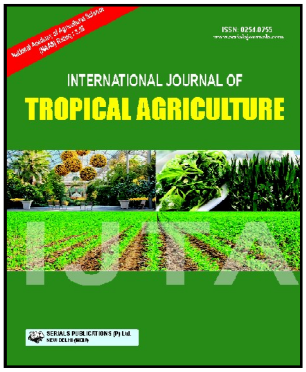

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Proximate Composition gelatin obtained from the skin and bone *Pangasianodon hypophthalmus*

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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 (skin and bone). The proximate composition of extracted gelatin from *P. hypophthalmus* skins and bone showed high value of protein and low value of moisture and fat content in skin gelatin as compared to bone gelatin. 88.51% in case of *P. hypophthalmus*.. Therefore it may be concluded that the protein content in gelatin extracted from skin and bone .

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). The growth in fish production has shown a cyclical pattern with an increasing long term trend15. 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. Processing discards from fisheries account for much as 70-85% of the total weight of catch and 30% of the fish waste is in the form of bones and skins20. Commonly, the fishery by-products are discarded as waste all over the world that causes serious environmental problems and economic losses 34,6. 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 byproduct 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. 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. 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.

MATERIAL METHOD

3.2.8 Determination of Proximate composition

3.2.8.1 Moisture content

The estimation of moisture content was done by following the method given by AOAC, (2012). In brief, 5 g of raw skin, bone and dry gelatin was weighed accurately in a pre-weighed petri dish. The dish containing the sample was placed in a hot air oven without lid. The temperature of the oven fixed at 100±5ÚC and the samples were kept for overnight (16 hours) drying. Dishes were taken out from the oven and cooled in desiccators at room temperature. Total moisture content was estimated with the formula given bellow:

$$Moisture\,(\%) = \frac{W_1 - W_2}{W_1 - W} \times 100$$

Where,

W = Weight (g) of empty dish

W_1 = Weight (g) of dish with material before drying

W_2 = Weight (g) of dish with material after drying

3.2.8.2. Protein estimation

Estimation of protein content of the raw skin, bone and dry gelatin 0.3 to 0.5g and fish of the moisture free gelatin sample was transferred into a digestion flask of 50 ml capacity. A few glass beads, a pinch of digestion mixture (8 part K_2SO_4 and 1 part $CuSO_4$) and 10ml concentrated sulphuric acid were added to the flask. It was digested over a burner until the solution turns colorless. The digest was transferred quantitatively into a 100 ml standard flask and made up to the mark. The 2ml of well-mixed made-up solution was transferred to the reaction chamber of the micro-kjeldahl distillation apparatus, 2 drops of phenolphthalein indicator and 40% sodium hydroxide were added till the indicator changes to pink. Distillation was done for 4 minutes and ammonia liberated was absorbed into 2% boric acid containing a drop of Tashiso's indicator. The amount of ammonia liberated was determined by titration with N/50 sulphuric acid. Percentage Crude protein was determined as:

% Crude protein = Nitrogen content X 5.4

5.4 is the nitrogen conversion factor as per (Eastoe and Eastoe ,1952)

Crude fat

The fat content was estimated by Soxhlet method (AOAC 2012). In brief, 5 g raw skin, bone and dry gelatin samples were weighed in a thimble and plugged with cotton. The thimble was then fixed in to Soxtech apparatus (Socsplus, Pelican India Ltd.). The fat was extracted with anhydrous ether (BP 40-60ÚC) for about 16 hours. After the extraction was complete, the thimble was removed and the solvent from the receiving flask was collected out by distilling it off, before it returned to the flask by siphoning. Thus, maximum possible amount of solvent was restored. Finally, the traces of solvent were removed from the flask by overnight drying it in oven at 100ÚC. After cooling the flask in the desiccators, its weighed was recorded. Final fat content was estimated by using the formula given bellow:

$$Crude\ fat\,(\%) = \frac{W_2 - W_1}{W} \times 100$$

Where,

W = Weight (g) of sample

W1 = Initial weight (g) of beaker

W2 = Final weight (g) of beaker

Crude ash

The estimation of ash content was done by following the method given by AOAC (2012). In brief, 5 g of sample was weighed accurately in a porcelain crucible and dried in a hot air oven overnight at temperature of 100±5°C. Sample were char dried completely by heating over a burner and incinerated in a muffle furnace at a temperature of 550±50°C with adequate air supply until it became completely white. After that the crucible was taken out and cooled in a desiccator at room temperature. Total ash content was estimated using the formula given below:

$$Total\ ash\,(\%) = \frac{W_2 - W}{W_1 - W} \times 100$$

Where,

W = Weight (g) of empty crucible

W_1 = Weight (g) of crucible with dried matter taken for test (sample wt.)

W_2 = Lowest weight (g) of the crucible with sample

RESULT AND DISCUSSION

Proximate composition of gelatin

4.4.1. Proximate composition and pH of extracted gelatin

The proximate composition of extracted gelatins is shown in Table 1. The gelatins extracted from both species showed high value of protein and low value of moisture and fat content in skin gelatins as compared to bone gelatins. The gelatin made from the skin of *P. diacanthus* and *P. hypophthalmus* had protein content of 89.15% and 88.51% respectively whereas the gelatin made from the bone *P. hypophthalmus* had protein content 87.44% and 85.62% respectively.

The proximate composition of skin and bone gelatin extracted from *P. hypophthalmus*. The moisture, ash, protein and fat content of *P. hypophthalmus* was significantly ($p<0.05$) different. The extracted skin gelatin showed high value of protein as compared to bone gelatin. *P. hypophthalmus* skin gelatin is 88.51% for *P. hypophthalmus* is bone 85.62%. It may be concluded that the protein content of the gelatin extracted from the skin gelatin higher than bone. Taheri *et al.* (2009) reported that the gelatin extracted from the skin and bone of Lizardfish (*Saurida tumbil*) was 83.94% and 81.89% respectively. Jakhar *et al.,* (2012) and Haddar *et al.,* (2011) reported that Blackspotted Croaker (*Protonibia diacanthus*) fish skin and Tuna (*Thynnus thynnus*) fish bone had protein content of 90.36% and 88.0% respectively. Protein content in gelatin preparation derived from skin and bones of young nile perch was 88.0% and 78.4% respectively (Muyonga *et al.,* 2004). Additionally, gelatin from skins of *Claris gariepinus*, *Catla catla*, *Pangasius sutchi* had protein content of 88.46%, 89.54% and 99.33% respectively (Kamble *et al.,* 2014).

The ash content of *P. hypophthalmus* and skin and bone gelatin observed as 2.10%, respectively. Taheri *et al.* (2009) also reported the ash content of 1.98% and 11.17% from Lizardfish (*Saurida tumbil*) skin and bone. Jakhar *et al.,* (2012) and Haddar *et al.*, (2011) assessed that Blackspotted Croaker (*Protonibia diacanthus*) fish skin and Tuna (*Thynnus thynnus*) fish bone had ash content of 0.93% and 0.68% respectively. Additionally, gelatin from skin of *Claris gariepinus*, *Catla catla*, *Pangasius sutchi* had ash content of 5.7%, 1.53% and 1.38% respectively (Kamble *et al.,* 2014). The difference in ash content may be due to mineral content in gelatin and variation in its extraction method (Jakhar *et al.*, 2012). Usually ash content up to 3.0% can be accepted in food application (GME, 2012). However, ash content in *P. hypophthalmus* bone is in the range of 3.50% which were higher than the recommended maximum limits indicating non viability of gelatin from bone for utilization as food ingredient.

In the present study, moisture content of gelatin extracted from *P. hypophthalmus* skin and bone was observed as 8.94%, 9.65% and 7.97% 9.57% respectively. Taheri *et al.* (2009) reported a moisture content of 10.07% and 8.27% of gelatin prepared from the skin and bone of lizardfish (*Saurida tumbil*). (Jakhar *et al.,* 2012; Haddar *et al.,* 2011) have reported that Blackspotted croaker (*Protonibia diacanthus*) skin and Tuna (*Thynnus thynnus*) bone had the moisture content of 8.43% and 7.7% respectively. Additionally, gelatin from skin of *Claris gariepinus*, *Catla catla*, *Pangasius sutchi* had ash content of 5.7%, 1.53% and 1.38%, respectively (Kamble *et al.,* 2014). Additionally, Gelatin from skins *Claris gariepinus*, *Catla catla*, *pangasius sutchi* of preparations had moisture content of 10.56%, 8.13% and 7.06% respectively (Kamble *et al.*, 2014). The moisture content in edible gelatin should be less than 15%. The gelatin extracted from the skin and bone of *P. hypophthalmus* and *P. diacanthus* are observed to contain moisture (Table 1) which is within the prescribed limit.

Generally gelatins are with less fat content (Cheow *et al.,* 2007). Fat content of gelatin extracted from *P. hypophthalmus* and *P. diacanthus* skin and bone was observed as 0.45%, 1.24% and 0.32% 0.63% respectively. It is reported that the gelatin from lizardfish (*Saurida tumbil*) skin and bone was 0.03% and 0.01%, (Taheri *et al.,* 2009). Jakhar *et al.,* (2012) and Haddar *et al.,* (2011) reported that Blackspotted Croaker (*Protonibia diacanthus*) fish skin and Tuna (*Thynnus thynnus*) fish bone had the fat content 8.43% and 7.7%. The presence of very low fat and ash content showed that the acid extraction process followed in the present study was appropriate in producing good quality gelatin.

Table 1
Proximate composition of extracted gelatin

Source of Gelatin		*Moisture (%)*	*Ash (%)*	*Protein (%)*	*Fat (%)*
P. hypophthalmus	**Skin**	8.94±0.04[b]	2.10±0.05[a]	88.51±0.09[c]	0.45±0.04[ab]
	Bone	9.65±0.20[c]	3.50±0.08[c]	85.62±0.10[a]	1.24±0.13[c]

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

CONCLUSION

The result shown that the extraction procedure of *Pangasianodon hypophthalmus And Protonibea dicanthus* was found to be very efficient and for production of good quality gelatin since, it got a good thermal donation values color and proximate compassion so could be used in food and pharmaceutical industry as replacement of mammalian gelatin.

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