

## Optimization of Fish Gelatin Extraction from *Pangasianodon hypophthalmus* and *Protonibea diacanthus* Skin and Bone

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### ABSTRACT

Demand of gelatin for food and pharmaceutical application is increasing because it is one of the most widely used food ingredients. The main present study is that extraction of fish gelatin from *Pangasianodon hypophthalmus* and *Protonibea diacanthus* (skin and bone). The gelatin was extracted by six different methods and best method was found<sup>21</sup>, further modified to achieve better gelatin yield and gel strength by Alkali digestion (0.23% Sodium hydroxide) and pre treatment followed by acid treatment first with 0.23 % Sulfuric acid and second 0.90% Citric acid for 45 min then extraction was followed at controlled temperature at 50 °C for 14 hrs and dried in hot air oven at 60 °C for 18 hrs. The skins of *P. hypophthalmus* and *P. diacanthus* yielded 17.29 and 17.52% gelatin, where as their bones yielded 14.16 and 14.24% gelatin respectively. Gel strength of gelatins from the skin and bones of *P. diacanthus* 320.79 and 290.11g respectively were found higher than that of *P. hypophthalmus* 309.52 and 282.09g respectively. The gelatin from *P. diacanthus* was found to possess the highest rheological properties compared to the other. It had the following Properties Viscosity skin 34.86 and bones 34.00 cP. Melting temperature skin 29.13 and bone 26.92 °C. Incidentally was found high gelation temperature in *P. hypophthalmus* skin 13.06 and bone 13.71 °C. Furthermore, the gelatin from skin and bone also showed a relatively good source yield, gel strength and rheological properties.

**Key word:** *Pangasianodon hypophthalmus*, *Protonibea diacanthus*, Yield, Gel strength, Viscosity.

### INTRODUCTION

Presently India is the second largest fish producing and second largest aquaculture nation in the world after China. The total fish production during 2015-16 was 10.79 million metric tonnes. In which contribution of inland and marine sector were 7.21 MMT and 3.58

MMT, respectively. The fish production during first three quarters of 2016-17 has also shown an increasing trend and is estimated to be 8.18 million tonnes (Provisional). The fish production has increased from 3.84 MMT in 1990-91 during 10.79 MMT in 2015-16 (P).

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The growth in fish production has shown a cyclical pattern with an increasing long term trend<sup>15</sup>. This contribution would have been much greater if the animal by-products had also been utilized efficiently. Efficient utilization of by-products has direct impact on the economy and environmental pollution of the country. Non-utilization or under utilization of by-products not only lead to loss of potential revenues but also lead to the additional cost of disposal of these products<sup>27</sup>.

Processing discards from fisheries account for much as 70-85% of the total weight of catch<sup>1</sup>, and 30% of the fish waste is in the form of bones and skins<sup>20</sup>. Commonly, the fishery by-products are discarded as waste all over the world that causes serious environmental problems and economic losses<sup>34,6</sup>. It is estimated that fish waste production is between 17.9 and 39.5 million tons per year, representing an important loss of valuable nutrients. The utilization of by-product is an important cleaner production opportunity for the industry, as it can potentially generate additional revenue as well as reduce disposal cost for these materials. A large use of fish processing wastes is in the production of fish meals and fish oils for aquaculture and animal feeds. There is a need for specialized aquaculture feed ingredients that can be blended with plant proteins to enhance the nutrition and palatability properties<sup>8</sup>. Besides, the utilization of by-catch and discards obtained from fishing and the wastes from fish processing industries for the production of gelatin fulfils the sustainable management policy of responsible fisheries<sup>51</sup>. These waste are excellent raw material 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<sup>13</sup>.

Although most of the commercial gelatins today are derived from mammalian sources, mainly from pigskin and cowhide but, for many socio-cultural reasons, alternative sources are increasingly in demand. Among such reasons for having alternatives are

religious beliefs such as Judaism and Islam, and diseases such as bovine spongiform encephalopathy (BSE), also known as “mad cow disease”, which has brought wide attention because of the apparent transmissibility and lethality to humans, and foot and mouth diseases<sup>12</sup>. The global demand for gelatin has shown an increasing trend in years. The recent reports indicate that the annual world production of gelatin is nearly 326,000 tonnes, with pig skin-derived gelatin according for the highest (44%) output, followed by bovine hides (28%), bovine bones (27%), and other sources (1%)<sup>55</sup>. Other sources, which include fish gelatin, accounted for around 1.5% of total gelatin produced action in 2007, but this percentage was double that in 2002, indicating that gelatin production from alternative non-mammalian species had grown importance<sup>19</sup>. This may be due to the shortage of the primary raw material mostly cattle hides, bones and pig skins<sup>57</sup>.

Not a naturally occurring protein, gelatin is derived from the fibrous protein collagen, which is the principal constituent of animal skin, bone, and connective tissue. Gelatin is produced via the partial hydrolysis of native collagen. During the manufacturing of gelatin, raw animal material is treated with dilute acid or alkali, resulting in partial cleavage of the crosslinks: the structure is broken down to such an extent that “warm-water-soluble collagen”, i.e. gelatin, is formed<sup>52</sup>.

Gelatin is a substantially pure protein food ingredient, obtained by the thermal denaturation of collagen, which is the structural mainstay and most common protein in the animal kingdom<sup>4</sup>. Gelatin is a high molecular weight polypeptide and an important hydrocolloid, which has proved popular with the general public and finds its uses in a wide range of food products largely because of its gelling and thickening properties. It differs from other hydrocolloids because most of them are polysaccharide, whereas gelatin is a digestible protein containing all the essential amino acids except tryptophan. The amino acid composition

particularly with respect to proline and hydroxyproline can vary from species to species, as a result of exposure to a wide range of environmental conditions, particularly temperature<sup>38</sup>.

The Pangasius catfish (*Pangasianodon hypophthalmus*) is a freshwater fish belongs to the order Siluriformes and is a member of family Pangasiidae. It is one of the important species of fish in tropical and subtropical country like India<sup>17</sup>. It has become an important fish for many countries like Vietnam, Indonesia, Malaysia and China. Total pangasius production in India during 2009-10 was 3,01,066 tonnes<sup>48</sup>. The *Pangasius hypophthalmus* fillet accounts for 33-38% and the left-over is the by-product. The large amount of waste was head, bones, skins and fat while fish oil take over 15.3% of fish weight. There was over 200 thousand tonnes of pangasius fish oil that were not enhanced the value and utilized effectively every year<sup>40</sup>. Traditionally, all byproducts are used for fishmeal production<sup>60</sup>. *Pangasius hypophthalmus* by-products have been used as raw materials for production of gelatin and collagen. These products have been proven to have nutraceutical and functional properties and have been widely used in food, cosmetics and medicine.

The Blackspotted croaker (*Protonibea diacanthus*) is a popular warm water marine fish which is well-accepted by consumers. This species belongs to the order Perciformes and is a member of family Sciaenidae. It is distributed all along the tropical regions of the Indo-West Pacific Ocean. Estimated landing of croakers in India was 1, 57, 793 tonnes in the year 2016<sup>14</sup>. These fishes are usually processed into skinless fillets in chilled form for export, resulting in the production of large quantities of skins and bones as waste material. The blackspotted croaker skins and bones as a prospective source to produce gelatin in good yield with desirable quality attributes comparable to commercially available mammalian gelatins as determined previously<sup>25</sup>. The utilization of skins and bones from blackspotted croaker for the extraction of

gelatin need to be addressed since it adds to the economic returns of the fishing industry and in turn reduces the pollution caused by the discarded skins and bones.

Therefore, the present study aims to determine the best method for extraction of gelatin from skin and bone resulting from fish processing waste and fish market waste using *Pangasianodon hypophthalmus* and *Protonibea diacanthus*. Six different method extractions were compeered in preliminary study to determine the best method depends on yield and gel strength. The methods were further optimized for use in gelatin extraction of fish skin and bones from *P. hypophthalmus* and *P. diacanthus* optimize, characterization, rheological and functional properties of gelatin extracted from skins and bones of the *P. hypophthalmus* and *P. diacanthus*.

## MATERIAL AND METHODS

### Raw material and chemicals

In the present study, freshwater and marine water species were chosen namely, Freshwater *Pangasinodon hypothlamus* and marine *Protonibia dicanthus*. The samples were bought randomly from the Ratnagiri fish market as a whole and transported with equal volume of ice to the laboratory and stored at -20°C until processed for further preparation. Acetic acid, calcium hydroxide, hydrochloric acid, lactic acid, sodium hydroxide, sodium chloride, Hydrogen peroxide, Citric acid and sulfuric acid were purchased from MERCK (India). All chemicals used were of analytical grade.

### Appropriate method to extract gelatin

Six different methods are used for gelatin extraction from published literature were considered. Along with these methods the best method of extraction was identified on the basis of yield and gel strength of the gelatin obtained. The following methods were used for the extraction of gelatin. The following six methods were used for the both fishes skin and bone gelatin extraction.

### Determination of Koli<sup>35</sup>,

The procedure described by Koli<sup>35</sup>, originally used for extracting gelatin from *Nemipterus*

*japonicas* and *Otolithes ruber*. Following this method the skins and bones of *Pangasinodon hypoptlumus* and *Protonibia dicathus* were used to extract gelatin. Thawed skin and bones were thoroughly cleaned with excess water to remove superfluous material. The clean materials then sequentially soak with 0.2% (w/v) sodium hydroxide, 0.2% (w/v) sulphuric acid and 1.0% (w/v) citric acid for 40 min. After each soaking treatment, the skins and bones were washed under running tap water until they had a pH of about 7 before transferring to new solution. This cycle were repeated three times with a total time of 2 h for each set of treatment. 1 kg of skin and bone was mixed with the 7 liter acid or alkaline solution for each treatment. After that, skins and bones were subjected to a final wash with distilled water to remove any residual matter. The final extraction was under temperatures at (45 °C) for 12 hr. After obtaining the clear extract, it were filtered with Whatman filter paper (no. 1), using a Buchner funnel. The filtrate was kept for drying in hot air oven at 60 °C for 16 h. The thin film of dried matter was powdered, weighed and packed in Zip pack bags, store at ambient temperature (25 ± 2 °C) for further study.

#### Determination of Ninan et al.<sup>44</sup>,

The procedure described by Method Ninan et al.<sup>44</sup>, originally used for extracting gelatin from fresh water fish Rohu (*Labeo rohita*), Common carp (*Cyprinus carpio*) and Grass carp (*Ctenopharyngodon idella*). Following this method the skins and bones of *Pangasinodon hypoptlumus* and *Protonibia dicathus* were used to extract gelatin. The clean and drain fish skins was given a pretreatment with an alkaline solution followed by an acid solution. Cleaned skins were soaking with 0.2% (w/v) sodium hydroxide solution for 45 min. It was followed by soaking with 0.2% (w/v) sulfuric acid for 45 min. This followed by soaking with 1.0% (w/v) citric acid. After each treatment, the skins were washing under running tap water until they had a pH near to 7. Each soaking and washing treatment was repeated for about two times. Then, the ratio of skin to alkali/acid

solution was 250 g wet weight of the skin to 1.5 L of solution. The skins were subjected to a final wash with distilled water before the final extraction. The final extraction were carried out in distilled water at controlled temperature of 45 °C by using a water bath (Bio-Technics') for 10 h. The ratio used was 250 g wet weight of the skin and bone to 1.5 L of distilled water. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No. 1); hot air oven dried, powdered and packed in air tight container.

#### Determination of Benjakul et al.<sup>7</sup>,

Method of Benjakul et al.<sup>7</sup>, originally used for extracting gelatin from the skins of big eye snapper, (*Priacanthus tayenus* and *Priacanthus macracanthus*). Following this method skins and bones of *Pangasinodon hypothlumas* and *Protonibia dicanthus* were used for extraction gelatin. To extract gelatin, initially the skins and bones were soaked in 0.025 M NaOH solution with a skins and bones to solution ratio of 1:10 (w/v). Then the mixture was stirred for 2 h at room temperature 25-28 °C. The alkaline solution was changed every hour to remove non-collagenous proteins and pigments. Alkaline-treated skins were then washed with tap water until neutral or faintly basic pH of wash water was obtained. The skins were then soaked in 0.2 M acetic acid with a skin-bone solution ratio of 1:10 (w/v) for 2 h with gentle stirring. The acid solution was changed every 40 min allow to swelling of the collagenous material in the fish skin-bone matrix. Acid-treated skins and bones were washed thoroughly as previously described. After swelling, the swollen fish skins and bones were soaked in 10 volumes (w/v) of distilled water (45 °C) for 12h with an occasionally stirring. The mixture was then filtered using two layers of cheesecloth. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (no.1); hot air oven dried, powdered and packed in air tight container.

#### Rahman et al.<sup>47</sup>,

Method of Rahman et al.<sup>47</sup>, originally used for extracting gelatin from the Yellowfin tuna

fish. Following this method gelatin was extracted from the skins and bones of *Pangasinodon hypophthalmus* and *Protonibia dicanthus*. In this method the skins and bones were washed in tap water and 0.5 M NaCl. They were then soaked in 0.1 M NaOH for 40 min. at 20 °C, rinsed with tap water and extracted in 0.1M acetic acid for 18 h at 50 °C. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No. 1), hot air oven dried, powdered and packed in air tight container.

#### Determination of Liu et al.<sup>39</sup>,

Method of Liu et al<sup>39</sup>, extracted gelatin from the channel catfish (*Ictalurus punctatus*). Following the same method, the skins and bones of *Pangasinodon hypophthalmus* and *Protonibia dicanthus* were treated with 10 volumes (w/v) of 0.1% calcium hydroxide solution for 72 h. The treated skin and bone was washed with distilled water and 1 M H<sub>2</sub>SO<sub>4</sub> was used to neutralize calcium hydroxide in the skin and bone. Then the skins and bones were washed again to remove deposition of calcium sulphate. Gelatin was subsequently extracted from skins and bones in distilled water at temperatures from 45 °C for 6 h. The extracted solution was filtered through Whatman No. 1 filter paper, concentrated with a rotary evaporator. The remainders of concentrated solution were hot air oven dried until moisture was less than

10% dry gelatin was then ground and packed in air tight container.

#### Determination of Grossman and Bergman<sup>21</sup>

The procedure described by Method Grossman and Bergman<sup>21</sup>, originally used for extracting gelatin from Tilapia skins *Oreochromis niloticus*. Following this method the skins and bones of *Pangasinodon hypophthalmus* and *Protonibia dicanthus* were used to extract gelatin. The fish thawed prior and remove the skin and bones were to the experiments. The accurately weighed 100 g of each of fish skins and bones were cleaned and washed with tap water to remove superfluous materials. The fish skins and bones were soaked in 0.25% (w/v) sodium hydroxide for 40 minutes. After washing out sodium hydroxide, two successive acid incubations were performed, each for 40 min, first in a sulphuric (0.25%, v/v) and then in a citric acid solution (1.1 %, w/v). The acid solutions were drained and then samples were washed with cold water till neutral pH. The final extraction of gelatin was performed in distilled water at 45 °C for 18 h. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No. 1), followed by hot air oven dried and made powder by pestle and mortar and packed in air tight container.

#### Yield of extracted Gelatin

The yield of gelatin was calculated based on wet weight of fresh skin using the following the formula described by Muyonga et al.<sup>42</sup>:

$$\text{Yield of skin gelatin} = \frac{\text{Weight of vacuum oven dried skin gelatin}}{\text{Wet weight of fresh skin}} \times 100$$

$$\text{Yield of bone gelatin} = \frac{\text{Weight of vacuum oven dried bone gelatin}}{\text{Wet weight of fresh bone}} \times 100$$

#### Determination of Gel strength (Bloom value)

The gelatin gel was prepared and the bloom value (gel strength) of gelatin gel was determined according to the method described by Wainewright<sup>61</sup>. The gel was prepared in bloom jar (SCHOTTGLAS) Bloom test vessel

dissolving a 6.67% (w/v) dry gelatin powder in distilled water at 60 °C. Then it was cooled for 15 minutes at room temperature and kept for 18 h at 7 °C for maturation. Gel strength was determined on Texture Analyzer (Pertan TexVol instrument) according to British Standard BS 757<sup>9</sup>, with a load cell of 5 kg,

cross-head speed 1 mm s<sup>-1</sup> and using a 0.5 inch diameter, flat bottomed plunger. The standard glass bloom jar was placed centrally under the plunger and the penetration test was then performed. The maximum force (in g) was determined till the probe penetrated into the gel to a depth of 4mm. Measurements were done in triplicate using similar jars and the maximum force of penetration was recorded and used for analysis of gel strength.

#### Determination of Viscosity

Viscosity of gelatin sample was determined according to the method of Cho et al.<sup>12</sup>. Gelatin solutions (10g/ 100ml) were prepared by dissolving the dry powder in distilled water and heating at 60 °C. 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±1 °C. The viscosity was read and reported in terms of cP.

#### Determination of Gelation/ Melting temperature<sup>61</sup>

The melting point measurement was done by a method modified from<sup>61</sup>. Gelatin solutions, 6.67% (w/w), were prepared and a 5ml aliquot of each sample was transferred to a small glass tube (borosilicate tube, 12 mm x 75 mm.). 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 °C for 15 min. The tubes were immediately cooled in ice-chilled water and matured at 10 °C for 18 h. Five drops of a mixture of 75% chloroform and 25% reddish brown dye (food color) was placed on the surface of the gel. The gels were put in a water bath at 10 °C and the bath was heated at the rate of 0.2 to 0.4 °C/min. The temperature of the bath was read using an electronic digital thermometer (Fisher Scientific, Germany). The temperature at which the dye drops began to move freely down the gel was taken as the melting point.

#### Statistical analysis

The yield and gel strength of gelatin extracted from *Pangasinodon hypoptilumus* and *Protonibia dicanthus*. All the tests were carried out at p<0.05 significance level. Statistical package used in the study was SPSS

version 22.0. Since the variances of yield and gel strength data obtained by different extraction method failed in the Levens test for homogeneity of variances, the non-parametric test namely Kruskal-Wallis H was followed to compare the means. For optimization method where variances are expecting low, to compare means of measurements one-way ANOVA was used. All data represented are the means of triplicates.

## RESULT AND DISCUSSION

#### Selection of most appropriate method

Six different methods for extraction were used for extracting gelatin from *P. hypophthalmus* and *P. dicanthus* skin and bone. The yield of gelatin extracted from *P. hypophthalmus* skins and bones were varied in between 5.25 to 16.96% and 4.12 to 12.47% respectively. While the gel strength of *P. hypophthalmus* skins and bones gelatin were varied in between 175.13 to 290.75g and 150.14 to 275.58g respectively (Table 1). The results of yield of *P. dicanthus* skins and bones gelatin were varied in between 8.47 to 18.41% and 5.92 to 13.41%. While the gel strength of *P. dicanthus* skins and bones were varied from 180.11 to 290.11 and 159.58 to 276.49g respectively (Table 2). Gel strength was measured by Texture analyzer.

Given that the six methods of extraction were fairly different, both the yields and the gel strengths expectedly varied widely. The statistical analysis for homogeneity of variance before applying any parametric test failed. Therefore, nonparametric test has to be followed for comparing the means of the yields and that of the gel strengths. Six values of yield and gel strength in triplicate were ranked in Kruskal-Wallis test. The highest rank obtained with the method No. 6 by Grossman and Bergman<sup>21</sup>. and was considered as the best method. Incidentally, this method was found to be the best for both yields, gel strengths of both the fish species. Therefore this method was further optimized to check if the yield and gel strengths can be further improved.

**Table 1: Results of yield and gel strength of extracted gelatin from *Pangasianodon hypophthalmus* skin and bone**

Sr. No.	Methods	Yield (%)		Gel strength (g)	
		Skin	Bone	Skin	Bone
1.	Koli, (2011)	13.18±0.51 <sup>a</sup> (14)	8.08±0.16 <sup>a</sup> (14)	240.59±0.004 <sup>a</sup> (14)	190.10±0.001 <sup>a</sup> (11)
2.	Ninan et al. (2009)	11.52±0.062 <sup>a</sup> (11)	7.19±0.07 <sup>a</sup> (11)	210.53±0.012 <sup>a</sup> (8)	185.12±0.003 <sup>a</sup> (8)
3.	Benjakul et al. (2009)	8.01±0.11 <sup>a</sup> (5)	5.13±0.12 <sup>a</sup> (5)	201.13±0.004 <sup>a</sup> (5)	180.15±0.001 <sup>a</sup> (5)
4.	Rahman et al. (2008)	8.58±0.056 <sup>a</sup> (8)	6.18±0.05 <sup>a</sup> (8)	215.06±0.215 <sup>a</sup> (11)	195.92±0.001 <sup>a</sup> (14)
5.	Liu et al. (2007)	5.25±0.06 <sup>a</sup> (2)	4.12±0.03 <sup>a</sup> (2)	175.13±0.006 <sup>a</sup> (2)	150.14±0.003 <sup>a</sup> (2)
6.	Grossman and Bergman (1992)	16.96±0.02 <sup>b</sup> (17)	12.47±0.06 <sup>b</sup> (17)	290.75±0.001 <sup>b</sup> (17)	275.58±0.358 <sup>b</sup> (17)

Values are given as Mean ± SD from triplicate determinations (n=3) and bracket values are mean of ranks by Kruskal-Wallis test.

**Table 2: Results of yield and gel strength of extracted gelatin from *Protonibe dicanthus* skin and bone**

Sr. No.	Methods	Yield (%)		Gel strength (g)	
		Skin	Bone	Skin	Bone
1.	Koli, (2011)	15.35±0.35 <sup>a</sup> (14)	9.24±0.06 <sup>a</sup> (14)	280.15±0.004 <sup>a</sup> (14)	239.79±0.60 <sup>a</sup> (14)
2.	Ninan et al. (2009)	13.90±0.02 <sup>a</sup> (11)	8.26±0.04 <sup>a</sup> (11)	260.16±0.004 <sup>a</sup> (11)	224.28±1.02 <sup>a</sup> (11)
3.	Benjakul et al. (2009)	10.62±0.03 <sup>a</sup> (8)	7.39±0.05 <sup>a</sup> (8)	200.12±0.001 <sup>a</sup> (8)	181.02±0.58 <sup>a</sup> (8)
4.	Rahman et al. (2008)	10.50±0.06 <sup>a</sup> (5)	6.62±0.03 <sup>a</sup> (5)	199.16±0.061 <sup>a</sup> (5)	169.13±0.58 <sup>a</sup> (5)
5.	Liu et al. (2007)	8.47±0.03 <sup>a</sup> (2)	5.92±0.020 <sup>a</sup> (2)	180.11±0.001 <sup>a</sup> (2)	159.58±1.15 <sup>a</sup> (2)
6.	Grossman and Bergman (1992)	18.41±0.02 <sup>b</sup> (17)	13.41±0.02 <sup>b</sup> (17)	290.11±0.001 <sup>b</sup> (17)	276.49±1.19 <sup>b</sup> (17)

Values are given as Mean ± SD from triplicate determinations (n=3) and bracket values are mean of ranks by Kruskal-Wallis test.

### Optimization of selected method

In the present study, the sodium hydroxide concentration was tried within a range of 0.20% to 0.27% given that the alkali concentration was made 0.20% to 0.27% to increase or decrease, the subsequent sulfuric acid ( $H_2SO_4$ ) treatment was also changed stepwise to neutralize the increased alkali concentration (Table 3A and 3B). Use of high concentrations of sulfuric acid and sodium hydroxide as well as citric acid resulted in lowest value of gel strength. This indicated that the gel forming ability of gelatin was sensitive to acid and alkali hydrolysis as both affected cross-linking in the gelatin<sup>28</sup>. The gel

strength decreased with the increased of temperature and extraction time show in table 10A and 10B. Although gelatin can be extracted more easily at a higher temperature and with a longer treatment time, this severe condition would break the bonding and result in the release of free amino acid cusses reduction of gel strength<sup>11</sup>. Alkaline treated skins and bones were washed with tap water until neutral pH. The alkaline solution was changed three times to remove non-collagenous proteins and pigments<sup>7</sup>. Since optimization was done with minor variations in the concentrations of acid and alkali, their duration of contact and temperature, the

variation in gel strengths and yields were expected to be low and thus the Levine's test indicated homogeneity. Therefore the one way ANOVA, a parametric test could be applied to compare the means of yields and gel strengths.

### **Yield of gelatin**

The yield of gelatins obtained from *P. hypothalmus* and *P. dicanthus* skin and bone in this study is presented in Table (1, 2 and 3A, 3B). Skins in general yielded more gelatin than the bones. The highest percentage of gelatin recovery was obtained from *P. hypothalmus* and *P. dicanthus* skin with 17.19% and 18.59% respectively. This was followed by *P. hypothalmus* and *P. dicanthus* bone with recovery of gelatin 14.16% and 14.24% respectively. Fish skins are especially suitable as a source of gelatin because they can be easily extracted at relatively moderate temperature, 50°C or lower<sup>18,20,22,37</sup>. Ratnasari et al.<sup>49</sup>, reported that the different kind of skin, acid concentration, pH condition, the rate of collagen break down when washing treatment and swelling process were among the possible reaction for the high of gelatin yield from the three species of fresh water fish. While the highest yield was obtained from Catfish (*Clarias gariepinus*) skin yield 21.79%<sup>1</sup>. Shyni et al<sup>55</sup>. obtained yield of shark skin gelatin, tuna skin gelatin, Rohu skin gelatin were 19.7%, 11.3%, 17.2%, respectively. Pangas catfish (22%), Asian redtail catfish 21.28%, Striped snakehead 20.25% and Nile tilapia 21.93% respectively<sup>49</sup>.

In this study yield was noticeably high when compared to those reported in other studies such as gelatin from the Nile perch young fish skin gave a lower total gelatin yield 12.5% than Nile perch adult fish skin 16% respectively. While Nile perch young fish bone 1.3% and Nile perch adult fish bone 2.4% respectively Muyonga et al<sup>42</sup>. Koli, (2011) also reported that yield of Tiger toothed croaker skin and Pink perch skin 7.56% and 5.57% respectively. While yield of Tiger toothed croaker bone and Pink perch bone 4.57% and 3.55%. *Pagrus* cat fish bone yield was 6.12%<sup>3</sup>. Tabarestani et al<sup>58</sup>, reported that the different structural and physical properties of gelatin for the wide diversity among the fish

species present in their skin. Furthermore, the higher susceptible of the collagenous material from fish skin to degradation is due to the lower content in intra and inter chain non reducible cross links<sup>32</sup>. assumed that the yield and quality of gelatin are influenced by the species to species and age to age of the fish, extraction process and pretreatment temperature. The gelling properties of gelatin are influenced by the source of raw material, which vary in proline and hydroxyproline contents<sup>29</sup>. The extraction yield of gelatin from skins ranged from about 5.5% to 21% of the starting weight of the raw material<sup>21,26,56</sup>. The variation in such values depends on the differences in both the proximate composition of the skins and the amount of soluble components in the skins as these properties vary with the species and the age of the fish. In addition, variation in the extraction method can also have an effect on yields. Reporting gelatin yield as dry gelatin weight compared to the weight of wet skin is common, but not very reliable<sup>42</sup>. Water content may vary because of different treatments to the skin (freezing, salting, scraping, draining, etc.).

### **Gel strength (Bloom value)**

Gel strength is one of the most important physical and functional properties of a gelatin<sup>32</sup>. Gel strength which depends on the Isoelectric point, pH, molecular weight distribution and amino acid content has been categorized as (<150), medium (150-220) and high (220-300), but gelatins with gel strength of 250- 260g are the most desirable and suitable for a wide range of application in the food industry especially in the processing of jellies, canned meat, marshmallows and yoghurts<sup>43,55,41</sup>.

In the present study shows that from *P. hypothalmus* and *P. dicanthus* skin and bone yielded gelatins with different bloom value (1, 2 and 3A, 3B). The gelatin derived from *P. dicanthus* skin was found to be the strongest with a bloom strength value of 320.79 g and weak bloom value of 309.52 g was obtained from *P. hypothalmus* skin. On other hand, gelatins of bones from both species had weakest bloom value i.e. 290.11 g and 282 g, respectively (fig. 6).

Jakhar et al<sup>25</sup>. reported the gel strength of black spotted croaker was varied between 110.2 to 439 g. Similarly in the present study the gel strength of gelatin extracted from *P. dicanthus* skin and bone was varied between 320.79 and 290.11 g respectively. Cho et al<sup>12</sup>. reported that the gel strength of Yellow fin tuna skin was 426 g and Zhou and Regenstein for Pollock skin gelatin 460 g. Tuna (*Thunnus thynnus*) head bone gel strength was found 109g<sup>24</sup>, Atlatntic salmon 108g and cod 71g<sup>2</sup>, Sin croaker 125g, Short fin scad 177g<sup>10</sup>, Cuttle fish 181g<sup>5</sup>, Big eye snapper 106g, Brownstrip red snapper 219g<sup>29</sup>, and young and adult Nile perch 217g and 240g, respectively<sup>42</sup>, Hadder et al<sup>24</sup>, shown that the gelatin gel from tuna head bones was 109g. Taheri et al.<sup>59</sup>,also shown that lizard fish skin gelatin showed higher gel strength 159.1g than the bone gelatin 135g respectively, As compared with previous studies the gel strength of skin and bone gelatin were

recorded lowest and highest in the present study.

In the present study the gel strength of gelatin extracted from *P. hypothalmus* skin and bone was varied between 320.79 and 290.11 g was followed by however this result was higher than the gel strength of warm water fish gelatin were Nile tilapia 328g Songchotikunpan et al.<sup>56</sup>, grass carp 267g Kansakala et al.<sup>31</sup>, Red Tilapia 487g See et al.,<sup>54</sup>, Silver cat fish 585 Yang et al.<sup>62</sup>, The gel strength *P. hypothalmus* skin and bone was varied between 320.79 and 290.11 g was followed in this study was recorded high when compared to those reported in other studies such as gelatin from red tilapia 128.1g<sup>26</sup>, grass carp 267g<sup>31</sup>, Catla 180.76<sup>30</sup>. The differences in the value of gel strength in all studies could be explained by difference in the manufacturing process used and intrinsic properties of collagen which vary among fish species.

**Table 3: A Different treatments given to skin and bone of *Pangasianodon hypophthalmus* for extraction of gelatin and its properties**

Sr. No.	Treatments										Properties of gelatin			
											Yield		Gel strength	
	A	B	C	D	E	F	G	H	I	J	Skin	Bone	Skin	Bone
1.	0.20	0.20	0.80	40	1:6	12	45	1:2	50	16	16.4±0.48 <sup>ab</sup>	12.13±0.59 <sup>a</sup>	280.13±1.00 <sup>c</sup>	259.59±0.55 <sup>c</sup>
2.	0.23	0.23	0.90	45	1:7	14	50	1:3	60	18	17.29±0.59 <sup>b</sup>	14.16±0.38 <sup>b</sup>	309.52±0.58 <sup>d</sup>	282.09±0.60 <sup>d</sup>
3.	0.25	0.25	1.00	50	1:8	16	55	1:4	70	20	15.20±0.66 <sup>a</sup>	12.00±0.35 <sup>a</sup>	276.04±0.57 <sup>b</sup>	256.93±0.61 <sup>b</sup>
4.	0.27	0.27	1.10	55	1:9	18	60	1:5	80	22	15.13±0.54 <sup>a</sup>	13.3±0.61 <sup>ab</sup>	262.00±1.54 <sup>a</sup>	241.68±1.74 <sup>a</sup>

Values are given as Mean ± Standard deviation of triplicate determinations; values in the same column with different superscripts differed significantly (p<0.05)

**Table 3:B Different treatments given to skin and bone of *Protonibea dicanthus* for extraction of gelatin and its properties**

Sr. No.	Treatments										Properties of gelatin			
											Yield		Gel strength	
	A	B	C	D	E	F	G	H	I	J	Skin	Bone	Skin	Bone
1.	0.20	0.20	0.80	40	1:6	16	45	1:2	50	16	15.83±0.53 <sup>ab</sup>	11.51±0.53 <sup>a</sup>	274.12±1.00 <sup>b</sup>	262.43±1.15 <sup>b</sup>
2.	0.23	0.23	0.90	45	1:7	18	50	1:3	60	18	18.59±0.53 <sup>c</sup>	14.24±0.57 <sup>b</sup>	320.79±0.58 <sup>a</sup>	290.11±0.00 <sup>d</sup>
3.	0.25	0.25	1.00	50	1:8	20	55	1:4	70	20	17.52±0.98 <sup>bc</sup>	11.98±0.61 <sup>a</sup>	289.48±0.55 <sup>c</sup>	275.26±0.94 <sup>c</sup>
4.	0.27	0.27	1.10	55	1:9	22	60	1:5	80	22	14.88±0.52 <sup>a</sup>	11.55±0.54 <sup>a</sup>	269.45±0.57 <sup>a</sup>	257.11±1.73 <sup>a</sup>

Values are given as Mean ± Standard deviation of triplicate determinations; values in the same column with different superscripts differed significantly (p<0.05)

### Treatments

A: NaOH concentration (%), B: H<sub>2</sub>SO<sub>4</sub> concentration (%), C: Citric acid concentration (%), D: Pretreatment time (Min.), E: Skin/Water ratio for pretreatment, F:

Extraction time (hrs), G: Extraction temperature (°C), H: Skin/Water ratio for extraction, I: Drying temperature (°C), J: Drying time (hrs)

## Viscosity

Viscosity is a second most commercially importance physical properties of gelatin<sup>63</sup>. Viscosity of extracted gelatins *P. hypophthalmus* and *P. dicanthus* skin and bone were shown in Table 4. Viscosity was found higher *P. dicanthus* skin (34.86cP) and bone (34.00cP). while as compared to *P. hypophthalmus* skin and bone gelatins (28.07cP) and (27.02cP) respectively. These results were obtained higher and similar than the commercial and fish gelatin. The viscosity obtained from pangas catfish, Asian redtail Catfish, Nile tilapia, striped snakehead and commercial gelatin were 36.5cP, 23.5cP, 19.3cP, 31.5cP and 39.5, respectively Ratnasari et al<sup>49</sup>. Muyonga et al<sup>42</sup>, also reported that gelatin extracted from adult fish skins at 50 °C also exhibited higher viscosity (42.3mSt) than bone gelatins (28.2 and 30.mSt, respectively, young and adult Nile perch fish). The relatively low viscosity of *P. hypophthalmus* and *P. dicanthus* skin and bone compared to other kind of gelatin obtained from skate (22.5cP), Rohu 6.06cP and yellowfin tuna 7.17cP, Ninan et al.<sup>44</sup>, black kingfish skin gelatin 13.53cP Killekar et al.<sup>33</sup>. The viscosity of gelatin from non defatted seabass skin 24.59mPas Sea-leaw et al<sup>53</sup>.<sup>50</sup>, obtained from the skin gelatin of *P. pangasius*, *C. batrachus*, *C. micropeltes* and *C. striata* are 3.63cP, 2.37cP, 1.87cP and 3.17cP<sup>35</sup>. also reported viscosity of croaker skin and bone were 10.53cP 8.30cP, while those of Pink perch were 8.47cP and 6.8cP. The difference in viscosity of gelatins could be due to the differences in chain length or molecular size distribution. With lower average molecular weight of gelatin, the lower gel strength and viscosity of the solution were obtained<sup>63</sup>.

## Melting temperature

A melting point of *P. hypophthalmus* and *P. dicanthus* skin and bone gelatin found is shown in Table 4. The extracted of Melting point from *P. hypophthalmus* and *P. dicanthus* skin and bone were 26.15 °C, and 29.13 °C. While bones were 26.17 and 26.92 °C respectively. Koli et al<sup>36</sup>. obtained that melting

point of gelatin extracted from tiger toothed croaker and pink parch skin 20.36 °C, 19.23 °C respectively where as the respective where melting point for bones were 19.5 °C and 19.0 °C. (Kamble et al.<sup>30</sup>, reported that the melting point of catla skin gelatin extracted at 45 °C was found to be 24.50 °C<sup>50</sup>. reported that the melting point of gelatin of *P. pangasius* (29 °C). *C. bartrachus* (18.8 °C) *C. micropeltes* (29.67 °C), and *C. striata* (29.8 °C) respectively, but lower than those of commercial gelatin of bovine (33.8 °C). This finding is also lower than that reported by (Karim and Bhat, 2009) that fish gelatin melting temperature ranged between (11-28 °C). Gudmundsson<sup>23</sup>, according to environmental temperature affected the melting temperature of the gelatin produce. Different type of gelatin also resulted in different physico-chemical properties infusing thermal and rheological characteristics; melting temperature Norzih et al.<sup>45</sup>. Kamble et al<sup>30</sup>. Fish gelatin with lower melting temperature had a better release of aroma and offered stronger flavor and useful in the product development to control the texture and flavored release during mastication<sup>13</sup>. reported that melting point incres with the maturation time and it has been observed that the levels of imino acids ( proline and Hydroxyproline) contribute to the melting point characteristics<sup>23</sup>. This finding is also lower than that reported by<sup>32</sup>, that fish gelatin melting temperature ranged between (8-25 °C).

## Gelation temperature

Gelation temperature has been observed Table 4 that *P. hypophthalmus* skin and bone 13.06 and 13.71 °C respectively. However, in the *P. dicanthus* skin and bone gelatin were 11.83 and 12.45 °C respectively. In the *P. hypophthalmus* bone was found significantly higher gelation temperature<sup>50</sup>. reported that the gelling point of gelatin of *P. pangasius* (11.67°C), *C. bartrachus* (10 °C), *C. micropeltes* (9.68 °C) and *C. striata* (10.67 °C), respectively, but lower than those of commercial gelatin of bovine (15.67 °C). Ratnasari et al<sup>49</sup>. also reported the gelling temperature of Pangas cat fish gelatin (12 °C),

were higher compared to that Asian redtail catfish 10 °C), Nile tilapia (10 °C), and Striped snakehead (11 °C), but lower than commercial gelatin (15 °C), respectively<sup>16</sup>. reported the gelling time for *Carcharhinus amblyrhynchos* (shark) at (20 °C) was found to be 1 minutes whereas for *Sphyraena barracuda* (Sheela) was 20 minutes. The extracted gelatin has higher gelling temperature compared with

commercial gelatin due to the presence of impurities. Gudmunsddon<sup>23</sup>, according to environmental temperature affected the gelation temperature of the gelatin produce. Different type of gelatin also resulted in different physico-chemical properties infusing thermal and rheological characteristics; gelation temperature Norzih et al.<sup>45</sup>.

**Table 4: Rheological and functional properties of gelatin extracted from *P. hypophthalmus* and *P. dicanthus***

Source of gelatin		Viscosity (cP)	Melting temperature (°C)	Gelation temperature (°C)
<i>P. hypophthalmus</i>	<b>Skin</b>	28.07±0.15 <sup>a</sup>	26.15±0.34 <sup>a</sup>	13.06±0.13 <sup>ab</sup>
	<b>Bone</b>	27.02±0.39 <sup>a</sup>	26.17±0.44 <sup>a</sup>	13.71±0.83 <sup>b</sup>
<i>P. dicanthus</i>	<b>Skin</b>	34.86 ± 0.87 <sup>b</sup>	29.13±0.21 <sup>b</sup>	11.83±0.34 <sup>a</sup>
	<b>Bone</b>	34.00 ± 1.27 <sup>b</sup>	26.92±0.44 <sup>a</sup>	12.45±0.53 <sup>ab</sup>

Values are given as Mean ± Standard deviation of triplicate determinations; values in the same column with different superscripts differed significantly ( $p<0.05$ )

## CONCLUSION

On the basis of present study it was concluded that *P. hypophthalmus* and *P. dicanthus* skin and bone could be utilization extraction of gelatin among these six methods tried the best method of extraction was identified on the yield and gel strength of the gelatin and the method was subjected to minor modification to optimize parameter for these parameter raw materials. By using optimum parameters for extraction of good quality gelatin could be extracted from skin and bone both species.

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