Extraction and Characterization of Gelatin from Black Tilapia (*Oreochromis niloticus*) Scales and Bones

Salamiah Zakaria and Nurul Hidayah Abu Bakar

Abstract**—** Gelatin extracted from fish sources is one best alternative to replace the porcine and bovine gelatin which can lead to the religious concerns and several diseases. Warm-blooded fish gelatin has similar properties to mammalian sources. The best parts of the fish are the scales and bones which are rich in collagen which can be the great source of the gelatin. In this study, gelatin was extracted from the scales and bones of the warm-blooded fish namely black tilapia (*Oreochromis niloticus*) were used. The extraction was done by heating the scales and bones in distilled water for 1.5 h at 70°C. The results showed that the scales gelatin give higher yield and foaming properties compared to the bones gelatin. However, the trends of emulsifying properties that consists of Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI) for both scales and bones gelatin are similar. Both scales and bones gelatin have similar functional groups that contained in the most gelatin which is amino group. However the types of amino groups presence are differ in both gelatins.

Keywords**—**black tilapia, bones, fish gelatin, scales.

I. 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. Nowadays, gelatin is one of the trending usages especially in culinary area [1]. 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 [2]. Therefore, the abundance sources of fish byproduct such as bones, scales and skin can be a great sources of gelatin. Fish scales and bones are more preferable in the extraction of gelatin because it yields large amount of gelatin due to high content of amino acids (proline) compared to fish skin. The gel strength properties is almost the same with the commercial pigskin and bones [3]. The retaining rate of hydroxyproline collagen of the fish scales is 96.10% [4]. With this background, the present study was undertaken to extract the gelatin from the bones and scales of the of warm-w ater fish which is black tilapia and understand its functional properties.

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II. Materials and Method

A. Sample Preparation

Black tilapia fish was bought from a supplier at Jitra, Kedah. The fish was stored directly in the refrigerator. The scales of black tilapia had been removed by using a knife while the bones were grinned by using a blender.

B. Gelatin Extraction

14.3 g of the scales had been washed with tap water for 1 h to remove superfluous material. Then, the scales were soaked in 100 mL of 0.4 (w/v) NaOH for 4 h to remove the non-collageneous protein. After that, the scales were rewashed with running tap water for 1 h. The scales were then soaked in 100 mL of 0.4 (v/v) HCl for 4 h. The pH of the scales was neutralized by washing the scales using tap water. Finally the gelatin extraction from the scales was carried out with distilled water at 1:1 ratio (g:mL) at 70°C for 1.5 h. After that, the gelatin extracted was filtered with two layers of cheese clothes. To remove the content of water, the filtrate was evaporated by using rotary evaporator for 30 min. Later, the filtrate was dried in hot-air oven at 50°C for 18 h. The gelatin film produced was stored in a desiccator for further use. All steps were repeated with the black tilapia's bones.

The yield of gelatin extracted can be calculated by using (1).

$$\%\ yield = \frac{mass\ of\ dried\ gelatin}{mass\ of\ clean\ scales\ or\ bones} \times 100 \quad (1)$$

C. Foaming Properties

Foaming properties were evaluated by foaming capacity (FC) and foaming stability (FS). The gelatin samples were added in 50 mM potassium phosphate buffer at pH 7.5 until the final concentration was 0.3% (w/v, gelatin sample). 5 ml of each sample was homogenized in a 15 mL plastic centrifuge tube and it was centrifuged for 1 min. The foaming capacity was calculated by the percentage of the volume increase of the protein dispersion when mixing while the foaming stability was determined by the percentage of the remaining foam after 15 min. FC and FS can be calculated by using (2) and (3) respectively.

$$FC\,(\%) = \frac{volume\ of\ solution\ after\ homogenized - volume\ of\ solution\ before\ homogenized}{volume\ of\ solution\ before\ centrifuged} \times 100 \quad (2)$$

$$FS(\%) = \frac{volume\ of\ solution\ after\ 15\ min - volume\ of\ solution\ before\ 15\ min}{volume\ of\ solution\ before\ 15\ min} \times 100 \quad (3)$$

D. Emulsifying Properties

Emulsifying properties were evaluated by emulsion capacity and emulsion stability. The gelatin solutions were prepared by different concentrations. Firstly, the gelatin sample was dissolved in 50 mM potassium phosphate buffer containing 0.3 M NaCl at pH 7 until the final concentrations were 0.05%, 0.1% and 0.2% (w/v, gelatin sample). Later, 2.0 ml of sunflower oil was mixed with 8.0 ml of each gelatin solution. The mixture was stirred by using magnetic stirrer for 24 h. After that, 5µL aliquot of the emulsion was diluted in 5 mL of sodium dodecyl sulphate (SDS) solution (0.1% w/v). Then, the solution was tested by Spectronic 200 and the absorbance was measured at 500 nm. The emulsion capacity was determined by calculating the Emulsion Activity Index (EIA) as shown in (4).

$$EAI = \frac{2 \times 2.303}{C \times \emptyset \times 10^4} \times A_{500} \times Dilution \quad (4)$$

Where, A_{500} represents the absorbance at 500 nm, C represents the concentration of protein (g/ml) before emulsion, ø represents the oil volume fraction (v/v) of the emulsion.

The emulsion stability was predicted by leaving the emulsion for 15 min at 25°C, later diluted with 5 ml SDS solution (0.1% w/v). The absorbance was also measured at 500 nm, emulsion stability index was calculated by using the following expression:

$$ESI = \frac{A_{15}}{A_0} \times 100 \quad (5)$$

Where A_{15} is the turbidity measured at 500 nm of the emulsion at 15 min, A_0 is the turbidity measured at 500 nm of the emulsion after 0 min.

E. Fourier transform infrared (FTIR) spectroscopic analysis

Gelatin samples were subjected to FTIR analysis using Thermo Electron Corporation Nicolet 380 FTIR spectrometer. by preparing a 500 mg KBr pellet containing 2–6 mg of the sample. Spectra were acquired in the IR range of 4000-500 $cm^{-1.}$

III. Results and Discussion

A. Yield

Yields of gelatin extracted from the scales and bones of black tilapia were 16% and 5% respectively. According to Karim and Bhat [5], the extraction yield of fish gelatin is less than mammalian gelatin. The ranges mean yield of gelatin extracted from fish is between 6% and 19% (grams of dry gelatin per100 g of clean skin). The low yield might be due to the loss of collagen during extraction, leaching during washing steps or incomplete hydrolysis of the collagen [6] and differences in collagen content, their composition in the scales, bone, skin, as well as their matrix [7].

B. Foaming Properties

Foaming capacity and foaming stability become important parameters to characterize the functional properties of proteins. For example, the size of bubbles in the foam influences the texture and appearance of food products. Gelatin is one of the most widely used protein foaming agent. The good protein foaming agent should stabilize foams rapidly and effectively at low concentration and become an effective foaming agent over the pH range that exists in various foods.

The foaming properties of both gelatins were tabulated in Table I. From the result obtained, it showed that the foaming properties of scales gelatin was higher than the foaming properties of bones gelatin. Both foaming capacity and foaming stability of the scales gelatin were higher than that of bones gelatin. From this result, it can be concluded that gelatin from the scale of black tilapia can act as better foaming agent as compared to gelatin extracted from its bones.

TABLE I
FOAMING PROPERTIES OF SCALES AND BONES GELATIN

	Foaming Capacity (%)	Foaming Stability (%)
Scales Gelatin	60	25
Bones Gelatin	10	5.45

B. Emulsifying Properties

Emulsifying properties of food proteins are described as emulsion capacity or emulsion activity, which reflect the ability of the protein to aid formation and stabilization of newly created emulsions and the ability of the proteins to impart strength to emulsion for resistance to stress [8]. Gelatin is surface-active and acts as an emulsifier in oil-in-water emulsion. The emulsifying properties was generated due to the

presence of hydrophobic areas on the peptide chain [5]. The EAI and ESI for both gelatin at different gelatin concentration (0.05%, 0.10% and 0.2%) were depicted in Fig.2 and Fig.3 respectively. The emulsifying activity index (EAI) is a measurement of the area interface stabilized over unit weight of protein (m^2/g) that relates to the ability of protein to coat an interface [9]. From the analysis, regardless of scales and bones, the EAI value decreased with increasing the gelatin concentration. Similar results were reported by Khiari nd co-workers [10] for mackerel and blue whiting bones gelatin. The protein concentration affects the value of EAI whereby the low protein concentration gives higher EAI value due to the ability of the protein to diffuse and adsorbs at the oil-water interface [11]. The high protein concentration resulting low EAI value because the diffusion of protein become limited due to the distraction of activation energy barrier [10]. Scales gelatin showed higher EAI for concentrations 0f 0.05% and 0.10% gelatin solution. While at 0.20% of gelatin concentration, bones gelatin showed significantly higher EAI than scales gelatin. This possibly resulted from the difference in the intrinsic properties, composition and conformation of the different gelatins [11].

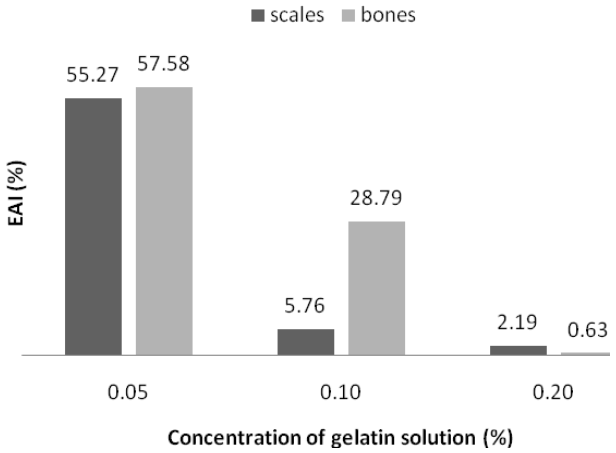

Fig.1 Emulsifying activity index of scales and bones gelatin

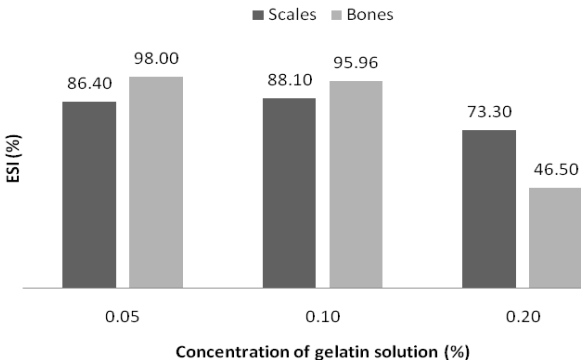

Fig.2 Emulsifying stability index of scales and bones gelatin

From Fig.2, the ESI for bones gelatin was decreased gradually with increasing concentration of the gelatin solution. The similar trend was also reported by Jridi and co-workers [12] in their study of gelatins extracted from alkali-pretreated skin of cuttlefish (*Sepia officinalis*) using pepsin. The stabilization of emulsion against coalescence/flocculation is greatly dependent on the force of electrostatic repulsions between the adsorbed proteins on the interfacial protein film [12]. However the ESI for scales gelatin was increased from 86.36% (in 0.05% solution) to 88.1% (in 0.10% solution) then it decreased to 73.33% in 0.2% of gelatin solution concentration.

D. Fourier transform infrared (FTIR) spectroscopic analysis

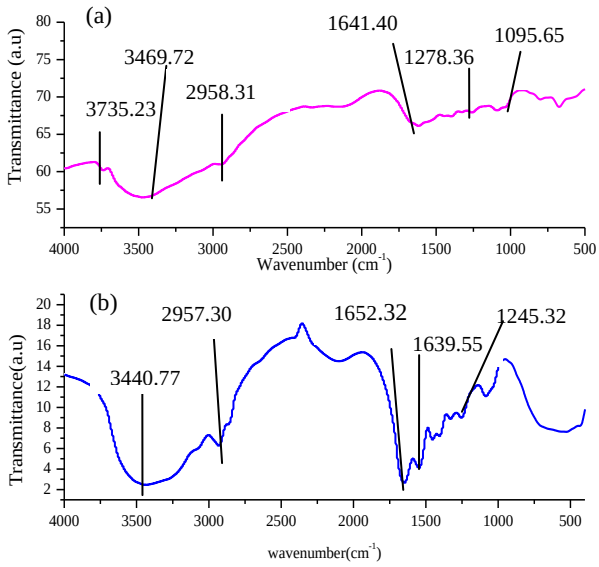

Fig.3 FTIR spectra of (a) scales gelatin and (b) bones gelatin

FTIR spectroscopy has been used to monitor the functional groups and secondary structure of gelatin. Based on the FTIR spectra in Fig.3 it can be seen that both gelatin samples had major peaks in the amide region. In scales gelatin (Fig. 3(a)), the FTIR spectra showed the presence of amide peaks that consists of Amide-I and Amide-III at 1641.40 cm^{-1} and 1278.36 cm^{-1} respectively. Amide-I peaks was the result from C=O stretching vibration coupled with C–N stretch and CCN deformation [8].The presence of phosphate stretching was observed at 1095.65 cm^{-1} indicated the presence of calcium salt in the scales gelatin sample [4].The spectra of bones gelatin in Fig.3b, shows the presence of Amide A at 3340.77cm^{-1} which arise from the stretching vibrations of the N-H group [12]. This gelatin was also consist of Amide-I, Amide-II and Amide-III which can be observed at 1652.32 cm^{-1}, 1539.55 cm^{-1} and 1245.32 cm^{-1} respectively.

IV. Conclusion

From the study, it can be concluded that the scales gelatin showed more advantages compared to bones gelatin. This can be seen in the percentage yield of scales gelatin was greater than that of bones gelatin. In foaming properties, the scales gelatin showed higher FC and FS compared to bones gelatin.

According to FT-IR analysis, the scales and bones gelatins exhibited different characteristics whereby scales gelatin showed the presence of Amide-I and Amide-III while bones gelatin consist of Amide-A, Amide-I, Amide-II and Amide-III. It was observed that scales and bones gelatin showed the same trend in the emulsifying properties of scales and bones gelatin where the value of EAI and ESI for both gelatins decreased with increasing gelatin concentration.

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