristinsson & 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 discards are excellent source of raw materials for the preparation of gelatin. Conversion of this waste into value-added products to yield additional income has both economic and environmental benefits for the fish industry (Choi & Regenstein, 2000).

Gelatin is a heterogeneous mixture of high molecular weight water soluble protein derived from collagen by heat denaturation. Gelatin is a gelling protein, which has widely been applied in the food and pharmaceutical industries. Traditional sources of gelatin are mainly pig skin and cow hide. For socio-cultural reasons, alternative sources are increasingly in demand (Gomez-Guillen et al., 2002). Gelatin from marine sources has been investigated as a possible alternative to bovine gelatin (George et al., 2009: 2011; Jayappa et al., 2013). One major advantage of marine gelatin sources is that they are not associated with the risk of outbreaks of bovine spongiform encephalopathy (mad cow disease) and religious reasons.

The shrimp trawling operations result in the landings of low value bycatch which are often thrown back to the sea. Malabar sole is an important low cost fish along the West Coast of India though there is no targeted fishery for the species. With increase in targeted fishery for shrimps, this species is also being heavily fished (Nair, 2007). Total catch of sole fish during year 2011 and 2012 was 61298 and 61859 t, respectively (CMFRI, 2013). Therefore, present study on the extraction and determination of the rheological properties of gelatin obtained from the skins of sole fish was undertaken.

Materials and Methods

Sole fish (*Cynoglossus macrostomus*) was collected from Ratnagiri Mirkarwada landing centre and skin was removed manually. The skin was washed and stored at –20ºC until further use. Gelatin was extracted following the procedure described by Koli et al. (2011). The ratio of skin to washing liquid used was 1 kg skin (wet weight) to 7 l of acid or alkali

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**E-mail: santoshmetar@gmail.com*

solution for each treatment. The skin 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, 45 and 50°C for 12 hrs as better yield and quality of gelatin is obtained at this range of temperature (Koli et al., 2011). The clear extract obtained was filtered with Whatman filter paper (no.1) using a Buchner funnel. The filtrate was then kept in tray and dried in oven at 60°C for 16 hrs. The thin film of dried matter was powdered, weighed packed in zip lock bags and stored at ambient temperature (25± 2°C) for further study.

The yield of gelatin was calculated using the following formula:

Yield of gelatin (%) =
(Weight of dry gelatin / Weight of fresh fish skin) ×100

Proximate composition was done as per AOAC (2005). The gelatin gel was prepared and the bloom value (gel strength) of gelatin gel was determined according to the method described by Wainewright (1977). Hydroxyproline content of gelatin was determined according to the method of Bergman & Loxley (1963) with a slight modification. Samples were hydrolyzed with 6 M HCl at 110°C for 24 h in reflex condenser and filtered 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 to a test tube and isopropanol (0.2 ml) 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 1.3 ml of Ehrlich's reagent solution (a mixture of solution 2 g of p-dimethylalamine benzaldehyde in 3 ml of isopropanol) was added. The mixture was heated at 60°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 558 nm using a spectrophotometer (Thermo spectronic, UV 10 rom 0628). Hydroxyproline standard solutions, with concentration ranging from 10 to 60 ppm, were also run simultaneously. Hydroxyproline content was calculated and expressed as mg g^{-1} sample.

The melting point measurement was done by a method described by Wainwright (1977) with slight modifications. 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, 45 and 50°C ±1°C (Cho et al., 2006). For determination of pH, the method of BS 757 (1975) was used. The method of Yasumatsu et al. (1972) was used to determine emulsifying capacity and stability.

Data was analyzed using appropriate statistical methods (Snedecor & Cochran, 1967; Zar, 1999). The differences between the treatments were determined by ANOVA. The differences between the means of treatments ($p<0.05$) were subjected to Standard Newman Kuels test.

Results and Discussion

The percentage yield of sole fish gelatin at different level of temperature is shown in the Table 1. The yield percentage at 45°C was significantly higher ($p<0.05$) than at 40°C but did not differ ($p>0.05$) from 50°C. The result showed that yield was found to be maximum (8.32%) at 45°C temperature and minimum (6.9%) at 40°C. The yield of gelatin have been reported to vary among fish species mainly due to differences in the collagen content, the composition of skin as well as the nature of skin matrix (Killekar et al., 2012; Cheow et al., 2007). Variations in the yield have also been reported due to differences in the diverse extraction methods followed (Gomez-Guillen et al., 2002; Jamilah & Harvinder, 2002; Muyonga et al., 2004).

Table 1. Yield of extracted gelatin from skin of Sole fish at different temperatures.

Factor	Extraction Temperature		
	40°C	45°C	50°C
Yield (%)	6.9±0.02[a]	8.32±0.03[b]	7.47±0.04[b]

The figures are based on three replicates and represented as Mean+SEM. The means with different superscripts [a,b,c] differed significantly ($p<0.05$)

Proximate composition of raw material and extracted gelatin are shown in Table 2. The gelatins extracted from skin of sole fish at different temperatures (40, 45 and 50°C) showed high values of proteins and low values for moisture, ash and fat content. Gelatin obtained at 45°C extraction temperature contained higher content of protein than other temperatures i.e. 88.73%. The protein content of gelatin varied from 72.63 to 89.7% for various fishes (Muyonga et al., 2004; Wangtueai & Noomhorm, 2009; See et al., 2010; Koli et al., 2011;

Killekar et al., 2012). Moisture, fat and ash content of all samples was well below the limit prescribed for edible gelatin i.e. 15% (GME, 2012).

In present study, gel strength of sole fish skin gelatin extracted at different temperatures (40, 45 and 50°C) were found to be 332.53 g, 342.86 g and 336.48 g, respectively. The gel strength was significantly ($p<0.05$) higher at 45°C when compared to 40°C and 50°C (Table 3).

The bloom value obtained in this study were higher than that of black king fish (222 g) (Killekar, et al., 2012), red tilapia (128.11 g) and black tilapia (180.76 g) (Jamilah & Harvinder, 2002), sin croaker (124.94 g) and short fin scad (176.92 g) (Cheow et al., 2007), Nile pearch (*Lates niloticus*) (229 g) (Muyonga et al., 2004), lizard fish (*Saurida spp.*) scales (268 g) (Wangtueai & Noomhorm, 2009), croaker (170g), big eye snapper (108 g) (Binsi et al., 2009) and pink perch (140 g) (Koli et al., 2011) reported and lower than that of yellow fin tuna (426 g) (Cho et al., 2005) and common carp (Ninan et al., 2011). 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 to be widely varying *viz.,* 124 to 426 g, compared to 200-300 g for bovine or porcine gelatin (Karim & 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 & 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 to 25% for warm-water fish gelatins, and 17% for cold water fish gelatins (Muyonga et al., 2004).

Viscosity of Sole fish skin gelatin extracted at the same shear rate at different temperatures (40, 45 and 50°C) was found to be 14.2 cP, 14.7 cP and 14.4 cP, respectively (Table 3). Viscosity is the second most important commercial property of gelatin after gel strength (Ward & Courts, 1977). The viscosity of sole fish gelatin was significantly ($p<0.05$) higher at 45°C compared to 40 and 50°C at the concentration of 6.67% (w/v). Viscosity is partially controlled by molecular weight and molecular size distribution (Sperling, 1985). Killekar et al. (2012) reported a viscosity value of 13.53 cP for black kingfish skin gelatin at 45°C. Jamilah & 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. Muyonga *et al.,*(2004) reported that the viscosity of Nile perch (*Lates niloticus*) was found to be 21.6 cP at 50°C temperature. Wangtueai & Noomhorm, (2009) reported viscosity of lizard fish (*Saurida* spp.) scales were found to be 4.22cP. Pranoto (2006) reported viscosity of tilapia and snapper gelatin as 8 cP and 3.67 cP, respectively. Koli et al. (2011) reported viscosity of tiger-toothed croaker and pink perch gelatin were found to be 10.53 cP and 8.47 cP, respectively.

The pH of extracted gelatin varied between 3.86 to 5.18 (Table 3). It has been reported that Type B gelatin with alkali pre-treatment showed pH in the range of 4.7 to 5.7 (Baziwane & He, 2003). Employing the same treatment, Cole (2000) observed that the viscosity is minimum and gel

Table 2. Proximate composition of sole fish and skin gelatin extracted at different temperatures.

Proximate composition	Sole fish raw skin	Sole fish skin gelatin at different temperature		
		40°C	45°C	50°C
Protein (%)	23.45±0.03	86.57±0.08^{a}	88.73±0.04^{b}	87.74±0.04^{c}
Fat (%)	2.76±0.08	1.51±0.06^{b}	1.30±0.10^{a}	2.15±0.08^{b}
Moisture (%)	70.79±0.01	9.78±0.03^{a}	8.26±0.3^{b}	7.66±0.02^{b}
Ash (%)	3.03±0.05	2.12±0.04^{a}	1.70±0.03^{b}	2.45±0.06^{c}

The figures are based on three replicates and represented as Mean+SEM. The means with different superscripts ^{a,b,c} differed significantly ($p<0.05$)

Table 3. Functional properties of sole fish gelatin

Sr. No.	Functional Properties	Extraction Temperature		
		40°C	45°C	50°C
1	Gel strength/ Bloom value (gm)	332.53±0.05[a]	342.86±0.09[b]	336.48±0.2[c]
2	Viscosity (cP)	14.2[a]	14.7[b]	14.4[b]
3	pH	3.86±0.05[a]	4.35±0.05[b]	5.18±0.04[c]
4	Isoionic point (pl)	3.5[a]	4[b]	3.5[a]
5	Hydroxyproline content (mg g^{-1})	6.32±0.08[a]	6.57±0.04[b]	5.98±0.08[c]
6	Melting point (°C)	19.6±0.15[a]	20.2± 0.09[b]	19.8±0.17[a]
7	Emulsifying capacity (%)	46.25±0.06[a]	58.84±0.05[b]	56.25±0.06[c]
8	Emulsifying stability (%)	17.64±0.01[a]	29.41±0.06[b]	20.02±0.20[c]

The figures are based on three replicates and represented as Mean+SEM. The means with different superscripts [a,b,c] differed significantly ($p<0.05$)

strength is maximum at pH 5.0 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 & Harvinder, 2002). Killekar et al. (2012) reported the pH of black kingfish was 4.81. Pranoto, (2006) reported the pH of tilapia and snapper were 4.23 and 4.83, respectively. Ninan et al. (2011) reported the pH range of 4.05-4.42 for rohu, common carp and grass carp, respectively. The pH range for snakehead and red tilapia skin were 5.39 and 5.50 (See et al., 2010).

The isoionic points of gelatin extracted from skin of sole fish were found to be in the range of 3.5 to 4 (Table 3). Generally, raw materials used to extract gelatin are pre-treated with either dilute acid or alkali solution. These two pre- treatment methods produce two types of gelatins, *viz.,* Type A and Type B. Type A gelatin, produced by the acid pre-treatment is reported to have pI range from 7 to 9; whereas Type B gelatin produced by the alkali processing, has a pI range of 4.8–5.1 (Cole, 2000). Stainsby (1987) has reported that two different types of gelatin each with differing characteristics can be produced, depending on the method in which the collagens are pre-treated. The lower pI for bone gelatins may be attributed to the prolonged exposure of bones to acid treatment during demineralization, as de-amidation of asparagines and glutamine occur during prolonged exposure of collagenous material to alkali, leading to a decrease in pI value (Eastoe & Leach, 1977). In this experiment, the skins of sole fish were pre-treated with alkali solution resulting in gelatin having pI value of about 3.5 to 4 which is in accordance with the value reported for Type B gelatin (Cole, 2000).

In the present study, hydroxyproline content of sole fish skin gelatin extracted at different temperatures (40, 45 and 50°C) was found to be 6.32 mg g^{-1}, 6.57 mg g^{-1} and 5.98 mg g^{-1}, respectively (Table 3). Hydroxyproline content was significantly ($p<0.05$) higher at 45°C for sole fish gelatin compared to 40 and 50°C which were lesser than the gelatin extracted from skate skin, 8.44 mg g^{-1} (Cho et al., 2006) and cod skin, 8.30 mg g^{-1} (Gomez-Guillen et al.,2002). Skate and cod having leathery skin which can yield more hydroxyprolin content. Gelatin with high levels of imino acids (hydroxyproline and proline) 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).

Killekar et al. (2012) reported a hydroxyproline content of 8.34 mg g^{-1} in black kingfish skin gelatin. Koli et al. (2011) reported that hydroxyproline content in tiger-toothed croaker skin and bone gelatins was 7.77 mg g^{-1} and 7.51 mg g^{-1}, respectively, while in pink perch skin and bone gelatin it was 7.63 mg g^{-1} and 7.41 mg g^{-1}, respectively. The hydroxyproline content of lizard fish (*Saurida* spp.) scales and adult nile perch were found to be 3.92 and 9.76 mg g^{-1} respectively (Wangtueai & Noomhorm, 2009; Muyonga et al., 2004). Strength of gelatin gel is influenced by amino acid compo-

sition 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 & Regenstein, 2000).

Melting point of sole fish skin gelatin extracted at different temperatures (40, 45 and 50°C) were found to be 19.6, 20.2 and 19.8°C, respectively (Table 3). The melting point of sole fish gelatin was significantly higher ($p<0.05$) at 45°C compared to 40 and 50°C. 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°C and 32.3°C, respectively (Gudmundsson, 2002). The melting points observed in the present study were far higher than those reported for cold water fishes such as cod (13.8°C) and hake (14°C) (Gomez-Guillen et al., 2002). However, these melting points were lower than that of black kingfish (22.1°C) (Killekar et al., 2012), tilapia and snapper (25.5 and 24.3°C) (Pranoto, 2006), tuna (24.3°C) (Cho et al., 2005), Nile perch (24.7-26.3°C) (Muyonga et al., 2004), black tilapia and red tilapia (28.9 and 22.45°C) (Jamilah & Harvinder, 2002) which were warm water fish. Ninan et al. (2011) reported the melting point in grass carp, rohu and common carp were 29.1, 28.13 and 28.27°C respectively. Similarly, Duan et al., 2010 reported a higher value of 24.7°C for grass carp skin. Fish gelatin with lower melting temperature had a better release of aroma and offered stronger flavour and may prove useful in the product development to control texture and flavour release during mastication (Choi & Regenstein, 2000). The melting point increase with the maturation time and it has been observed that the levels of amino acids (proline and hydroxyl proline) contributed to the melting point characteristics of skin (Gudmundsson, 2002).

Emulsifying capacity of sole fish gelatin extracted at different temperatures (40, 45 and 50°C) was found to be, 46.25, 58.84 and 56.25%, respectively while emulsifying stability was found to be, 17.64, 29.41 and 20.02%, respectively. Killekar et al. (2012) reported emulsifying capacity and stability of black kingfish at 45°C as 55.66 and 32.5% respectively. There is a growing trend within food industry to replace synthetic emulsifiers with more natural counterparts (Garti, 1999). Protein extracted from a variety of natural sources can be used as emulsifiers in food because of their ability to facilitate the formation of stable emulsion and impart desirable functional characteristics to the food system (Dickinson & Helene, 2001; MaClements, 2004). The functional properties of gelatin depend on several factors including the method of preparation and the intrinsic characteristics of collagen (Badii & Howell, 2006). Wassava et al., (2007) reported emulsifying capacity of Nile perch, grass carp in the range of 20-21%.

There are different types of food grade emulsifiers and biopolymers which could be used in various food applications. Mechanism of generating the emulsion system is attributed to adsorption of peptides on the surface of freshly formed oil droplets during homogenization and formation of a protective membrane that inhibits coalescence of the oil droplet (Dickinson et al., 1988; Avena-Bustilos et al., 2006). A major potential advantage of proteins as emulsifiers in foods is their ability to protect lipids from iron catalysed oxidation (Surh et al., 2005). At pH values below their isoelectric point (pI), proteins form positively charged interfacial membranes around oil droplets, which electrostatically repel any Fe^{2+} and Fe^{3+} ions present in the aqueous phase, thereby preventing oxidation of polyunsaturated lipids within the droplets (Surh et al., 2005).

It can be concluded that sole fish skin can be used as a material for gelatin extraction because of its high yield, protein content, gel strength and viscosity. The extracted gelatin showed the superior functional properties, and hence that can be used in food industry as an alternative to mammalian gelatin.

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