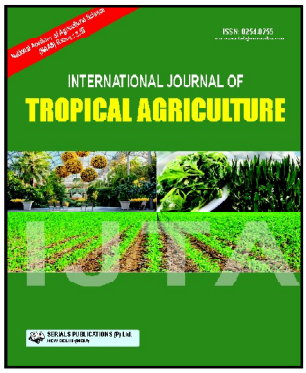

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Proximate composition analysis of gelatin obtained from skin and bone *Protonibea dicanthus*

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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 P.dicanthus (skin and bone). The protein content in gelatin produced from the bone of *P. diacanthus* was 87.44% while the content was. Therefore it may be concluded that the protein content in gelatin extracted from skin and bone of *P. diacanthus* as.

Keyword: gelatin, proximate composition, *P. dicanthus*

INTRODUCTION

The global demand for gelatin has been increasing over the years. The major source of collagen for the manufacture of gelatin is porcine skins, cattle hides and bones. Recent reports suggest the annual world production 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%). Unfortunately, these sources of gelatin present religious and safety oriented concerns for various consumer communities (Both Judaism and Islam forbid to consume any pork related products, while Hindus do not consume cow related products (Zhou., 2007). However, the outbreak of bovine spongiform encephalopathy (BSE) and the foot-and-mouth disease (FMD) crisis have also resulted in anxiety among users of collagen and gelatin products from land-based animals (Helcke.,2000). Therefore, alternative sources, especially fish processing wastes including skin, bone or scale, have been paid increasing attention for gelatin extraction. These sources are good substitute for mammalian gelatin. The waste from fish processing after filleting can

account for as much as 75% of the total catch weight (Shahidi 1994). About 30% of such waste consists of skin and bones with high collagen content. This waste is excellent raw material for the preparations of collagen and gelatin. Thus, preparations of gelatin from marine by-products not only satisfy the kosher and halal requirements and consumers concern for BSE, but also increase economic returns for the fishing industry.

MATERIAL METHOD

Determination of Proximate composition

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:

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

Where,

W = Weight (g) of sample

W_1 = Initial weight (g) of beaker

W_2 = 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±5ÚC. Sample were char dried completely by heating over a burner and incinerated in a muffle furnace at a temperature of 550±50ÚC 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

The proximate composition of extracted gelatins is shown in Table 1 and . The gelatins extracted from 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 protein content of 89.15% respectively whereas the gelatin made from the bone of *P. diacanthus* and *P. hypophthalmus* had protein content 87.44% .

Table 1
Proximate composition of extracted gelatin

Source of Gelatin		*Moisture (%)*	*Ash (%)*	*Protein (%)*	*Fat (%)*
P. diacanthus	**Skin**	7.97±0.12^{a}	2.56±0.11^{b}	89.15±0.10^{d}	0.32±0.11^{a}
	Bone	9.57±0.12^{c}	2.36±0.16^{ab}	87.44±0.12^{b}	0.63±0.08^{b}

Proximate composition of gelatin

The proximate composition of skin and bone gelatin extracted from *P. diacanthus* is shown in Table 1 The moisture, ash, protein and fat content of *P. diacanthus* skin and bone was significantly ($p<0.05$) different. The extracted skin gelatin showed high value of protein as compared to bone gelatin. *P. diacanthus* skin gelatin has protein content of 89.15% and 88.51%. *P. diacanthus* bone gelatin has protein content of 87.44% It may be concluded that the protein content of the gelatin extracted from the skin and bone of *P. diacanthus* is higher than that of *P. hypophthalmus*. 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 (Ardekani *et al.,* 2013;).

The ash content of *P. hypophthalmus* and *P. diacanthus* skin and bone gelatin observed as 2.10%, 3.50% and 2.56%, 2.36% 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 (Ardekani *et al.,*). 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* and *P. diacanthus* 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 (Ardekani *et al.,* 2013;). Additionally, Gelatin from skins *Claris gariepinus*, *Catla catla*, *pangasius sutchi* of preparations had moisture content of 10.56%, 8.13% and 7.06% respectively (Ardekani *et al.*, Mahmoodani *et al.*, 2014b). The moisture content in edible gelatin should be less than 15% (GME, 2012). The gelatin extracted from the skin and bone of *P. hypophthalmus* and *P. diacanthus* are observed to contain moisture (Table 11, fig 9) which is within the prescribed limit. (GME,2012).

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.

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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