



Gelatin Extraction from the Bangladeshi Pangas Catfish (*Pangasius pangasius*) Waste and Comparative Study of Their Physicochemical Properties with a Commercial Gelatin

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Abstract- Production of gelatin from the aquatic source is gradually replacing the mammalian sources because of some socio-cultural and religious issues. The *Pangasius pangasius* catfish is a native species and very popular in Bangladesh due to their availability and cheap price. The perspective of this study was to extract the gelatin from the skin and bones of this catfish and compare them with commercial gelatin. Gelatin was extracted by applying the acid-base extraction process and the resultant gelatins were evaluated based on some physical properties. The gelatin yield was found significantly higher from the skin sample ($10.85 \pm 0.93\%$) than the bone ($5.23 \pm 0.39\%$) of *Pangasius*. The extracted skin gelatin had higher moisture and fat content than the bone and Commercial gelatin, while the ash content was significantly higher in bone gelatin. Protein content was noted in skin gelatin ($81.34 \pm 3.45\%$), bone gelatin ($73.44 \pm 2.58\%$), Where commercial gelatin ($92.38 \pm 3.89\%$). Skin gelatin exerted significantly higher ($p < 0.05$) viscosity (4.62 ± 0.3 mPa.s) than the extracted bone gelatin (3.11 ± 0.24 mPa.s) and lower than the commercial gelatin (5.76 ± 0.34 mPa.s). The melting and setting temperature of this catfish skin and bone gelatin were very near to each other and significantly lower than the commercial gelatin. Skin gelatin had exerted higher water holding capacity (2.36 ± 0.11 ml/g), fat binding capacity (3.23 ± 0.05 ml/g), foaming capacity ratio (1.88 ± 0.07), and foam stability (1.51 ± 0.04). Both the skin and bone gelatins were acidic. In this comparative study, it was noticed that the skin gelatin had better physical properties than the bone gelatin of native *Pangasius* catfish. *Pangasius* skin may be recognized as a potential aquatic source of edible gelatin with good yield and desirable physical properties comparing with commercial gelatin.

Indexed Terms- Gelatin, *Pangasius pangasius*, viscosity, foaming capacity.

I. INTRODUCTION

Gelatin, a very widely used biopolymer, extracted from the controlled partial hydrolysis of collagen, which is the most common protein in the animal kingdom. Smaller peptides are produced from the collagen fibrils during this hydrolysis process [1, 2]. Gelatin is commonly used in food processing as a food additive because of its wide range of functionalities. It is also applied in bio-degradable packaging as it has the film-forming capability [3]. It is a very popular hydrocolloid which is widely used in different food processing due to its gelling and thickening properties. Gelatin is compositionally different from the other hydrocolloids which are mostly polysaccharides, whereas gelatin is a digestible protein [2]. Gelatin can be derived from various sources such as- pig skins or cowhides, bones, fish wastage, some insects (*Aspongopus viduatus*, *Agonoscelis pubescens*) [2]. Gelatin extracted from the pigskin and cowhide have some restrictions for use as a food additive due to some sociocultural and religious reasons and so the demand of halal gelatin from the alternative sources is increasing day by

day. Nowadays the gelatin market is affected by the BSE (bovine spongiform encephalopathy) or “mad cow disease” and FMD (foot and mouth disease). In that case, the use of the fish fillets, skins, and bones may be the best alternative to the mammals for halal gelatin production both for the Muslims and Hindus [4, 5]. Production of the gelatin from the fish wastage is increasing day by day thus it has no such restriction as to the mammalian sources. It is also advantageous in case of economic consideration of a fish processing industry by valorizing the wastage to the value-added gelatin [4]. According to a previous study, a considerable amount of waste is produced in a fish processing industry and it was reported as 30% of the waste part was the fish skins and bone [6]. The physicochemical properties of the produced gelatin depend on various factors such as- species origin (mammalian, fish, or insect), waste origin (bones, skins), animal age, extraction condition [3]. *Pangasius pangasius* is a freshwater catfish belonging to the family Pangasiidae which is native to the south-east Asian countries- Bangladesh, India, Pakistan, Myanmar, and Nepal [7]. It is one of the world’s fastest-growing freshwater species in aquaculture [8]. It is becoming a popular food due to its tender and white flesh, absence of fishy odor, firm cooked texture and high nutritive value and that’s why fillet of this catfish is traded to all over 100 countries worldwide [9]. Aquaculture of pangas catfish has been enlarged in Bangladesh [10]. Production of gelatin from the waste of pangas catfish will reduce the industrial waste of the fish processing industry. To meet up the increasing demand in food market, native *Pangasius* can be used as a massive potential source considering the cheap cost and availability of the raw material. The goal of this study was to observe the yield capacity of the gelatin from the *Pangasius pangasius*, native to the Bangladeshi fresh water source and evaluation of the physical properties of the derived gelatin.

II. MATERIALS AND METHODS

The research work was completed in the laboratory of Food Engineering and Tea Technology; Chemical Engineering and Polymer Sciences department, Shahjalal University of Science and Technology, Sylhet, Bangladesh.

2.1 Sample preparation

Pangas fishes were collected from the local market of Sylhet (Fig 1). Skins and bones of these fishes were used as raw materials. Three solutions, 0.2% (w/v) sodium hydroxide (NaOH), 0.2% (v/v) sulfuric acid (H₂SO₄) and 1.0% (w/v) citric acid (C₆H₈O₇) were prepared and kept at 4 °C for 12 hours.

2.2 Extraction of gelatin from the sample

Gelatin extraction from the pangasius skin and bone was accomplished by following the method developed by Grossman and Bergman [11]. The fishes were cleaned with water before the experiment followed by removing the skins with a sharp knife. Then they were boiled in water for 30 minutes. The flesh was removed, and bones were collected. Skins were cut into squares (1cm×1cm) before being stored at -20 °C until further use. The raw samples (skins & bones) were rinsed continuously under running tap water to remove superfluous materials. Then they were soaked in 0.2% (w/v) NaOH for 40 minutes. After washing out sodium hydroxide, the skin samples were treated by two consecutive acid incubations. The incubation period was 40 min for each treatment. Firstly, it was treated in sulfuric acid solution (0.2%, v/v) and then in citric acid solution (1.0%, w/v). But for bones, ethylenediaminetetraacetic acid (EDTA) treatment was given after sulfuric acid incubation. The bones were soaked in EDTA (0.25N) solutions at an inherent pH of 7.66 for two different periods of shaking 12 hours and 4 hours. Then it was treated with acetic acid. After each soaking treatment, the samples were washed properly with water until they had a pH of about 7. Each soaking treatment was repeated multiple times. The ratio of raw samples to every solution was 1:10. Finally, extraction was accomplished in distilled water (1:3 ratio) at 55 °C for 16 h. After that, the extracted gelatin solution was filtered with the Whatman No-1 filter paper. The filtrate was collected and evaporated for 30 min

in a rotary evaporator. The concentrated solution was dried using a temperature-controlled oven at 55 °C for ± 48 hours. The dried gelatin samples were stored in an airtight pack for further analysis.

2.3 Characterization of extracted gelatin

2.3.1 Determination of yield of gelatins

The yield of gelatin was measured by using this formula according to Zakaria and Bakar [6].

$$\text{Yield} = \left[\frac{(\text{mass of dried gelatin})}{(\text{mass of cleaned skins or bones})} \right] \times 100 \quad (1)$$

2.3.2 Determination of Moisture, Ash, and Protein content

The moisture and ash content of the gelatin samples were measured by AOAC method [12]. Protein content in the fish skin, bone, and the gelatin samples were measured by the Kjeldahl method by estimating the total nitrogen [12]. Nitrogen conversion factor 5.8 was used in this method.

2.3.3 Determination of fat content

The fat content in the extracted gelatin was measured by following the method developed by Ravichandran and Parthiban [13]. One gram of each gelatin sample was added to 25 mL of chloroform-methanol (2:1) solution in a separating funnel. Then, 5mL of NaCl (0.9%) solution was added with this mixture and shook properly. It was allowed to stand until the separated layers are observed. The chloroform layer was carefully collected in a pre-weighed dry beaker and evaporated in a boiling water bath. The beaker containing fat was dried and weighed again. From the difference in weights, the percentage of fat present in the sample was calculated gravimetrically and expressed as a percent.

$$\text{Total extracted fat (\%)} = \left[\frac{(\text{weight of dry beaker with fat}) - (\text{Weight of dry beaker})}{(\text{weight of sample})} \right] \times 100 \quad (2)$$

2.3.4 Determination of pH

A 6.67% (w/v) gelatin solutions were prepared at 45 °C in a water bath and the pH of the samples were measured with a pH meter (Consort C5010, Belgium). The pH meter was calibrated with buffer 4.0 and buffer 7.0 solution [12].

2.3.5 Viscosity

The viscosity of the gelatin samples were measured by following AOAC method [14]. For this analysis, gelatin solution 6.67% (w/v) was prepared by dissolving gelatin in distilled water and heating in a temperature-controlled water bath at 60 °C for 30 min. Finally, the viscosity of 20 ml gelatin solution was measured by using a Brookfield viscometer at room temperature and expressed in mPa s.

2.3.6 Melting Temperature

The melting point of the sample was measured according to the method developed by Ninan et al. [15]. A 6.67 % gelatin solution was prepared. An aliquot (5 ml) of each sample was taken in a small test tube and heated at 60 °C for 15 min in a water bath. Then it was allowed to cool down in an ice bath and matured at 10 °C for 18 h. Then, five drops of a mixture of chloroform and methylene blue dye (3:1) were added to the gel and placed in the water bath at 10 °C and the water heated at the rate of 0.2 °C per min. Finally, the melting point was recorded as the temperature at which the drops (chloroform-methylene blue dye) start to move into the gel.

2.3.7 Setting Temperature

The setting temperature of the sample was measured by using the method followed by Ninan et al. [15]. For this analysis, 10% (w/w) gelatin solutions were prepared, and they were placed in a temperature-controlled water bath having 40 °C. The temperature of the bath was then gradually reduced at a rate of 0.2 °C per min. A thermometer was set into the sample and lifted out at 30 s intervals. The setting temperature was recorded at which temperature gelatin solution was not dripped from the tip of the thermometer.

2.3.8 Water Holding and Fat Binding Capacity

Water holding and fat binding capacity of the gelatin samples were measured according to the method followed by Joanna et al. [16]. For this analysis, 1 g of gelatin was added with 50 ml of distilled water for measuring water holding capacity and 10 ml of sunflower oil for measuring fat binding capacity. Then they were held at room temperature for 1h. The gelatin solutions were mixed with a vortex mixer for 5 s every 15 min. The gelatin solutions were then centrifuged at 450 g for 20 min in a centrifuger (Model-416G, Gyrozen, Korea) and the supernatant was filtered with filter paper (Whatman No. 1). The volume recovered was measured carefully. Finally, the amount of absorbed water/oil (ml/g) was measured from the difference of the initial volume of distilled water/sunflower oil added to the gelatin sample and the volume of the supernatant.

2.3.9 Foaming Properties

Foaming properties were measured as foaming capacity (FC) and foam stability (FS) according to the method followed by Ninan et al. [15]. For this analysis, 1g of each gelatin sample was taken and a solution was prepared by dissolving in 50 ml distilled water at 60 °C. Then this solution was homogenized at 10,000 rpm for 5 min in a homogenizer. The homogenized solution was poured into a 250 ml measuring flask. FC was calculated as the volume ratio of foam liquid. FS was measured from the ratio of the initial foam volume to the foam volume after 30 min.

2.4 Statistical Analysis

The analyses were done in triplicates and all the data were analyzed using one-way ANOVA with Minitab 18 software and Excel 13. The Tukey test was used to determine the significant difference ($p < 0.05$). Data were expressed as means \pm standard deviation (SD) of three independent measurements.

III. RESULTS AND DISCUSSIONS

3.1 Yield of gelatin

The yields of gelatin from skin and bone of our native *Pangasius pangasius* were found as $10.85 \pm 0.93\%$ and $5.23 \pm 0.39\%$ respectively in this study. Significantly higher ($p < 0.05$) yield was observed from the skin sample than the bone sample (Table 1).

The hard bones were needed extreme treatment for the breakdown of collagen. The result from this study was near to the findings of Hong et al., which reported that the yield of gelatin from the *Pangasius sutchi* skin was 10.78 % [17]. The yield of the gelatin from the skin of our native *Pangasius pangasius* is less than the *Cyprinus carpio* skin (12.00%) and much higher than the tilapia skin (5.39-7.81%) and *Priacanthus hamrur* fish skin (4%) reported in other studies [16, 18, 19]. In another study, yield (6.12%) was reported when pineapple liquid waste was used to extract the gelatin from the bone of *Pangasius catfish* [20]. The yield of gelatin may vary due to the species of the fish and extraction condition

(pretreatment, temperature, time). Incomplete hydrolysis of the collagen of fish skin and bone during the extraction process may be responsible for the lower yield [17].

Table 1: Different physical properties of the gelatin samples.

Properties	Skin gelatin	Bone gelatin	Commercial gelatin
Yield (%)	10.85±0.93 ^a	5.23±0.39 ^b	-
pH	3.66±0.26 ^b	4.02±0.31 ^b	6.02±0.11 ^a
Viscosity (mPa.s)	4.62±0.25 ^b	3.11±0.16 ^c	5.76±0.09 ^a
Melting temperature (°C)	28.6±0.57 ^b	28±1.0 ^b	32±1.0 ^a
Setting temperature (°C)	18±1.0 ^b	17±1.0 ^b	24.4±1.6 ^a
Water holding capacity (ml/g)	2.36±0.11 ^b	1.73±0.06 ^c	2.63±0.11 ^a
Fat binding capacity (ml/g)	3.23±0.05 ^a	2.7±0.17 ^b	3.46±0.15 ^a

Note: Values with the same superscripts in the same row indicate no significant difference ($p < 0.05$).



Fig. 1: Collected fresh *Pangasius* catfish



Fig. 2: (A) commercial gelatin, (B) extracted *Pangasius* skin gelatin, (C) bone gelatin

3.2 Moisture Content

The moisture content of the extracted gelatin samples is represented in Fig. 3. *Pangasius* skin gelatin contained higher moisture (11.16±1.98%) than the bone gelatin (8.27±1.63%). The moisture content of commercial gelatin was found as 5.91±0.72% in this current study. The results of this study were near to the findings of Mahmoodani et al., who were reported the moisture content of the *Pangasius sutchi* bone gelatin as (9.2±0.15%) [21]. In another study by Pradarameswari et al., the moisture content of *Pangasius* skin gelatin was found as 11.20±1.18% at 45 °C extraction temperature, which is about similar to the findings of the current study [22]. Comparatively higher moisture content in the extracted skin gelatin was noticed probably due to the presence of higher moisture content in raw skin and the hygroscopic characteristics of the dried gelatin [17].

3.3 Ash Content of Gelatin

The amount of ash in extracted gelatin samples found from this study are represented in Fig. 3. It denotes significantly ($p < 0.05$) much higher ash content in extracted bone gelatin (3.18±0.25%) and skin gelatin

($2.13 \pm 0.15\%$) than the studied commercial gelatin ($1.45 \pm 0.27\%$). The amount of ash was found to be maximum in bone gelatin which is possibly due to the highest concentration of minerals in raw bone samples. A previous study mentioned that the quality of the gelatin depends on its ash content [21]. A low amount of the ash in gelatin indicates its high quality and gelatin containing maximum 2.6% ash may be acceptable for the food processing purpose. Significantly different results were observed by Pradarameswari et al., who were found the ash content of *Pangasius pangasius* skin gelatin as $0.83 \pm 0.06\%$ at $55\text{ }^\circ\text{C}$ extraction temperature [22]. Ash content 2.4% was reported in the case of African catfish skin gelatin which is near to our native *Pangasius* skin gelatin [23].

3.4 Fat Content of Gelatin

Fat is a very important element that can determine the quality of the extracted gelatin. Generally, gelatin contains a very lower amount of fat. Fig 3 represents the highest value of fat ($2.47 \pm 0.79\%$) in the extracted *Pangasius* skin gelatin. The fat percentages of *Pangasius* bone gelatin and the commercial gelatin were $0.69 \pm 0.13\%$ and $0.43 \pm 0.14\%$, respectively. The significant difference of the fat content in skin and bone gelatin is due to the presence of the fat in the raw sample. A lower fat composition in the extracted gelatin samples may also be due to the washout of maximum lipid content during the extraction in acid solution. Another factor responsible for the loss of fat content of gelatin is the immersion of the sample in a strong metal base [22]. Our results are compatible with the findings of Hong et al., who were found the fat content (2.63%) in *Pangasius* catfish skin gelatin and it results from the lipid content (10.65%) of the raw skin sample [17]. Fat content (3.99 %) of the gelatin extracted from the carp skin was reported in another study which is much higher than our *Pangasius* bone and skin gelatin (0.69-2.47%) [16].

The proximate compositions of skin, bone, and commercial gelatins are graphically represented in the Fig. 3.

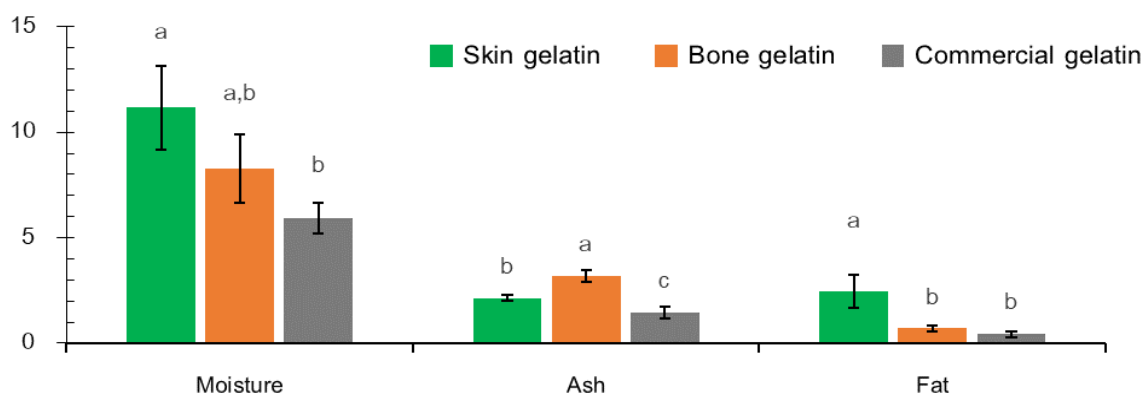


Fig. 3: Comparison of proximate composition between *Pangasius* skin gelatin, bone gelatin, and commercial gelatin. Values with the same superscripts within the samples indicate no significant difference ($p < 0.05$).

3.5 Protein Content

In this study, protein content in the fish skin, bone, extracted gelatin samples, and commercial gelatin were evaluated, and the results are represented in Fig 4. The native *Pangasius* skin was found to contain $21.09 \pm 3.61\%$ crude protein and the gelatin extracted from the skin contains a higher amount of protein

81.34±3.45%. The bone sample had a lower protein composition (12.86±2.19%). The extracted bone gelatin also contained a lower amount of protein (73.44±2.58%), Where 92.38±3.89% protein was noted in the studied commercial gelatin.

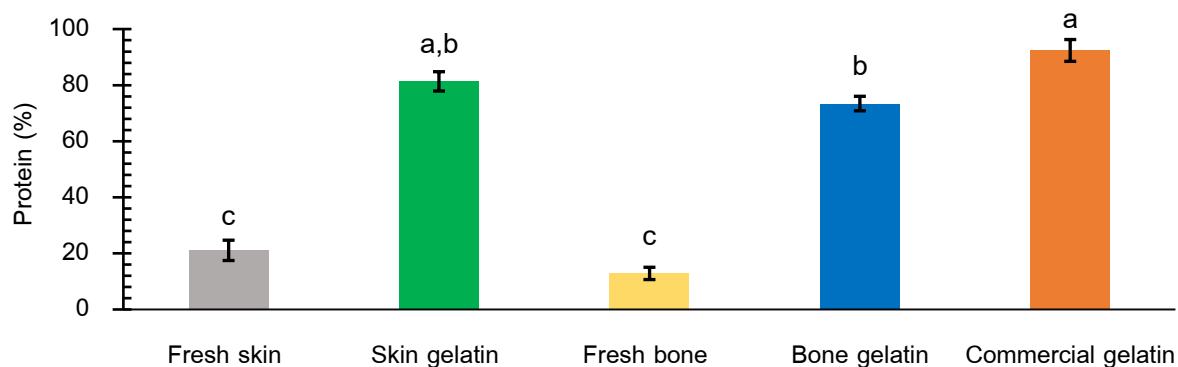


Fig. 4: Protein content in raw material, extracted gelatin, and commercial gelatin. Values with the same superscripts within the samples indicate no significant difference ($p < 0.05$).

This results significantly varies from extracted skin gelatin from catfish (87.81±2.84%), snakehead catfish (75.63±1.05%), red tilapia (89.70±3.13%), cold water fish (92.07±1.26%), and bovine (95.86±4.38%) [17]. The protein content of gelatin depends on its extraction condition and they found (88.11±5.48 to 89.61±7.85%) protein in pangasius skin [22]. The highest protein composition of extracted gelatin was observed when the carp (*Cyprinus carpio*) skins were pre-treated with weak alkali (82.44±0.49) and it was lowest (75.86±0.52) when washed with sodium chloride [16].

3.6 pH

The pH is a very important factor that indicates the extraction process and determines the extracted gelatin's functional quality. In this present analysis, Skin gelatin of *Pangasius pangasius* was noted as the most acidic (pH-3.66±0.26) comparing with bone gelatin (pH-4.02±0.31) and commercial gelatin (pH-6.02± 0.11) (Table 1). The pH of the extracted gelatin solution is effected by the chemical treatment used in the extraction process [16]. The pH of the *Pangasius pangasius* skin gelatin (4.47±0.33) was found by Pradarameswari et al., which is significantly different from our current study. The lower or higher acidity of the extracted gelatin results from the development of swelling of skin collagen. Curing materials are trapped within the amino acid bonding structure of the collagen protein during swelling and become insoluble in the neutralization period [22].

3.7 Viscosity

The Table 1 represents the viscosity of our native *Pangasius* skin and bone gelatin comparing with the commercial gelatin. Skin gelatin viscosity (4.62±0.25 mPa.s) was significantly higher ($p < 0.05$) than the extracted bone gelatin (3.11±0.16 mPa.s) and lower than the commercial gelatin (5.76±0.09 mPa.s) (table 1). Like our findings, higher viscosity of the skin gelatin was also noticed in Lizardfish and Nile Perch than their bone gelatin [24, 25]. The viscosity of the extracted gelatin depends on the extraction temperature and it was found to decrease with the increasing temperature from 45 to 55 °C [22]. It is also depended on the pH of the extraction condition [26]. The previous studies were reported the viscosity of most of the commercial gelatins as in the range of 2.0 to 7.0 mPa.s [18]. The viscosity of

the native *Pangasius* skin gelatin found from this current study was comparatively higher than the snakehead (3.40 ± 0.16), Red Tilapia (1.73 ± 0.09) Cold Water Fish (1.55 ± 0.09), Bovine (3.32 ± 0.26) skin gelatin found from other study [17]. Viscosity is a very important physical property that contributes to manufacturing certain food and its stabilization. Gelatin with high viscous property is also needed in pharmaceutical applications [21].

3.8 Melting and Setting Temperature

Extracted skin gelatin and the bone gelatin had a melting temperature of 28.6 ± 0.58 °C and 28 ± 1.0 °C, respectively. Significantly higher ($p < 0.05$) melting temperature was detected for commercial gelatin (Table 1). As found from the other studies, fish gelatin has a lower melting temperature than mammalian gelatin. The studied melting temperature of skin gelatin was higher than the Rohu (28.1 °C) and Common carp (28.2 °C), Nile perch gelatin (21.4 – 26.5 °C), tilapia skin gelatin (22.5 – 28.9 °C) [15, 18, 24]. The melting point was near to the pangas catfish (28 ± 1.22 to 30 ± 1.22) °C found in other study [22]. Amino acid content, molecular weight, maturation temperature affect the melting point of gelatin [18]. The setting temperature of skin gelatin (18 ± 1.0) and bone gelatin (17 ± 1.0) was also significantly lower than that of the commercial gelatin (24.4 ± 1.6). The setting temperature of the skin gelatin was near to the common carp skin gelatin (17.9 °C), tilapia gelatin (18.2 °C) and less than Grass carp gelatin (20.5 °C), Nile perch gelatins (19.5), and mammalian gelatin (31.6 – 31.8 °C) [15, 24]. Imino acid composition of the raw material correlates with the setting temperature of the extracted gelatin. Imino acid was typically ~ 24 % for mammals and 16 – 18 % for most fish species previously found in different studies [15].

3.9 Water Holding and Fat Binding Capacities

The water holding capacity of skin gelatin (2.36 ± 0.11 ml/g) was comparatively higher than the extracted bone gelatin (1.73 ± 0.06 ml/g) and lower than the commercial gelatin (2.63 ± 0.11 ml/g) (Table 1). Differences in the water holding capacity may be due to the amount of hydrophilic amino acid present in the protein matrix, the number of polar groups within the particle, pH, temperature, and protein concentration [15]. The water holding capacity of this native pangasius skin gelatin was much higher than rohu and common carp skin, while near to pink perch skin gelatin found in other studies [15, 27]. The water-binding property of gelatin helps in reducing drip loss and impairs the juiciness in frozen fish or meat products when thawed or cooked [16]. Fat binding capacity was also higher in skin gelatin than bone gelatin. A maximum fat binding capacity was observed in commercial gelatin. It depends on the exposure of the hydrophobic group in the protein matrix which attracts nonpolar groups and increases fat binding capacity [15].

3.10 Foaming Properties

Fig. 5 represents the foaming capacity (FC) and foam stability (FS) of the extracted pangasius skin and bone gelatin comparing with the commercial gelatin. Skin gelatin had higher foaming capacity (1.88 ± 0.07) and foam stability (1.51 ± 0.04) than the extracted bone gelatin (FC- 1.69 ± 0.04 , FS- 1.32 ± 0.03). A similar type of result was exhibited in the gelatin extracted from the black tilapia skin and bone [6]. Comparatively lower foam formation in bone gelatin may be due to the aggregation of proteins that affects interactions between the protein and water needed for foam formation [15]. Among them, foam capacity was much higher in commercial gelatin (2.68 ± 0.02). Foam stability is also significantly higher in commercial gelatin than the fish gelatin. The foaming properties of the gelatin

depend on the hydrophobic areas of the peptide chain [15, 16]. The foaming capacity of our native *Pangasius* skin gelatin was higher than the carp gelatin (FC- 1.58-0.95) [16].

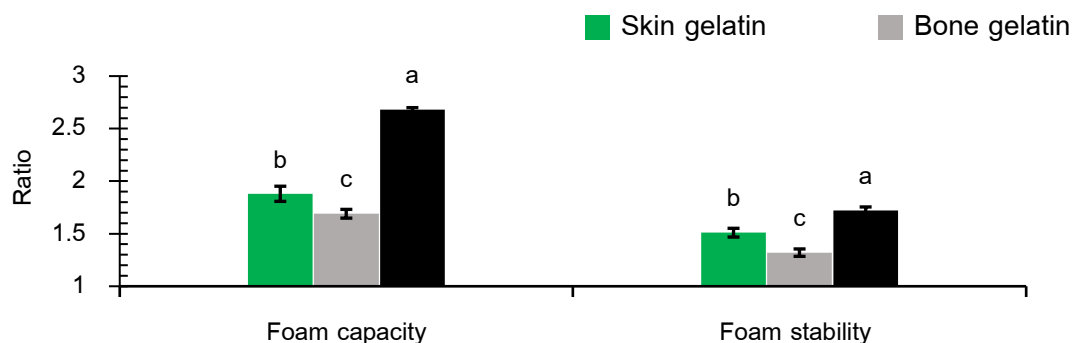


Fig. 5: Foaming capacity (FC) and foam stability (FS) of the extracted gelatin and commercial gelatin. Values with the same superscripts within the samples indicate no significant difference ($p < 0.05$).

IV. CONCLUSION

This comparative study showed the physicochemical properties of the skin gelatin are near to the commercial gelatin. This skin gelatin can be used in food industrial applications. Extracted gelatin having higher viscous property can also be used in gelatin film formation and stabilization of the emulsion. The yield of gelatin from the Bangladeshi native *Pangasius pangasius* skin sample was at a satisfactory level. There is an economical potential of this wastages from this fish can be utilized properly in value-added gelatin production.

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