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Preliminary Characterization on Physical Properties of Selected Marine Fish Skins as Alternative Sources of Halal Gelatin

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Abstract

Recently, an alternative gelatin from the fish skins in *halal* market has obtained high demands in many industries. This occurrence has made by-products from the fish such as skins, bones and viscera become valuable and significantly reduced environmental concerns. In this study, there were two types of marine fish used; *Euthynnus affinis* (*Tongkol*) and *Decapterus maruadsi* (*Selayang*) to extract out gelatin from their skins. The extraction methods were utilized two pre-treatment processes using acidic and alkaline solutions; sulphuric acid, citric acid and sodium hydroxide to elucidate the quality of obtained gelatin from the different fish skins. There were no significant differences ($p>0.05$) in their pH, melting point and emulsifying capacity at values of 3.25 ± 0.08 and 3.49 ± 0.04 for pH, 24.0 ± 0.0 (^{0}C) and 23.5 ± 0.5 (^{0}C) for melting point and 42.59 ± 1.85% and 35.19 ± 1.85% for emulsifying capacity respectively for the *E. affinis* and *D. maruadsi* gelatin. Significant differences ($p<0.05$) were existed in yield, viscosity and emulsifying stability respectively for the *E. affinis* and *D. maruadsi* gelatin at values of 6.08 ± 1.29% and 1.56 ± 0.86% for yield, 0.05 ± 0.00 (cP) and 0.03 ± 0.00 (cP) for viscosity and 42.59 ± 1.85% and 33.33 ± 0.00% for emulsifying stability. Throughout the results of gelatin characterizations, *E. affinis* gelatin had showed better physical properties compared to *D. maruadsi* gelatin.

Keywords: Fish gelatine; Gelatin physical properties; *Halal* gelatine; Marine fish

Introduction

Gelatin is a protein polymer where mainly derived from the most abundant collagen (Type I); mostly present in skin and bone of animal. It is one of the most popular ingredients that broadly used in many industries include food, cosmetic, pharmaceutical and photography manufacturing. It have been used as thickeners or stabilizers for dairy and jelly products in many food industries [1]; as encapsulation materials in pharmaceutical industries while in photographic industries as film formation materials [2]. In recent market nowadays, most commercial gelatin is sourced from the beef and pork skins, hides and bones. According to [3], the gelatin present in the world market mostly derived from the mammalian by-products; which consist of pig skins (42.4%), bovine hides (29.3%), bones (27.6%) and other sources (0.7%). Even porcine and bovine gelatin are widely used, unfortunately they are bringing some issues related to health and religious.

Bovine gelatin is having health issue regarding to the high risk of spreading bovine spongiform encephalopathy (BSE) or known as mad cow disease. In religious issues, Hindus cannot consume cow-related products and Muslims have been prohibited to consume pork-related products or *non-halal* products [3].

In order to meet the demands of *halal* market, fish gelatin started to become as alternative sources of gelatin in recent years. According to [4], nearly 30% of fish wastes produced during fish filleting process in industry may cause waste and pollution. Therefore, the used of fish by-products for gelatin can significantly overcome the environmental issues on massive amounts of fish wastes from the fish processing industries. In Malaysia, marine fish are constantly cheap in price and can easily get from the local market since the area of East Coast and South China Sea have provide a bulk of pelagic marine fish catch [5]. *Euthynnus affinis* or *Tongkol* and *Decapterus maruadsi* or *Selayang* were among the common fish sold at wet market or cool storage market. Moreover, according to [6] the protein content in *E. affinis* is about 18-23%, while in *D. maruadsi* is about 16% [3]. Thus, with these significant percentages of solubilized protein in both fish, it could benefit for gelatin extraction purposes.

Gelatin can be extracted using different kinds of pre-treatment methods such as thermal, acidic, alkaline and enzymatic pre-treatments. However, the most preferred method is combination of acid and alkaline pre-treatment because it can give better quality of gelatin compared to the direct thermal treatment; which can produces a gel inferior quality [3]. Additionally, this combination method of pre-treatment is the better way for treating the fish skins. According to [7,8], a mild acid extraction medium not only can remove non-collagenous proteins but also can gain a good result of gelatin yield and gel property. Hence, the aims of this present work are to extract out gelatin from the skins of *E. affinis* and *D. maruadsi* through acidic and alkaline pre-treatment methods and elucidate the relationship between the physical properties of gelatin from both fish skins.

Materials and Methods

Fish Skins Preparation

Fresh fish were purchased from the local wet market in Kuala Lumpur. The fish skins were mechanically removed from its flesh using a sharp knife. After that, the fish skins were rinsed under a running tap water for about three times to remove any superfluous material. Later, the clean fish skins were drained and stored in the freezer at 4.0^{0}C.

Gelatin Extraction

The gelatin extraction procedures were conducted according to the methods of [1] with a slight modification. Firstly, 50.0 g of total fish skin cuts were cut into 2.0 to 3.0 cm and rinsed under a running tap water. After the cleaning process is done, the fish skin cuts were ready to proceed for the pre-treatment processes. The pre-treatment processes were started by soaking the cleaned fish skin cuts into 700.0 mL of 0.2% (w/v) sodium hydroxide (NaOH) for about 40 minutes (at room temperature) to remove all non-collageneous proteins. Then, the alkaline-treated fish skin cuts were rinsed thoroughly with distilled water. After that, the fish skin cuts were soaked into 700.0 mL of 0.2% sulphuric acid (H_2SO_4) for 40 minutes to increase swelling process. Once the process is completed, the fish skin cuts were soaked again into 700.0 mL of 1.0% (w/v) citric acid ($C_6H_8O_7$) for another 40 minutes and rinsed thoroughly with distilled water. The final extraction was carried out with soaking of fish skin cuts into 800.0 mL of distilled water at temperature of 45.0ºC for about 18 hours. After 18 hours, the extracts of solubilized gelatin were separated from the residual skin fragments by series of filtration through a *Whatman filter paper (No.1).* The filtrates were then freeze-dried until dried gelatin is formed. The dried gelatin were weighed and stored at room temperature for further usage in characterization analysis.

Physical Properties Characterization of Gelatin

Percentage of yield: Fish skins gelatin that obtained from the extractions was weighed to determine the percentage of gelatin yield according to [1] based on the following equation:

$$\text{Gelatin (\%)} = \frac{\text{Dried gelatin (g)}}{\text{Wet fish skins (g)}} \times 100\%$$

pH value: pH values of gelatin are determined at room temperature (27.0±1.0^{0}C) by providing gelatin solution at percentage of 6.67% (w/v).

Melting point: Initially, 6.67% (w/v) gelatin solution was prepared before 5.0 mL of it is transferred into a small borosilicate tube; according to the method of [7]. Test tube was covered and placed in a water bath at 60.0^{0}C for about 15 minutes. After that, the tube was subjected to cool immediately in ice-chilled water and maintained at 10.0^{0}C for another 18 hours. Next, five drops of a mixture

that containing 75.0 mL of chloroform and 25.0 mL of red dye (food colouring type) were placed on the surface of the gel in the tube. Later, the tube was heated at a rate of 0.2^{0}C per minutes from its initial or previous temperature of 10.0^{0}C. The melting point was read out at the temperature which the dye drops began to move freely down the gel.

Viscosity: The viscosity of gelatin solution was determined according to the method of [9]. The dried gelatin powders were dissolved in distilled water at ratio of 1:14 to reach about 6.67% (w/v). After that, the gelatin was heated at 60.0^{0}C and then 6.0 mL of it was filled into a syringe. The stopwatch had measured the time when the solution (liquid) is started to flow out from the syringe. The flowing time were recorded and calculated using the following equation for its viscosity determination in centipoise unit (cP):

$$\text{Viscosity of gelatin} = \frac{\text{liq x time (liq) x vis}}{\text{water x time (water)}}$$

density (liq) = density of sample
time (liq) = flow time for sample
vis (water) = 0.467 centipoise (60.0^{0}C)
density (water) = 1.0 g/mL
time (water) = flow time for water

Emulsifying Capacity and Stability: At beginning, 0.5 g of gelatin sample was mixed with 25.0 mL of cold distilled water at 4.0^{0}C and 25.0 mL of sunflower oil for emulsification purposes; according to the method of [10]. After that, the samples were dispersed using a vortex mixer. The blended samples were equally transferred into two centrifuge tubes with total volume of 25.0 mL. One centrifuge tube was directly centrifuge at 2000 rpm for 10 minutes. Another tube was pre-treated in 80.0^{0}C water bath for 30 minutes and cooling down at 25.0^{0}C before subjected to centrifuge at 2000 rpm for 10 minutes. After the centrifugations were completed, the height of emulsified layer over the height of whole layer was used to calculate the percentage (%) of emulsifying capacity and stability based on the following equations:

$$\text{Emulsifying cap. (\%)} = \frac{\text{Height of layer}}{\text{Height of whole layer}} \times 100$$

$$\text{Emulsifying stab. (\%)} = \frac{\text{Height of layer (pre-treated tube)}}{\text{Height of whole layer}} \times 100$$

Data Analysis

All data were obtained from the duplicated samples of tests and analyzed using Statistical Package for Social Science (SPSS) version 16.0; through One-Way Analysis of Variance (ANOVA). Significance difference ($p<0.05$) between the attributes were distinguished using *t*-test.

Results and Discussion

All results of gelatin characterizations on their yield percentage, pH value, melting point, viscosity, emulsifying capacity and stability were stated in the (Table 1).

	Euthynnus affinis	***Decapterus maruadsi***
Yield (%)	6.08^a ± 1.29	1.56^b± 0.86
pH	3.25^a ± 0.08	3.49^a ± 0.04
Melting point (^{0}C)	24.00^a ± 0.00	23.50^a ± 0.50
Viscosity (cP)	0.05^a ± 0.00	0.03^b ± 0.00
Emulsifying Capacity (%)	42.59^a ± 1.85	35.19^a ± 1.85
Emulsifying Stability (%)	42.59^a ± 1.85	33.33^b ± 0.00

Table 1: Results of Characterization on Various Physical Properties of Gelatin from the Skins of *Euthynnus affinis* and *Decapterus maruadsi.*

Note: Different superscript alphabets $^{a-b}$ in the same row indicate significant difference ($p<0.05$)

Yield of Gelatin

Based on the gelatin percentages, there was significant differences ($p<0.05$) between 6.08 ± 1.29 % of *E. affinis* gelatin and 1.56 ± 0.86 % of *D. maruadsi* gelatin. According to [2], the gelatin from sole fish, megrim fish, cod fish and hake fish had showed higher values with 8.3%, 7.4%, 7.2%, 2.6% and 6.5% respectively; compared to *E. affinis* and *D. maruadsi* gelatin. On the other hands, [9] were managed to obtain 5.39% yield of gelatin from the black *tilapia* fish; which was significantly lowered than *E. affinis* gelatin yield in this study. The differences in the gelatin yields were existed possibly due to the species and size of animals, characteristics of their skins, collagen content and extraction methods according to [11].

pH Value

The pH of *E. affinis* and *D. maruadsi* gelatin were showed no significantly differences (*p>0.05)* at values of 3.25 ± 0.08 and 3.49 ± 0.04 respectively. The texture profile of gelatin that correlated with its high *bloom* strength was greatly influenced by pH value of 5.0 for Type B-gelatin; produced from the alkaline pre-treatment of collagen tissues [12]. Thus, both *E. affinis* and *D. maruadsi* gelatin had considerably exhibited moderate gel strength as close to pH 5.0 compared to other studies using marine and freshwater fish; cod fish species (pH 2.7-3.9) and freshwater fish; red *tilapia* fish (pH 3.1) according to [12] and [9] respectively. In addition, pH of gelatin is influenced by the types and chemicals strength used during the pre-treatment processes according to [13]. Therefore, pH values of both *E. Affinis* and *D. maruadsi* gelatin can be improvised in the pre-treatment techniques during the extraction process.

Melting Point

The result of melting point of gelatin indicated that *E. affinis* and *D. maruadsi* gelatin at 24.00 ± 0.00^{0}C and 23.50 ± 0.50^{0}C was not significantly different (*p>0.05*). However, the melting point results from other marine fish (cod species) had reported as low as 8.0^{0}C-10.0^{0}C according to [9]. Additionally, the change of melting point may due to the content of imino acids; proline and hydroxyproline that responsible in stabilizing the structure of gelatin [11]. Thus, it can simply be explained that low melting point may cause by the low contents of proline and hydroxyproline. The structures of both *E. affinis* and *D. maruadsi* gelatin can be uttered as moderately stable since their melting points are considerably high; although the chemical analysis on amino acid contents is not performed in this study.

Viscosity

The viscosity for *E. affinis* gelatin was showed higher than the *D. maruadsi* gelatin with their respective values of 0.05 ± 0.00 (cP) and 0.03 ± 0.00 (cP) and significantly different (*p<0.05*). Through the commercial standpoint, the viscosity range of gelatin was generally between 2.0 to 7.0 (cP); which obviously showed that *E. affinis* and *D. Maruadsi* gelatin were categorized as a low in viscosity. Nevertheless, gelatin viscosity can be significantly increased by the production of gelatin between the pH of 3.0 to 10.5 [9] since the differences of viscosity are influenced by molecular size distributions and pH, according to [14].

Emulsifying Capacity and Stability

Based on emulsifying capacity results, there was no significant difference (*p>0.05*) between *E. affinis* and *D. maruadsi* gelatin at values of 42.59 ± 1.85% and 35.19 ± 1.85% respectively. However, significant difference (*p<0.05*) was existed in the results of emulsifying stability at the values of 42.59 ± 1.85% and 33.33 ± 0.00% for *E. affinis* and *D. maruadsi* gelatin respectively. Furthermore, based on the previous study on the gelatin by [10]; black kingfish or a yellowtail fish subspecies like *E. affinis* showed higher value of emulsifying stability (55.66%) but lower value of emulsifying capacity (32.5%) than *E. affinis* in this study. These results may have been due to the greater hydrophilic and hydrophobic regions which act as emulsifiers in a mixture of oil and water. Rapid migration of protein molecules to fat droplets and emulsifying efficiency would increase as well; due to the high solubility and hydrophobic protein molecules during the dispersal phase [15,16]. In addition, a high content of hydrophobic amino acid residue results in effective distributions of hydrophilic-hydrophobic amino acids that improve gelatin emulsifying properties, according to [16].

Conclusion

Results from the present study are clearly demonstrated the skin of *E. affinis* is a prospective source to produce *halal* gelatin in considerably good yield with the desirable characteristics in physical properties; comparable to *D. maruadsi* skin. The next steps would focus on the analysis of chemical properties, sensory evaluations and gel strength of *E. affinis* gelatin for elucidating its quality entirely.

References

1. Irwandi J, Faridayanti S, Mohamed ESM, Hamzah MS, Torla HH, et al. (2009) Extraction and characterization of gelatin from different marine fish species in Malaysia. International Food Research Journal 16: 381-389.

2. Gomez-Guillen MC, Turnay J, Ferna M, Ulmo N, Lizarbe MA, Montero P (2002) Structural and physical properties of gelatin extracted from different marine species: A comparative study. Food Hydrocolloids 16: 25-34.

3. Herpandi N, Huda N, Adzitey F (2011) Fish bone and scale as potential sources of *halal* gelatin. Journal of Fisheries and Aquatic Science 6(4): 379-389.

4. Chee PE (1995) Tuna fisheries interaction in Malaysia: Status of interaction of Pasific Tuna Fisheries in 1995.

5. Ghaly AE, Ramakrishnan VV, Brooks MS, Budge SM, Dave D (2013) Microbial biochemical technology fish processing wastes as a potential source of proteins, amino acids and oils: A critical review. Journal of Microbial Biochemical Technology 5(4): 107-129.

6. Jiang H, Tong T, Sun J, Xu Y, Zhao Z, et al. (2014) Purification and characterization of antioxidative peptides from Round Scad (*Decapterus maruads*i) muscle protein hydrolysate. Food Chemistry 63: 154-158.

7. Jamilah B, Tan KW, Umi Hartina MR, Azizah A (2011) Gelatin from three cultured freshwater fish skins obtained by liming process. Food Hydrocolloids 25: 1256-1260.

8. Zhou P, Regenstein JM (2005) Food chemistry and toxicology effects of alkaline and acid pretreatments on Alaska Pollock skin gelatin extraction. Journal of Food Science 70(6): 392-396.

9. Jamilah B, Harvinder KG (2002) Properties of gelatins from skins of fish-black *tilapia* (Oreochromis mossambicus) and red tilapia (*Oreochromis nilotica*). Food Chemistry 77: 81-84.

10. Killekar VC, Koli JM, Sharangdhar ST, Metar SY (2012) Functional Properties of Gelatin Extracted from Skin of Black Kingfish (*Ranchycentron canadus*). Indian Journal of Fundamental and Applied Life Sciences 2(3): 106-116.

11. Nazri MAR, Azzainurfina SSKAJ (2012) Extraction, phytochemical characterization and sensory quality of chicken feet gelatin. Borneo Science 30: 1-13.

12. Gudmundsson M, Hafsteinsson H (1997) Gelatin form cod skin as affected by chemical treatments. Journal of Food Science 62: 37-47.

13. Cho SM, Kwak KS, Park DC, Gu YS, Ji CI, Jang DH, et al. (2006) Processing optimization and functional properties of gelatin from shark (*Isurus oxyrinchus*) cartilage. Food Hydrocolloids 19: 573-579.

14. Greener J, Contestable BA, Dale MD (1987) Interaction of anionic surfactants with gelatin: Viscosity effects. Macromolecules 20(10): 2490-2498.

15. Rawdkuen S, Thitipramote N, Benjakul S (2013) Preparation and functional characterization of fish skin gelatin and comparison with commercial gelatin. International Journal of Food Science and Technology 48: 1093–1102.

16. Gomez-Guillen MC, Pérez-Mateos M, Gómez-Estaca J, López-Caballero E, Giménez, B, et al. (2009) Fish gelatin: A renewable material for developing active biodegradable films. Trends in Food Science and Technology 20: 3-16.