A Study on Extracting Gelatin from Bigeye Tuna (*Thunnus Obesus)* Skin: An Alternative to Mammalian Gelatin

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Introduction

Gelatin is not a naturally occurring protein, obtained by partially hydrolysis of native collagen (Karim, and Bhat, 2009). It is a colorless or slightly yellow, nearly tasteless and odorless compound with translucent property. Gelatin is widely used in food, pharmaceutical, photographic and cosmetic industries (Karim and Bhat, 2009). Currently, gelatin is produced using beef bone, hide, and pig skin and pig bone. However, Bovine spongiform encephalopathy (BSE) disease as well as religious concerns is negatively affecting the gelatin market. There is also a high competition for this kind of mammalian sources among producers. Therefore, it is important to introduce alternative sources rich in collagen for production of gelatin. Fish skin, which is enriched with collagen, has a potential to be used for extraction of gelatin (Badii and Howell, 2006). Furthermore, fish skin is concerned as a major byproduct of the fish-processing industry, causing waste accumulation and pollution. Utilization of this collagenous fish waste minimizes environmental pollution, while it adds value to fish based by-product sector. For the present study, Bigeye tuna (*Thunnus obesus*) skin was used, since Bigeye tuna is one of the most commercially important tuna fishery resource in Sri Lanka. The study aims to extract fish gelatin as an alternative to mammalian gelatins, using appropriate methodology and characterization of physical, chemical and functional properties of extracted gelatin.

Methodology

The cleaned fish skin samples were chopped in to small pieces and washed with running tap water for about 10 minutes. Six treatments with three different NaOH and H_2SO_4 concentrations at two different time combinations were selected for final experiment after conducting preliminary experimental trials. First 30 g each of six samples were soaked in different concentrations of Sodium Hydroxide (w/v) for two different time combinations (S1- 0.1 % for 24 hrs, S2- 0.2 % for 24 hrs, S3 -0.3 % for 24 hrs,S4- 0.1 % for 36 hrs, S5-0.2 % for 36 hrs, S6-0.3 % for 36 hrs) separately. Then each pretreated skin samples were rinsed with running tap water and allowed to drain using muslin cloth. Each partially treated sample was again treated with different diluted H_2SO_4 concentrations (w/v) for two different time combinations (S1-0.1 % for 24hrs, S2-0.2 % for 24 hrs, S3-0.3 % for 24 hrs, S4-0.1 % for 36 hrs, S5-0.2 % for 36 hrs, S6-0.3 % for 36 hrs) separately. Each treated skin samples were again rinsed with tap water and allowed to drain using muslin cloth separately. Treated samples with different acid, alkaline time combinations were performed using distilled water (1:2 w/v) in a water bath at 60 °C for 05 hours for gelatin extraction separately. Finally, differently treated gelatin solutions were filtered through 2 layers of muslin cloth to remove residual skin parts and final products were oven dried at 90 °C for 06 hrs.

Then the final products were analyzed for different parameters. Yield was expressed as a percentage (%) of the wet weight of the fish skin used. Gel strength was determined by using a Texture Analyzer (53205 Digital fruit firmness tester). The melting point was determined by preparing 6.67 % (w/v) gelatin solutions and maturating in a refrigeration temperature at 07 °C for 16-18 hrs. Then melting points of final products were recorded by increasing the temperature in a water bath until the gelatin samples are dissolved (Karim and Bhat, 2009).

Color of each gelatin sample was recorded by keeping all samples against a white background and comparing them by visual observation. Odour was recorded by sensory evaluation. pH was measured using EUTECH 510 pH meter. Moisture content (oven drying procedure), crude protein level (kjeldhal method) and crude lipid content (ether extraction), were performed for all six samples and commercial gelatin (AOAC, 1990). The results of final products were analyzed using ANOVA by MINITAB 16 statistical software at 0.05 significant level.

Results and Discussion

There is a significant difference ($p<0.05$) for yield and gel strength of final samples according to treatment methods. The maximum yield (19.67 %) (w/v) was obtained for S1and minimum yield (16.03 %) was obtained for S6 (Table 01). Gel strength and gel melting point are the major physical properties of gelatin (Karim and Bhat, 2009). Highest gel strength (260 Bloom) was recorded for S1 sample, while lowest gel strength (30 Bloom) was obtained for S6 (Table 01). According to the results, gel strength of final treatments decreased with increasing concentration of acid and alkaline solutions. According to research finding of Sarabia et al. (2000), when hydroxyproline content of product is higher, gel strength of the gelatin also becomes higher. In the present study, hydroxyproline content of samples can decline with increasing acid and alkaline concentrations. As a result, there is a potential to decline gel strength with greater acid and alkaline concentrations of treatments. Holzer (1996) has revealed that gelling strength of commercial gelatins ranges from 100 to 300, but gelatins with Bloom values of 250–260 are the most desirable. Since only one final product (S1) is within that desirable range, this treatment can be concerned as suitable treatment for gelatin production. Bloom value of gelatin produced using yellow fin tuna skin has been reported as 426 (Cho *et al.*, 2005).This value is superior compared to all final products of present study. There is a significant variation between the average melting points and final gelatin samples. The greatest melting point (24.2 °C) was recorded for S1 gelatin sample, while lowest melting point (19.1 °C) was reported for S6 (Table 1).The main differences in the properties of mammalian and fish gelatins are that fish gelatins have lower melting temperatures (Leuenberger, 1991). Greatest melting point (24.2 °C) of present study (S1) is similar to previous result (Cho et al., 2005) recorded for melting point (24.3 °C) of gelatin extracted using yellow fin tuna skin.

Table 1. Yield, gel strength and melting point of the big eye tuna gelatin samples.

Character	S1	S2	S3	S4	S5	S6
Yield (%)	19.670* ±0.422	19.003* ±0.943	16.530* ±0.400	19.133* ±0.820	17.500* ±1.059	16.033* ±0.326
Gel strength (Bloom)	260.00^{a} ±10.00	123.34^{a} ±5.77	83.34^{a} ±11.55	76.67^{a} ±5.77	33.34^{a} ±5.77	30.00^{a} ±0.00
Melting point (°C)	24.033^{b} ± 0.208	21.667^{b} ±0.306	21.333^{b} ±0.306	22.000^{b} ±0.500	19.300^{b} ±0.300	19.000^{b} ±0.458

*^{ab} - Significantly different at 0.05 level

Gelatin samples which were obtained after heat treatment had different color variation from pale yellow color to amber color, while commercial gelatin is of pale yellow color. All final gelatin products prepared were found to have a mild but easily perceivable fishy odor. pH values of all the resulted gelatin samples were slightly acidic and ranged between 4-6. Maximum moisture content (15.2 %) and protein level (82.17 %) was recorded for S1, while minimum moisture level (13.6 %) and protein percentage (74.32 %) was for S5. Greatest lipid content (1.273 %) was reported for S4 and minimum (0.97%) was for S1. According to results of physicochemical and functional properties of final gelatin products, mainly gel strengths, final yields and nutritional quality are not in satisfactory level for all the final products except one treatment (S1). Therefore, it can be recommended that Bigeye tuna fish skin treated using 0.1 % NaOH

and H_2SO_4, concentration with a soaking time of 24 hours at 60 C hot water extraction for 05 hours as the best treatment for gelatin production, due to its desirable gel strength, maximum yield, highest melting point and satisfactory nutritional quality (highest protein level and lowest lipid content). When compared best treatment with commercially available bovine gelatin, crude protein level is relatively low in gelatin extracted using Bigeye tuna skin (Table 02). Also this treatment records comparatively higher lipid content rather than market available gelatin. Average value of pH of fish gelatin of present study was slightly acidic (5.53) compared to mammalian gelatin.

Table 2. The physical, chemical and functional properties of big eye tuna gelatin compared to bovine gelatin.

Properties	Big eye tuna Gelatin	Bovine gelatin
Gel strength (g)	260 Bloom	200 Bloom
Melting point	24.2 ^{0}C	33.8 ^{0}C
Moisture content	15.2 %	14.0 %
Crude protein	82.17 %	88.40 %
Crude lipid	0.97 %	0.26 %
pH	5.53	6.50
Odor	Mild fishy odor	No odor
Color	Amber	Pale yellow

Gel strength of fish gelatin is greater than bovine gelatin and melting point of product is comparatively lower (Table 02). Lower melting point results in faster dissolution in the mouth with no residual 'chewy' mouth feel (Karim and Bhat, 2009). It is a beneficial character in food industry. Since fish skin is generally discarded as waste by processing plants, cost of raw material for Bigeye tuna gelatin is lower compared to commercial gelatin. Moreover, utilization of fish skin for gelatin industry is an environmental friendly solution to overcome the waste accumulation in terrestrial and aquatic environments. Further investigations are needed to develop nutritional quality and physical properties of Bigeye tuna gelatin.

Conclusion

Bigeye tuna fish skin treated using concentration of 0.1 % NaOH and H_2SO_4, with a soaking time of 24 hrs at 60 C hot water extraction for 05 hours is recommended as most appropriate method for gelatin production. In future, Bigeye tuna fish skin can be used as a new alternative to mammalian gelatin with further developments.

References

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