Solid State Science and Technology, Vol. 25, No 2 (2017) 79-85
ISSN 0128-7389 | *http://journal.masshp.net*

THE PROPERTIES OF GELATIN FROM THE SKIN OF TERUBUK FISH (*TENUALOSA TOLI*)

Salamiah Zakaria**,** Harizah Syahirah Zainal and Wahida Abdul Rahman

Faculty of Applied Science, Universiti Teknologi MARA, Perlis Branch, Arau campus, 02600 Arau, Perlis, Malaysia

Corresponding author: salamiah882@perlis.uitm.edu.my

ABSTRACT

Gelatin was successfully extracted from the skin of terubuk (*Tenualosa toli*) by alkaline and acid pre-treatment method with the yield of 25.12%. The functional group, pH value, and sensory, foaming and emulsifying properties were studied. The FTIR spectra showed that the gelatin has major absorption bands in the amide region. The pH of the gelatin was in acidic range. The sensory evaluation showed that the gelatin has a strong but not offensive odour with the mean sensory of 2.55. The gelatin has low foaming capacity as well as foaming stability with 10% and 6% respectively. The low foaming capacity could be due to the lower percentage of negatively charged amino acids. For emulsifying properties, it showed that the increased of the concentration of gelatin solution the decreased of emulsifying activity index (EAI) as well as the emulsifying stability index (ESI). This might because of at high protein concentration, the diffusion is limited due to the activation energy barrier. Meanwhile, at low protein concentration favours higher EAI because of the ability of the protein to diffuse and adsorb at the oil-water interface.

Keywords: fish gelatin; Tenualosa toil; functional properties

INTRODUCTION

Gelatin is a clear and tasteless protein and has a rheological property of thermo-reversible transformation between sol and gel which has been widely utilized in food, pharmaceutical, and photographic industries. Gelatin is widely used in food especially in bakery products. The stabilizing properties of gelatin is very useful in making marshmallow, cream fillings, whipping cream and icing because it can help to maintain the structure of sugar crystal. Generally, most of the commercial gelatins can be obtained from skin, scale, bones, ligament and tendon of porcine or bovine. The used of porcine and bovine gelatin is vitally limited by religious concerns. For example, Muslims are prohibited to consume all pork related products and Hindus, they are prohibited to consume of all cow-related products. In addition, bovine gelatin has a potential risk of spreading bovine spongiform encephalopathy (BSE), widely known as a mad cow disease and foot-and-mouth disease (FMD). Due to this religious reasons and health concerns, therefore, the study of gelatin from fish, such as skin, bone and

scales, is of interest. The fish processing industries generated a large amounts of waste every year and the cost of the fish waste disposal was very high. The fish processing produced a large quantity of waste and it was reported that the waste after filleting was 75% of the total fish weight and the remaining 30% of the waste was the fish bones and skins [1]. Therefore, the abundance sources of fish byproduct such as bones, scales and skin can be a great sources of gelatin. The gel strength properties of fish gelatin is almost the same with the commercial pigskin and bones [2]. With this background, the present study was undertaken to extract the gelatin from the skin of terubuk (*Tenualosa toli)* fish and understand its properties.

EXPERIMENTAL

Raw Material.
Terubuk fishes (*Tenualosa toli*) were bought from Lembaga Kemajuan Ikan Malaysia (LKIM), Perlis. After that it was placed in freezer in order to freeze it. After the evisceration process, the skins of the terubuk fish were peeled with a sharp scalpel. Then, the fillets were removed from the fish manually.

Gelatin Extraction.
The skin gelatin was extracted by using the method as described by Shyni et al. [3] with slight modification. 60g of fish skins were rinsed thoroughly in excess water 30 min. Then it were soaked in 0.2% (w/v) sodium hydroxide with a skin/solution ratio of 1:10 (w/v) for 2 hr at ambient temperature. The solution were changed for every 1 hr to remove the non- collagenous proteins and pigments. After that, the skins were washed with tap water until the wash water was neutral. Then the treated skins were soaked in 0.2% (w/v) acetic acid with a skin/solution ratio of 1:10 (w/v) at 4°C for 24 hr. The solution was changed for every 12 hr to swell the collagenous material in fish skin and the treated skins were rinsed with distilled water between each soakings. Then it was washed with tap water until pH of wash water become neutral. After that, the fish skins were extracted with distilled water at 45°C for 12 hr with a skin/solution ratio of 1:10 (w/v). The extract was filtered with cheese cloth and freeze dried at - 15°C, 0.014 mbar for 24 hr.

Properties of the Gelatin

The pH Value of the Gelatin.
The pH value the skins gelatin were determined using method described by Alfaro et al. [4] . 1% gelatin solution was prepared under constant stirring for 30 min at 60°C. The solution was allowed to cool at room temperatureand the pH value was measured by using pH meter.

Sensory Evaluation.
Sensory evaluation were determined using the method as described by Muyonga et al. [5], with a slight modification. The evaluation was conducted using 20 members panel. Sample was prepared by dissolving 0.5 g of gelatin in 7 ml of distilled water, to obtain a

solution containing approximately 6.67% gelatin in test tubes with screw caps. The mixture was swirled and let to stand for 30 min at room temperature to allow the gelatin to absorb water. The samples were held in water bath at 50°C, with the screw caps lightly closed. Panelist were instructed to remove screw caps, sniff the contents and identify the odour they perceived as well as indicate the odour intensity, using a six point scale (0 = no odour, 1 = very mild and only perceiveable on carefull assessment, 2 = mild but easily perceivable, 3 = strong but not offensive, 4 = strong and offensive , 5 = very strong and very offensive).

Fourier Transform Infrared (FTIR) Spectroscopy.
The gelatin structural properties were measured using FTIR spectroscopy (PerkinElmer Spectrum Version 10.4.4) with ATR mode. The gelatin sample were placed on the plate for analysis and the spectrum were recorded in the range of 4000-650 cm^{-1}.

Foaming Properties of Gelatin.
Sample was added into distilled water until the final concentration is 1 % (w/v). Then, 5 mL of the sample was added into a 15 mL plastic centrifuge and homogenized in a centrifuge at a speed of 5000 rpm for 30 min. The foaming stability (FS) was determined after 15 min by the percentage of the remaining foam. As for foaming capacity (FC), it was calculated by the percentage of the volume increase of the protein dispersion when mixing.

$$\text{FC (\%)} = \frac{V_T - V_0}{V_0} \times 100 \qquad \text{FS (\%)} = \frac{V_t - V_0}{V_0} \times 100$$

where V_T is the total volume after homogenizing (mL), V_0 is the volume before homogenizing and V_t is the volume remain after 15 min.

Emulsifying Properties of Gelatin.
The gelatin solution with concentration of 0.1%, 0.2% and 0.5% were prepared. A mixture of gelatin solution (6 mL) and 2 mL of sunflower oil were homogenized at a speed of 5000 rpm for 30 min at room temperature. 1 μL of the emulsions was then pipetted out at 0 and 10 min and further diluted with 5 mL of 0.1% sodium dodecyl sulphate solution. The dispersion was measured at absorbance of 500 nm using UV-Vis spectrophotometer. The emulsion activity index (EAI) and emulsion stability index (ESI) were calculated using the following formula:

$$\text{EAI } (m^2/g) = \frac{2 \times 2.303 \times A \times DF}{1 \times \phi \times C}$$

where A = absorbance at 500nm, DF = dilution factor (100), 1 = path length of cuvette (m), ø = oil volume fraction and C = gelatin concentration in aqueous phase (g/m^3).

$$\text{ESI (min)} = \frac{A_0}{A_0 - A_{10}} \times 10$$

Where, A_0 = absorbance at 500nm at 0 min, A_{10} = absorbance at 500nm at 10 min and 10= 10 min

RESULTS AND DISCUSSION

Percentage Yield.
The percentage yield skin gelatin was 25.12%. Percentage yield of the terubuk's skin gelatin showed that it was slightly higher than skin gelatin from Pangas catfish (22%), Asian redtail catfish (21.28%), Striped snakehead (20.25%) and Nile tilapia (21.93%) which reported by Ratnasari et al. [6]. It was also much higher compared to the percentage yield of skin gelatin from eel reported by Nurul and Sarbon, (2015) [7] which was 11.98%. The possible reasons of high percentage yield of gelatin were due to the different kind of skin, acid concentration, the rate of collagen break down when washing treatment and swelling process [6]. Nurul and Sarbon [7] suggested that the differences in percentages may be because of variations in acid extraction methods or differences in chemical composition of skins, which all of these properties vary according to species and age of the fish.

The pH Value of the Gelatin.
The pH of the gelatin solution was 5.33. In the present study this skin gelatin is in acidic range. this pH value is lower to the value reported by Ratnasari et al., [6] for Pangas fish (5.8), Asian redtail (5.9), Nile tilapia (5.7) and Striped snakehead (5.8). To be compared with the pH value described by Venkateshwarlu et al.,[8] for Blackspotted Croaker (5.5), pH value of skin gelatin from terubuk is still lower.The acidic pH values of gelatin obtained was influenced by the washing treatment [6,8]. Shyni et al., [3] stated that the differences in pH value obtained was due to the different pretreatments used during the extraction which involved both alkaline and acid treatments. Furthermore, the type and strength of acids used during extraction process also affected the pH value of gelatin obtained [9]

Functional Groups. The amide band, which is known as a characteristic pattern of gelatin was determine by using FTIR

Table 1 showed the major peaks observed in the FTIR spectrum of the skin gelatin. FTIR spectroscopy has been used to monitor the functional groups and secondary structure of gelatin . Amide-I represent C=O stretching/hydrogen bonding coupled with COO. The absorption in the amide-I region is probably the most useful for infrared spectroscopic analysis of the secondary structure of proteins. Its exact location depends on the hydrogen bonding and the conformation of protein structure. In the present study Amide I band are in the range of 1600-1700 cm^{-1}. In addition, absorption band of Amide II was noticeable at the wavenumbers of 1538.61 cm^{-1}. The lower wavenumbers were detected by Duan et al., [10] for the acid soluble collagen extracted from skin of

carp (1540.00 cm^{-1}). The normal range of Amide II bands position is 1550-1600 cm^{-1} and the position is shifted to lower frequency, 1540 cm^{-1} which indicated that the existence of hydrogen bonds in each collagen.The Amide III bands was observed at 1235.41 cm^{-1} [10].The Amide A and Amide B band positioned was recorded at 3291.02 cm^{-1}. and 2923.03 cm^{-1} respectively [7] while Amide I band was recorded at the frequency of 1632.18 cm^{-1} [10].

Table 1: Major peaks observed in the FTIR spectrum of the skin gelatin

Functional group	**Wavenumber range (cm^{-1})**	**Assignment**
Amide A	3291.02	NH stretching
Amide B	2923.03	CH_2 assymetrical stretching
Amide I	1632.18	C=O stretching
Amide II	1538.61	CN stretching with NH bending
Amide III	1235.41	CN stretching

Sensory Evaluation.
From the result of the sensory evaluation, it was observed that this terubuk‘s skin gelatin had strong but not offensive odour with the mean sensory of 2.55. The difference of odour for each gelatin could be affected by living environment of the fish. Furthermore, the depth of habitat where the fish live, types of materials, level of pollution, types of plankton living around and others are the environmental factors that might influence the characteristic of the gelatin [11].

Foaming Properties.
Foaming properties of a protein may be affected by the source, intrinsic properties, the composition and conformations of the protein solution. The determination of foaming properties in the food industry has an important effect on the processing and the quality of some products [12] . This foaming properties are important properties of gelatin for commonly used food such as marshmallows [13] . The foaming capacity (FC) and foaming stability (FS) of the gelatin are shown in Table 2. It showed that the gelatin has 10% of FC and 6% FS. The lower FS could be due to the lower percentage of negatively charged amino acids [12].

Emulsifying Properties.
Due to the suface active properties, gelatin acts as emulsifying, foaming, and wetting agent in food, technical, pharmaceutical and medical application. Emulsifying activity index (EAI) and emulsifying stability index (ESI) for the gelatin are tabulated in Table 2. From the results obtained, it showed that the increased of the concentration of gelatin solution decreased the EAI as well as the ESI. The same results also obtained by Khiari et al., [12] for gelatin extracted from mackerel and blue whitting bones. They also mentioned that an important parameter that influence the emulsifying activity is the protein concentration. At high protein concentration, the diffusion is limited due to the

activation energy barrier. Meanwhile, at low protein concentration favours higher EAI because of the ability of the protein to diffuse and adsorb at the oil-water interface. The results of emulsfying properties showed that skin gelatin from terubuk was stable due to the high value of ESI.

Table 2 The foaming and emulsifying properties of the gelatin.

Foaming Properties		**Emulsifying properties**		
FC (%)	**FS**	**Concentration (%)**	**EAI (m^2/g)**	**ESI (min)**
10	**6%**	0.1	34.60	23.74
		0.2	18.34	22.72
		0.5	8.34	21.30

CONCLUSION

The yield of the gelatin extracted from the skin of terubuk was higher compared to the skin of other fish skin in several studied. This terubuk fish skin is a prospective source to produce gelatin. It showed some potential to be used in application in the industry as the replacement of mammalian gelatin.

ACKNOWLEDGMENT

Thanks to Faculty of Applied Science, Universiti Teknologi Mara Perlis for the facilities and equipment used.

REFERENCES

[1] M.C.Gòmez-Guillen, J. Turnay,M.D. Fernandez-Diaz, N. Ulmo, M.A. Lizarbe and P. Montero. *Food Hydrocolloids,* **1** 25–34 (2002)
[2] Herpandi, N.Huda, F.Adzitey, *Journal of Fisheries and Aquatic Science,* **6** (4) 379-389 (2011)
[3] K. Shyni, G.S. Hema,G.Ninan,S.Mathew,C.G.Joshy, and P.T. Lakshmanan, *Food Hydrocolloids,* **39** 68-76 (2014)
[4] A.T. Alfaro, F.C. Biluca, C. Marquetti, I.B. Tonial, and N.E. De Souza, *Food Research International* **65** 416-422 (2014)
[5] J.H. Muyonga, C.G.B.Cole, and K.G.Duodu, *Food Hydrocolloids,* **18** 581-592 (2004)
[6] I. Ratnasari,S.S.Yuwono, H. Nusyam, and S.B. Widjaanarko, *International Food Research Journal,* **20** (6) 3085-3091 (2013)
[7] A.G. Nurul, and N.M.Sarbon, *International Food Research Journal,* **22** (2) 572-583 (2015)
[8] G.Venkateshwarlu, J.K. Jakhar, A.D. Reddy, S. Maharia, H.M. Devi, and G.V.S.Reddy , *Archieves of Applied Science Research,* **3** 1353-1358 (2012)
[9] B. Jamilah, and K.G. Harvinder, *Food Chemistry,* **77** 81-84 (2002)
[10] R.Duan, , J. Zhang, X.Du, X. Yao, and K. Konno, *Food Chemistry,* **112** 702-706

(2009)
[11] J. Irwandi, S. Faridayanti, E.S.M. Mohamed, M.S. Hamzah, H.H. Torla, and Y.B. Che Man, *International Food Research Journal,* **16** 381-389 (2009)
[12] Z. Khiari, D. Rico, A. Martin-Diana, and C. Bany-Ryan, *Food Chemistry*, **139** (1-4) 347-354 (2013)
[13] R.Balti, M. Jridi, A. Sila, N. Souissi, N. Nedjar-Arroume, D. Guillochon, and M. Nasri , *Food Hydrocolloids*, **25** 943-950 (2011)