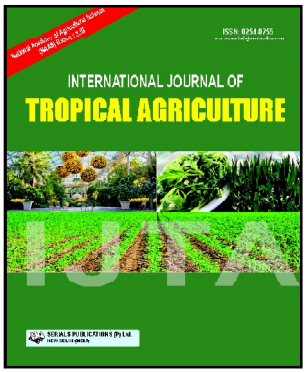

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Rheological and Functional Properties of gelatin extracted from the skin and bone of *Pangasianodon hypophthalmus*

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Abstract: Gelatin is a versatile product and has established strongholds in food, photography and pharmaceutical industries. In the present study, Isoelectric point (pI) of extracted gelatin was found higher in skin of *P. hypophthalmus* 5.57 pI, while it was lower in the gelatin extracted from bone 5.30 pI. It was observed that emulsifying capacity of *P. hypophthalmus* skin gelatin was recorded highest (59.11%) as compared to bone gelatin (57.23%). The emulsifying stability of these extracted gelatins was also experimented where skin gelatin (55.26%) has shown better stability than the bone gelatin (53.47%). Moreover the foaming property of skin gelatin was recorded higher (15.19%) than the bone gelatin (14.25%). The Water holding capacity of gelatin extracted from skin was found to be better (2.42ml/gm) than the bone gelatin. In all the selected parameters, gelatin extracted from the skin of *P. hypophthalmus* has shown the best result compared to bone.

Keywords: Pangasianodon hypophthalmus, Gelatin , Isoelectric point ,Emulsifying stability , Foaming, Water holding capacity.

INTRODUCTION

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 (FAO, 2016). 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 (Rao *et al.*, 2013). 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 (Luc *et al*., 2013). Traditionally, all byproducts are used for fishmeal production (Thuy *et al*., 2014). *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.

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 (Bailey and Paul, 1998). 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 (Ladislaus *et al*., 2007).

The properties of gelatin gels depend on the source and pretreatment of the raw material and parameters of the process. They are also affected by the concentration of gelatin, pH, presence of interacting compounds and gel maturation time and temperature . Gelatin displays multiple functional roles in food processing and formulations. The functional properties of gelatin can be divided into two groups (Schriecher and Gareis, 2007). The first has properties that are associated with gelling, for example, gel strength, gelling time, setting and melting temperatures, viscosity, thickening, texturizing and water binding. The second group relates to the surface behavior of the gelatin, for example, emulsion formation and stabilization, protective colloidal function, foam formation and stabilization (such as in marshmallow), film formation and adhesion/cohesion (Schriecher and Gareis, 2007).

The gelatin produced from the skins of fish living in cold-waters does not gel at room temperature-its gelling temperature is below 8–10^{0}C. Cold-water fish gelatin can also be used in applications that do not require a high Bloom value or gelling, relying instead in its other properties, such as prevention of syneresis and texturization. Cold-water fish gelatin can be used in frozen or refrigerated products that are consumed quickly following removal from the fridge or defrosting (Norland, 1990). Low gelling temperatures also offer new potential applications for fish gelatin. Gelatins with low melting points could also be used in dry products (such as for micro-encapsulation), and in fact, one of the major applications of fish gelatin is in the microencapsulation of vitamins and other pharmaceutical additives such as azoxanthine. Fish gelatin may also be used in the microencapsulation of colorants. Díaz-Calderon *et al*, (2014) described a method for microencapsulation of food flavors such as vegetable oil, lemon oil, garlic flavor, apple flavor, or black pepper with warm-water fish gelatin (150–300 Bloom). The microencapsulation process is conducted at temperatures of 33–35^{o}C by complex conversion to form the microencapsulated capsules. Most encapsulators have developed the expertise to handle fish gelatin in the sophisticated process, and minor volumes of fish gelatin are used to make soft-gel capsules. The use of fish gelatin soft capsules is most common in nutrition supplements.

Warm-water fish gelatin can have a Bloom value of 200–250 g. Tuna, for instance, is regarded as a good source, but the skin can be fatty, and gelatin must be fat-free. Tuna or tilapia gelatins have a melting point of 25–27^{o}C, and therefore, these gelatins are suitable

for products stored at low room temperatures. These gelatins more closely resemble bovine or pig gelatin, which melts at 32–35°C. A previous sensory study on gelatin gel desserts suggested that fish gelatin with lower gel melting temperatures had a better release of aroma and offered a stronger flavor (Choi and Regenstein, 2000). By increasing the concentration of gelatin or by using gelatin mixtures, desserts made from fish gelatins would be more similar to desserts made from high Bloom pork skin gelatin (Zhou and Regenstein, 2007). Warm-water fish gelatin grades can therefore more readily compete in the traditional gelatin markets.

Fish is a major nutritional source for humans providing a major fraction of the animal protein and essential fatty acids. However, they are highly perishable in nature and spoil quickly compared to other meat products as they contain relatively large quantities of free amino acids and volatile nitrogenous bases (Sallam, 2007). During storage, chemical and enzymatic reactions cause initial loss of freshness and further microbial spoilage rapidly degrades the flesh quality. Therefore, demand has been increased to look for new methods and technologies to maintain high quality of seafoods until it reaches its final destination. Application of edible coating was developed to preserved the quality of seafood and extend its shelf life (Feng *et al*., 2016). A coating provides a barrier to water and oxygen, their by reducing purge, color deterioration, and rancidity. Specifically, collagen and gelatin have been used as coatings on fish meat products to reduce purge, preserve color, decrease deterioration of aroma, improve sensory score, slow spoilage, and act as an antioxidant (Antoniewski and Barringer, 2010).

MATERIAL METHOD

Determination of Isoelectric point (Ying *et al.*, 2009)

Gelatin solution (0.1% w/w) were titrated with 0.1 M NaOH and 0.1 M HCL, and the zeta potential at a given in pH were recorded by Malvern Zetasizer Nano ZS,UK. The titration temperature was 25°C, and the decreasing pH intervals were 0.1-0.5 unit pH. The added amount of acid and alkali were less than 100ì in 100ml, imposing a negligible reduction in the original gelatin solution concentration. The changes of zeta potential were plotted against the change of pH for the sample.

Emulsifying capacity and stability

The method of Yasumatsu *et al*., (1972) was used to determine emulsifying capacity and stability. Emulsions were prepared with 1g of each sample, 50 ml of cold distilled water (4 °C) and 50 ml of sunflower oil. The gelatin samples were dispersed with a homogenizer/ blender. Each blended sample was divided equally into 50 ml centrifuge tubes. One centrifuge tube was directly centrifuged at 4000g for 10 min while the other was centrifuged under the same conditions after heating in a water bath at 80 °C for 30 min and cooling to room temperature (25 °C). The height of the emulsified layer, as a percentage of the total height of material in the unheated tubes, was used to calculate the emulsifying activity and stability, using the following formulae:

$$Emulsion\ capacity\ (\%) = \frac{Height\ of\ emulsion\ layer}{Height\ of\ whole\ layer} \times 100$$

$$Emulsion\ stability (\%) = \frac{Height\ of\ emulsion\ layer\ after\ heating}{Height\ of\ whole\ layer} \times 100$$

3.2.16. Foaming Properties

The method of Miller and Groniger (1976) was used to determine foaming properties. The gelatin powder (1g) was added to 100 ml of distilled water and homogenized for 1 min at high speed (Homogenizer-Remi Elektrotechnik Ltd). The mixture was carefully transferred into a 250ml calibrated beaker for volume measurement. The foam was calculated as the volume of mixture after blending compared to the original volume. The foaming stability was the ratio

of the foam capacity after 30 min divided by the original foam capacity.

3.2.17. Water holding capacity

Water holding capacity (WHC) was determined using the centrifugation method (Diniz and Martin., 1997). Duplicate samples (0.5g) of gelatin were dissolved in 20 ml of water in centrifuge tubes and dispersed with a vortex mixer for 30 s. The dispersion was allowed to stand at room temperature for 6 hours, and it was then centrifuged at 2800×g for 30 min. The supernatant was filtered with Whatman No. 1 filter paper and the volume recovered was accurately measured. The difference between the initial volume of distilled water added to the protein sample and the volume of the supernatant was determined, and the results were reported as ml of water absorbed per gram of gelatin sample.

RESULT

In the present study, the rheological and functional properties of *P. hypophthalmus and P. diacanthus* skin and bone gelatins are shown in Table 1. Isoelectric point (pI) of extracted gelatin was found higher in skin of *P. hypophthalmus* 5.57, while it was lower in bone of *P. diacanthus*. Bloom value was found significantly higher in *P. diacanthus* skin gelatin (315.92gm) as compared to other gelatins. As recorded, the Bloom value in case of *P. diacanthus* and *P. hypophthalmus* skin and bone gelatin were 315.92, 298.76 and 307.55, 290.60gm respectively. Melting temperature was found higher in *P. diacanthus* skin gelatin 29.13 °C as compared to other gelatins. Gelation temperature in case of *P. hypophthalmus* skin and bone were 13.06 and 13.71 °C and in *P. diacanthus,* the values were 11.83 and 12.45 °C respectively.

The values of emulsifying capacity and emulsifying stability are shown in Table 18A and observed that emulsifying capacity of *P. hypophthalmus* skin gelatin was recorded higher as compared to other gelatins. The value for viscosity and foaming percentage is shown in Table 1.1. Water holding capacity of *P. diacanthus* skin was recorded higher (3.15ml/gm) as compared to other gelatins in the present study.

Table 1
Rheological properties and Functional properties of extracted gelatin from *P. hypophthalmus* skin and bone

Source of gelatin		*Isoelectric point(pI)*	*Emulsifying Capacity (%)*	*Emulsifying Stability (%)*	*Foaming (%)*
P. hypophthalmus	**Skin**	5.57±0.15^{c}	59.11±0.68^{d}	55.26±0.43^{b}	15.19±0.45^{b}
	Bone	5.30±0.12^{a}	57.23±0.38^{c}	53.47±0.62^{b}	14.25±0.60^{b}

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

Table 1.1
B Functional properties of extracted gelatin from *P. hypophthalmus* and *P. diacanthus* skin and bone

Source of gelatin		*Water Holding Capacity(ml/gm)*
P. hypophthalmus	**Skin**	2.42±0.04^{b}
	Bone	2.31±0.03^{ab}

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

DISCUSSION

5.7.1. Isoelectric point (pI)

In the present study, the rheological properties of *P. hypophthalmus and P. diacanthus* skin and bone gelatins are shown in Table 17. Isoelectric point (pI) of extracted gelatin from skin of *P. hypophthalmus* was 5.57 considered highest while lowest value was recorded in case of gelatin extracted from the bone of *P. diacanthus* with a value of 4.83. The Isoelectric point (pI) of skin and bone gelatin of *P. hypophthalmus and P. diacanthus* was significantly different (P<0.05). This result is in concurrence with the findings of Ratnasari *et al.* (2013) who worked on the skin gelatin extracted from Pangas fish, Asian redtail cat fish, Nile tilapia and Striped snakehead where the recorded Isoelectric points were 5.1, 4.8, 5.3 and 4.8 respectively. Gudmundsson and Hafsteinsson (1997) recorded a isoelectric point of 6.2-6.4 in gelatin extracted from the skin of Cod fish and according to him, the isoelectric point of fish gelatin should be in the range of 6.2d"7.1. This suggests that the gel strength may be dependent on the isoelectric point (Gudmundsson and Hafsteinsson, 1997).

Emulsifying Capacity and Emulsifying Stability

Emulsifiers are surface active materials that adsorb to interfaces and facilitate the production of small droplets by lowering the interfacial tension during homogenization (Walstra, 2003). The amphoteric nature with the hydrophobic zones on the peptide chain make gelatin to behave as an emulsifier and it is being used in the manufacture of toffees and water-in-oil emulsions such as low fat margarine, salad dressings, and whipped cream (Baziwane and He, 2003).

In the present study, the functional properties of extracted gelatins of *P. hypophthalmus and P. diacanthus* skin and bone are presented in Table 18A. Emulsifying capacity of *P. hypophthalmus* skin and bone gelatin was 59.11 and 57.23%, while that of *P. diacanthus* skin and bone were 52.19 and 50.15% respectively. The Emulsifying Capacity of skin and bone gelatin of *P. hypophthalmus and P. diacanthus* was significantly different (P<0.05). Sahoo *et al.* (2015) reported that the emulsifying capacity of the extracted gelatin from Pacu skin at low temperature at 40 °C showed higher emulsifying capacity than extracted at higher temperature of 60 °C. Killekar *et al.* (2012) also reported that emulsifying capacity of black kingfish gelatin extracted at the temperature of 45 °C was found to be 55.66%. There is a growing trend within food industry to replace synthetic emulsifiers with more natural sources (Garti, 1999). Emulsifying capacity of croaker skin and bone were 55.70% and 40.50%, while those of Pink perch were 47.50% and 35.50% (Koli, 2011). Protein extracted from a variety of natural sources can be used as emulsifiers in food because of their ability to facilitate the formation, improve the stability, and produce desirable physico-chemical properties (Dickinson, 2003a; McClements, 2004).

The Emulsifying Stability properties of extracted gelatins from *P. hypophthalmus and P. diacanthus* skin and bone are presented in Table 19A. Recorded value of Emulsifying stability of gelatin extracted from skin and bone of *P. hypophthalmus* are 55.26 and 53.47% respectively, while the values for *P. diacanthus* were 46.05 and 45.67% respectively. Emulsifying stability of *P. hypophthalmus* skin gelatin recorded higher value as compared to other gelatins. The Emulsifying Stability of skin and bone gelatin of *P. hypophthalmus and P. diacanthus* was significantly different (P<0.05). Koli, (2011) also reported Emulsifying stability of croaker skin and bone with values of 35.70% 32.50%, while those of Pink perch were 32.40% and 25.00%. High Emulsifying stability of fish gelatin contributed to its high viscosity. The good emulsion property of the fish gelatin can be used in production of ice cream yoghurt, or other dairy products (Prommajak and Raviyan, 2013).

5.7.7. Foaming

Foaming properties are other important properties of gelatin for commonly used foods such as marshmallows (Zuniga and Aguilera, 2009). Foams with higher concentration of proteins were denser because of an increase in the thickness of interfacial film (Rawdkuen *et al.,* 2013). Foam capacities of gelatin are shown in Table 18 B. The recorded foaming values in *P. hypophthalmus* skin and bone gelatin were 15.19 and 14.25% respectively while the values for *P. diacanthus* skin and bone were12.10 and 12.00% respectively. The Foaming of skin and bone gelatin of *P. hypophthalmus and P. diacanthus* was significantly different (P<0.05). Koli, (2011) In the present study, the rheological and functional properties of *P. hypophthalmus and P. diacanthus* skin and bone gelatins are shown in Table 17, 18A and 18B. Isoelectric point (pI) of extracted gelatin was found higher in skin of *P. hypophthalmus* 5.57, while it was lower in bone of *P. diacanthus*. Bloom value was found significantly higher in *P. diacanthus* skin gelatin (315.92gm) as compared to other gelatins. As recorded, the Bloom value in case of *P. diacanthus* and *P. hypophthalmus* skin and bone gelatin were 315.92, 298.76 and 307.55, 290.60gm respectively. Melting temperature was found higher in *P. diacanthus* skin gelatin 29.13 °C as compared to other gelatins. Gelation temperature in case of *P. hypophthalmus* skin and bone were 13.06 and 13.71 °C and in *P. diacanthus,* the values were 11.83 and 12.45 °C respectively.

The values of emulsifying capacity and emulsifying stability are shown in Table 18A and observed that emulsifying capacity of *P. hypophthalmus* skin gelatin was recorded higher as compared to other gelatins. The value for viscosity and foaming percentage is shown in Table 18B. Water holding capacity of *P. diacanthus* skin was recorded higher (3.15ml/gm) as compared to other gelatins in the present study.

Water Holding Capacity

The functional properties of proteins in a food system depend in part on the water holding capacity (WHC) which refers to the ability of protein to imbibe water and retain it against a gravitational force within protein matrix (Koli *et al.,* 2012). The water holding capacities of *P. hypophthalmus* and *P. diacanthus* skin and bone gelatins are given in Table 18B. Water holding capacity of *P. diacanthus* skin was 3.15ml/gm considered higher than the values obtained for other gelatins in the present study. The Water Holding Capacity of skin and bone gelatin of *P. hypophthalmus and P. diacanthus* was significantly different (P<0.05). Koli *et al.* (2012) also reported that Water holding capacity of gelatin with values of 4.50 ml/gm and 3.00 ml/gm for skin and bone respectively taken from tiger toothed croaker was higher as compared to pink perch gelatin utilizing skin and bone. Higher water holding capacity is mainly related to the higher amount of hydrophilic amino acid and higher Hydroxyproline content (Shyni *et al.,* 2014).

CONCLUSION

In order to evaluate the waste from *P. hypophthalmus* a source of gelatin, it was extracted from skin and bones of which rheological and functional properties were examined. Similarly, the Isoelectric point, Emulsifying Capacity, Emulsifying Stability, , Foaming and Water Holding Capacity of gelatin extracted were in general greater than those of the gelatin from *P. hypophthalmus* and the values of skin gelatin was higher compared to bone gelatin . It can be concluded from the present study that *P. diacanthus* skin is a prospective source to produce gelatin with good yield and desirable functional properties comparable to commercially available mammalian gelatin.

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