Extracting of yellowfin tuna (*Thunnus albacares*) fish skin gelatin as influenced by alkaline concentration and extraction times

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Abstract. Yellowfin tuna (*Thunnus albacares*) skin is a by-product of the fish processing industry. Yellowfin tuna skin was used as materials for gelatin because it contains collagen. This study was conducted to determine the characteristics of yellowfin tuna skin gelatin (YSG). In this study, YSG was obtained by the extraction process in 0.01 M NaOH solution for 12 h. The results show that the yield of YSG was 12.49 % dry basis, with the gel strength of 291.73 g Bloom, viscosity of 8.6 cPs and melting point of 35 °C. The gel strength and melting point of YSG were lower than commercial bovine gelatin (CBG), which was 309.7401 g Bloom and 36.33 °C, respectively. However, the viscosity of YSG was higher than the CBG which was 5.6 cPs.

1. Introduction

Gelatin is defined as a denatured protein derived from collagen by thermal hydrolysis and the thermoreversible transformation between sol and gel determines the property of gelatin. Gelatin has been widely used in the food, pharmaceutical and photographic industries [1]. The worldwide production of gelatin in 2005 of which 44.9 % were from pigskin, 27.9 % from bovine hides, 27.2 % from bones and a few percent from other parts [2].

In fish industries, 30 % of the waste is in the form of bones and skins [3]. The study of gelatin from fish by-products, such as skin and bone, has increased for the replacement of mammalian resources [4]. Fish gelatin can be used to replace porcine and bovine gelatin which are a major constraint for consumers with the religion and socio-cultural background. For example, porcine gelatin which is prohibited for Muslims or Jews and bovine gelatin which is not consumed by Hindus and also usually not acceptable to Jews and Muslims. Moreover, frequent occurrences of bovine spongiform encephalopathy (BSE) affects the safety issue of bovine gelatin.

The most common use of gelatin is its thermally reversible gelling properties with water, as for example, in the production of table jellies. An aqueous solution of a few percent gelatin forms thermally reversible gels with water and the gel melting temperature (< 35 °C) is below body temperature, which gives gelatin products unique organoleptic properties and flavor release [5]. The thermal reversibility of this process gives the gelatin gel it’s unique “melt in mouth” quality. Other
gelling agents such as starch, alginate, pectin, agar and carrageenan are all polysaccharides from plant sources, but their gels lack the melt-in-the-mouth quality, elastic properties of gelatin gels. Gelatin is notable for its gelling properties and clean flavor profile. The gelatin gel has been described as having a sparkling and clear appearance with clean melt in the mouth texture that has yet to be duplicated by any polysaccharide [4].

The degree of collagen conversion into gelatin is related to the severity of both the pretreatment and the warm water extraction process, as a function of pH, temperature, and extraction time [6]. This research aimed to know the effect of NaOH concentrations in extraction process and extraction times on the quality of gelatin made of yellowfin tuna (*Thunnus albacares*) skin (YSG) to meet the standard of gelatin in food industry, to determine the characteristics of YSG and to compare YSG with commercial bovine gelatin (CBG).

2. Materials and Methods

2.1. Materials

Yellowfin tuna (*Thunnus albacares*) skin was provided by Indo Marlin, Pelabuhan Perikanan Muara Baru Jakarta. Mammalian gelatin extracted from the skin bovine was purchased from Sigma Chemical Co. All reagents used in this study were analytical grade.

2.2. Extraction of gelatin from yellowfin tuna skin

The yellowfin tuna skin gelatin (YSG) was extracted based on modified methods of previous studies [3, 6, 7, 8, 9, 10, 11]. Skin of yellowfin tuna (*T. albacares*) stored at 20 °C. Before use, the skins were allowed to thaw below 10 °C and skins were scraped manually by a scalpel. A part of fat on those skins was removed by vigorous stirring in chill water and then de-greased by tumbling in warm water (70 °C) for 10 min. Then the skin was cleaned in distilled water and cut into 2 × 2 cm squares using scissors. The cleaned skin was treated with a skin to solution ratio of 1:6 (v/w) of alkali solution (0.01 M; 0.03 M and 0.05 M NaOH) at room temperature for 12 h; 18 h and 24 h to remove the non-collagen protein and subcutaneous tissue after they were swollen. Alkaline treated skin was then washed with tap water until neutral or faintly basic pH (pH < 7.5) of wash water was obtained. To swell the collagenous material in the fish skin matrix, the alkaline treated skin was soaked in 0.075 M acetic acid with a skin to solution ratio of 1:6 (w/v) for 18 h at room temperature (25 °C). The treated skin was washed with distilled water until the wash water pH was greater than four. This required 6–7 washing cycles.

Gelatin was extracted from the bleached skin using distilled water (60 °C) for 4 h with a skin/water ratio of 1:4 (w/v) in a water bath shaker (Memmert, WB14, Germany). During extraction, the mixture was stirred continuously. Solubilized gelatin was separated from residual skin fragments by using two-layer filter cloth. The resultant filtrate was cabinet dried and the dry matter from cabinet dried process was ground and referred to as “gelatin powder”.

2.3. Measurements of physicochemical characteristics

2.3.1. Determination of yield. The yields of the gelatins obtained were calculated on both weight and protein basis[13] as follows:

(i) % Yield (wet wt basis) = (Dry wt of gelatin / wet wt of skin) × 100 %

(ii) % Yield (dry wt basis) = (Dry wt of gelatin) / (wet wt of skin - moisture content) × 100 %

(iii) % Yield (protein basis) = (Dry wt of gelatin / protein content of skin) × 100 %

2.3.2. Determination of gel strength. Gel strength was determined using a Texture analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, England). Gelatin was dissolved with distilled water (6.67 %, w/v) and agitated at 60 °C, 150 strokes·min⁻¹ for 30 min in a shaking water bath (SS40-D Shaking Bath, Grant Instruments Ltd., Cambridge, England) until completely dispersed. The gelatin solution
was filled in glass measuring bottles (40.1-mm dia 52-mm height, flat bottom) and then kept at 9–10 °C for 17–18 h. After cool maturation, the gel strength expressed in Bloom value was measured while the sample was still at 9–10 °C. A plunger with a 12.7 mm diameter had a penetration depth of 4 mm into the gelatin gels and a penetration speed of 1 mm·s\(^{-1}\) was used. The force in g at this temperature is the Bloom strength [13].

2.3.3. Determination of viscosity. The viscoelastic studies were performed on a Brookfield DVIII + Rheometer (Model RY, Middleboro, MA, USA) using a 6.67 % of gelatin solution at 60 °C equipped with an SC4-21 spindle at 100 rpm. Dry gelatin was dissolved in Milli-Q water and gently stirred at 60 °C in a shaking water bath for about 20 min before the viscosity measurements (centipoises) were taken directly using the above viscometer coupled to a water bath at 60 °C [14].

2.3.4. Determination of melting point. Solutions containing 6.67 % (w/v) gelatin were prepared in screw cap test tubes. The test tubes were filled and kept in the refrigerator (7 °C) for 16–18 h, and then were transferred into a cold-water bath (4 °C). The measurement was performed at a heating rate of 0.2 °C·min\(^{-1}\), using oil drop method. The amount of 0.5 % methyl-red chloroform was used as an indicator of melting point. The temperature, at which the gel melted, allowing the chloroform to start falling, was recorded as the melting point [15].

2.4. Statistical analysis

All the collected data were analyzed using Analysis of Variance (ANOVA) and Duncan’s Multiple Test to determine the significance level among the means using the statistical program analyses package [17].

3. Results and Discussion

3.1. Yield of gelatin

Yields of yellowfin tuna skin gelatins (YSG) extracted under treatment conditions at different of alkaline (NaOH) concentration combined with treatment time is shown in table 1.

| Treatments | Yield of YSG (%) |
|------------|------------------|
| 0.01 M; 12 h | 12.49\(^{f}\) |
| 0.03 M; 12 h | 12.02\(^{f}\) |
| 0.05 M; 12 h | 8.74\(^{e}\) |
| 0.01 M; 18 h | 12.12\(^{g}\) |
| 0.03 M; 18 h | 11.81\(^{e}\) |
| 0.05 M; 18 h | 8.6\(^{b}\) |
| 0.01 M; 24 h | 12.1\(^{g}\) |
| 0.03 M; 24 h | 9.76\(^{d}\) |
| 0.05 M; 24 h | 8.4\(^{a}\) |

*Values are the means of triplicates.
*Different superscripts in the same column indicate significant differences (p < 0.05).

The yields of YSG which used 0.01 M NaOH for the alkaline treatment combined with a treatment time of 12 h gave the highest total gelatin yield (12.49 % dry basis wt) compare to the other treatments. This suggested that the use of NaOH in a concentration of 0.01 M combined with a treatment time of 12 h provided the best conditioning treatment to remove non-collagen protein and subcutaneous tissue from tuna skin prior to gelatin extraction which resulted in better yield recovery.
Our findings are in line with previous work reported by previous researchers [11], who reported that the extraction conditions, including solvent, temperature and extraction time, might affect the yield of the extracted gelatin. Calcium hydroxide solution was used to remove impurities (proteoglycan, blood, mucins, sugars, fat, etc.) and change collagen to the optimal type for gelatin extraction [16]. Furthermore, using a prolonged extraction of whole cod skins, achieved a yield of gelatin between 11 % and 14 %, depending on the concentrations of the sodium hydroxide, sulfuric acid, and citric acid solutions used in the preliminary treatment of raw material [23].

3.2. Gelatin gel strength
Comparison of the gel strength of YSG extracted under various treatment conditions of different alkaline (NaOH) concentration combined with various treatment time is shown in table 2.

Table 2. Gel strength of yellowfin tuna skin gelatin (YSG) under various NaOH concentration and treatment time compared with bovine gelatin.

| Treatments | Gel strength of YSG (g Bloom) | Gel strength of bovine (g Bloom) |
|------------|------------------------------|---------------------------------|
| 0.01 M; 12 h | 291.73f | 309.7401g |
| 0.03 M; 12 h | 261.8897e | |
| 0.05 M; 12 h | 183.7049c | |
| 0.01 M; 18 h | 262.5762e | |
| 0.03 M; 18 h | 203.8145d | |
| 0.05 M; 18 h | 165.6546b | |
| 0.01 M; 24 h | 262.2446e | |
| 0.03 M; 24 h | 201.4912d | |
| 0.05 M; 24 h | 123.1543a | |

*Values are the means of triplicates.
*Different superscripts in the same column indicate significant differences (p < 0.05).

The gel strength of YSG which used 0.01 M NaOH for the alkaline treatment combined with a treatment time of 12 h gave the highest gel strength (291.73 g Bloom) compare to the other treatments, however, this value was slightly lower than that of Commercial Bovine Gelatin (CBG) (309.7401 g Bloom). In accordance with the result for the best yield, the use of 0.01 M NaOH combined with a treatment time of 12 h shows the best pretreatment condition prior to gelatin extraction. This result suggested that the removal of collagen protein in pretreatment step (using NaOH) is important for the gelatin extraction process and it might further affect the gel strength of obtained gelatin.

Gel strength is the most important attribute of gelatin and determines the quality of produced gelatin [18]. Furthermore, the difference in attribute of gelatin among species was possibly due to the different composition, particularly in terms of amino acid composition and size of protein chains [8]. It is likely that relatively limited amino acids and glycine content might have resulted in less organized triple helical structures and is largely responsible for lower bloom strength [24]. The difference in bloom strength among species was possibly due to the different composition, particularly in terms of amino acid composition and size of protein chains [25]. The low hydroxyproline content in fish skin gelatin was a major reason for the low gel strength of these gelatins. It is well established that hydrogen bonds between water molecules and free hydroxyl groups of amino acids in gelatin are essential for gel strength [26].

3.3. Gelatin viscosity
Comparison of the viscosity of YSG extracted under various treatment conditions of different alkaline (NaOH) concentration combined with various treatment time is shown in table 3.
The viscosity of yellowfin tuna skin gelatin (YSG) under various NaOH concentration and treatment time compared with bovine gelatin.

| Treatments       | Viscosity of YSG (cPs) |
|------------------|------------------------|
| 0.01 M; 12 h     | 8.6f                   |
| 0.03 M; 12 h     | 4.5f                   |
| 0.05 M; 12 h     | 4.1c                   |
| 0.01 M; 18 h     | 8.5f                   |
| 0.03 M; 18 h     | 4.3c                   |
| 0.05 M; 18 h     | 3.9b                   |
| 0.01 M; 24 h     | 8.2h                   |
| 0.03 M; 24 h     | 4.2d                   |
| 0.05 M; 24 h     | 3.8a                   |
| Viscosity of bovine (cPs) | 5.6g                   |

*Values are the means of triplicates.
*Different superscripts in the same column indicate significant differences (p < 0.05).

The viscosity of YSG which used 0.01 M NaOH for the alkaline treatment combined with treatment time of 12 h gave the highest viscosity (8.6 cP) compare to the other treatments. This suggested that the use of 0.01 M NaOH combined with a treatment time of 12 h gave the best pretreatment condition for gelatin extraction. The viscosity of YSG solution (8.6 cP) was higher than that of CBG (5.6 cP) and it shows a significantly different (p < 0.05). Viscosity is the second most important commercial physical property of a gelatin [19]. The viscosity of gelatin solutions varies with sources in terms of molecular weight and molecular size distribution of proteins [20]. Viscosity did not appear to be affected by the protein yield but was clearly correlated with the molecular weight distribution of the extracted proteins. The gelatin-containing smaller ratio of large molecule portion like β-chains exhibited lower viscosity value [21]. The viscosity of gelatin solutions varies with sources in terms of concentration, pH [27] as well as temperature [28] of the gelatin solutions.

3.4. Gelatin melting point

Comparison of the melting point of YSG extracted under various treatment conditions of different alkaline (NaOH) concentration combined with various treatment time is shown in table 4.

| Treatments       | Melting Point of YSG (°C) |
|------------------|---------------------------|
| 0.01 M; 12 h     | 35d                       |
| 0.03 M; 12 h     | 33.5e                     |
| 0.05 M; 12 h     | 31a                       |
| 0.01 M; 18 h     | 33bc                      |
| 0.03 M; 18 h     | 33bc                      |
| 0.05 M; 18 h     | 32.5b                     |
| 0.01 M; 24 h     | 33bc                      |
| 0.03 M; 24 h     | 33b                       |
| 0.05 M; 24 h     | 32.5b                     |
| Melting point of bovine (°C) | 36.33c                   |

*Values are the means of triplicates.
*Different superscripts in the same column indicate significant differences (p < 0.05).

The melting point of YSG which used 0.01 M NaOH for the alkaline treatment combined with a treatment time of 12 h gave the highest melting point (35 °C) compare to the other treatments. The
melting point of YSG (35 °C) was slightly lower than that of CBG (36.33 °C). An aqueous solution of a few percent gelatin forms thermally reversible gels with water and the gel melting temperature (< 35 °C) is below body temperature, which gives gelatin products unique organoleptic properties and flavor release [5]. The wide range of gelling temperatures is greatly influenced by the origin of the raw material used in the process [22].

4. Conclusion
The present study indicated that the optimal condition for YSG preparation was using 0.01 M NaOH for 12 h. The results show that the concentration of NaOH had a significant effect on yield, gel strength, viscosity and melting point.

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