

Extraction optimization and characterization of gelatine from fish dry skin of Spanish mackerel (*Scomberromorus commersoni*)

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Abstract. This work was to optimized gelatin extraction from dry skin of Spanish mackerel (*Scomberromorus commersoni*) using Response Surface Methodology (RSM). The aim of this study was to determine the optimal condition of temperature and time for extraction process and properties of the gelatin extracted from dry mackerel skin. The optimal condition for extraction was 59.71°C for 4.25 hours. Results showed that predicted yield by RSM was 13.69% and predicted gel strength was 291.93 Bloom, whereas the actual experiment for yield and gel strength were 13.03% and 291.33 Bloom, respectively. The gelatin extracted from dried skin were analyzed for their proximate composition, yield, gel strength, viscosity, color, and amino acid composition. The results of dried skin gelatin properties compared to the commercial gelatin. Gelatin extracted from the dried skin gave content lower moisture, ash and protein content but higher fat compared to commercial gelatin. This study also shows that the gelatin extracted from the dried skin gave higher gel strength and pH but the lower amino acid composition compared to commercial gelatin.

1. Introduction

The use of gelatin is wide enough in many applications, but there are still some obstacles for consumers to consume these products. In addition to the controversy and concerns of many people because of the taboo for Hindus to eat beef, a ban on the Jewish people to consume any products derived from pigs and Islam forbid pork, as well as the emergence of a security risk. This happens when the raw material of gelatin made from pig and the cow disease Bovine Spongiform Encephalopathy (BSE), foot and mouth disease (foot and mouth disease) and that can be transmitted through gelatin [1-2]. It is therefore very important to find the source of gelatin other than cows and pigs to meet the needs of gelatin in the country and to overcome various obstacles.

Raw material of gelatin can be derived from fish, especially the skin and a small portion of fish bones. Utilization of fish skin is also in line with efforts to reduce waste in the fish processing industry. Fish processing waste that is currently underutilized in the fishery industry and gelatin from fish skins would be a good way to add value to this waste product [3]. Gelatin derived from fish tend to be more secure and abundant raw materials. In addition, the utilization of fish skin as a raw material for gelatin can improve the value added to the waste treatment [2, 4].



Dried fish skin gelatin as a source of raw materials has been reported in recent studies, e.g. on cobia skin [5] and seawater fish skin [6]. They studied the effect of the two methods of Cobia skin preservation, i.e. drying and freezing on the properties of gelatin produced, in terms of gelatin yield, proximate composition, gel strength, viscosity, color and amino acid composition. While [6] investigated the gelatins properties extracted from fresh and sun-dried skins of seawater fish.

One type of fish skin that could potentially be used is the skin of Spanish mackerel fish that are found throughout the year in the waters of Indonesia. Spanish mackerel is one result of capture fisheries in marine waters, especially in East Kalimantan. The volume of marine capture fisheries production of mackerel in Indonesia were 132 705 tonnes in 2011, while the volume production of mackerel from East Kalimantan were 1 793 tonnes [7].

The high production of fish fillet dumped as the waste of processing. One of the processing industries used Spanish mackerel were the manufacturing industries of “amplang” crackers. The processing waste of these industries generally just thrown away. The skin of Spanish mackerel contains a large amount of collagen. It is suggested that the skin can be useful sources of gelatin.

Yield and quality of gelatine are highly dependent on the method of extraction was done. The quality was determined by the gel strength of gelatin which is the main characteristic of gelatin. While the quantity, the yield is an indicator of production efficiency. The method used for the extraction of gelatin from fish skin, in general, was the acid extraction. Collagen derived from fish skin collagen, which is a type of easily extracted by weak acid treatment, followed by heating process for dissolving collagen. Heating time and temperature on the extraction process is a thing that must be controlled to obtain the yield of gelatin with a better quality [8].

Optimization of temperature and extraction time was necessary for this research. Response surface methodology (RSM) was a method used in the optimization between the effects of temperature and extraction time on yield and gel strength as one of the determinants of the quality of gelatin. Therefore, the objectives of this study were to determine the optimum conditions for extracting collagen from the dried skin of Spanish mackerel using RSM and to compare its physicochemical and rheological properties with the commercial gelatin.

2. Experimental details

2.1. Materials

Raw materials to be used was the dried skin of Spanish mackerel obtained from some of the fish processing industry in Samarinda, East Kalimantan. The fresh skins were kept in a cool box and washed to remove any contaminants. Fish skin was cleaned from the remaining flesh was scraped with a spoon and also removed the bones and then washed with running water. The cleaned fish skin was dried under the sun for about 2-3 days until dried.

2.2. Pre-treatment

Dried fish skin was rehydrated first by soaked in the water (dried fish skin: water = 1: 4) during 4 hours. After that, the skins were dipped in warm water (60-70°C). Once cooled, cut into small sizes \pm 0.5 x 0.5 cm. Skins that has been cut into small pieces and then were treated with 0.05 M acetic acid with a ratio of fish skin: acetic acid = 1:4 (w / v) at room temperature for 10 hours. Then, the skins were washed to remove excess chemicals until neutral condition.

2.3. Gelatin extraction

The extraction of gelatin was carried out as described inMuyonga procedure [9]. First, the pre-treated fish skins were transferred to beakers and filled with distilled water at 1:3 (w/v) ratio. The extractions were carried out on control temperatures and time in accordance with the conditions that have been designed. The gelatin liquor was then filtered through four-layered cheesecloth to obtain gelatin filtrate. The filtrate was put on the trays and dried in a cabinet drier at 55°C for 48 h to obtain gelatin sheets. The sheets were ground to result in gelatin granules. The gelatin granules were packed in a

plastic and kept in a refrigerator temperature until used for analysis. The yield and gel strength was used as a parameter to determine the optimum conditions for extraction.

2.4. Yields and proximate composition analysis

Yield was expressed in a percentage (%), calculated by weighing the resulted gelatin granules divided by the weight of fish skin in dry basis (DB), after considering the moisture content. The proximate compositions of gelatin granules were analyzed in terms of moisture content, crude protein, crude lipid and ash content following the AOAC method [10].

2.5. Determination of gel strength

The gel strength was carried out according to [11], determined by using Universal Testing Machine INSTRON Model 4510 (INSTRON Co, Canton, Mass, USA), with load cell 5kN, cross-head speed 1 mm/s, equipped with a 1.27-cm diameter of fat-faced cylindrical Teflon plunger. Gel sample was prepared by dissolving gelatin in distilled water to make concentration 6.67% and then kept at 9-10°C for 17-18 h for maturation. Gel strength was expressed in Bloom, obtained from maximum force (g) when the plunger had penetrated 4 mm into the gelatin gel.

2.6. Determination of viscosity

The viscosity of gelatin solution was expressed in centipoises (CPS), measured by using a rotary Viscometer (BOHLIN Instruments Ltd., Gloucestershire, UK). Gelatin solution (6.67%) was prepared by dissolving gelatin granules in distilled water at 60°C, and the viscosity measurement was conducted at a temperature of 40-50°C.

2.7. Determination of color

To determine the color, the colorimeter (Minolta Cr-300 series, Japan) was used. L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) value were measured.

2.8. Amino acid analysis

Gelatin samples were hydrolyzed in HCl 6N at 110°C for 24 h, and the hydrolyzates were analyzed by an amino acid analyzer.

2.9. Experimental design and analysis of data

Optimum conditions for the extraction of dried fish skin gelatin were determined using Response Surface Methodology (RSM). A central composite design (CCD) was used to determine the effects of the independent variables on the yield and gel strength of gelatin extraction. The design consists of a 2^k factorial, where k is a factor that was observed (X) at least two factors with the addition of axial points (α) for two factors is 1.414 ($\alpha = 2k / 4$) [12]. The two factors were hot water extraction temperature in °C (X1) and extraction time in hours (X2). The experiment was conducted at 13 experimental points. The experimental design is shown in Table 1. The experimental data from different treatments was analyzed using Minitab statistical software (Version 15.1). The fitting was done to 2nd order model for each response. This model can be expressed by the following equation:

$$Y_i = \beta_0 + \sum_{i=1}^k \beta_i X_i + \beta_{ii} X_i^2 + \sum \sum_{i < j} \beta_{ij} X_i X_j \quad (1)$$

where Y is dependent variable (yield and gel strength), β_0 is the constant, β_i, β_{ii} , and β_{ij} are regression coefficients and X_i, X_j are levels of the independent variables.

3. Results and discussion

3.1. Optimization experimental design

The experimental results using RSM with CCD model are presented in Table 1. Two independent variables used in this research that the extraction temperature (X1) and extraction time (X2) with five levels for each variable. While the dependent variable is the yield (%) and gel strength (Bloom).

Table1. Central composite design for optimizing the extraction condition and responses of the dependent variables of gelatin to changes in the independent variables.

Run no.	Variable levels		Response	
	X1	X2	Y1	Y2
1	-1	-1	9.66	208.67
2	1	-1	7.20	105.00
3	-1	1	9.84	169.63
4	1	1	10.45	103.32
5	-1.414	0	5.62	73.46
6	1.414	0	8.08	80.62
7	0	-1.414	10.06	200.95
8	0	1.414	11.91	218.62
9	0	0	13.00	308.76
10	0	0	14.00	264.96
11	0	0	14.74	306.69
12	0	0	13.15	290.17
13	0	0	13.20	292.44

X1 (extraction temperature, °C), X2 (extraction time, h), Y1 (yield, %), Y2 (gel strength, Bloom).

3.2. Conditions for optimum responses

For the optimization of gelatin extraction, the two independent variables were of extraction temperature (X1) and extraction time (X2) was set in range while the dependent variables were fixed at maximum. The optimal solutions of extraction temperature were (59.7 °C, X1) and extraction time were (4.25 h, X2). The predicted values of yield and gel strength were 13.69% and 292 Bloom, while the experimental values of extraction yield and gel strength were 13.03% and 291 Bloom, respectively.

The yield obtained based on the weight of dried skin with moisture content of fish include in its calculations. In preliminary studies, the results obtained for the water content of Spanish mackerel dried skin was 13.24% (WB). The results indicated the yield of the resulting optimization of the dried skins extraction was 13.03% (DB) that obtained at 59.71°C for 4.25 hour treatments.

High and low yields also affected by the loss of proteins that occur during immersion in acid solution (swelling process) and during the extraction process with a high temperature or too low. The low yield is also associated with the loss of extracting collagen through leaching during the series of washing steps or due to incomplete hydrolysis of the collagen [13]. The yield of fish gelatin is lower than mammalian gelatin, which ranges from 6-19%. Wide range of yield due to differences in collagen content in the raw material [9].

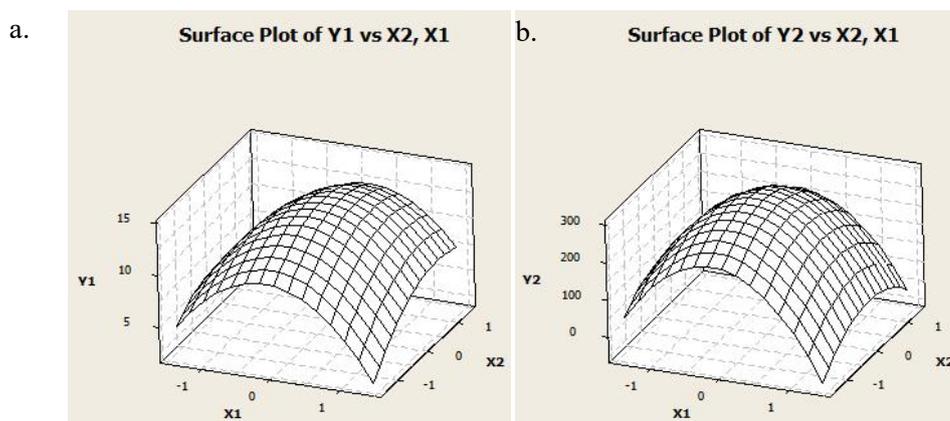


Figure 1. Surface and contour plot optimization of mackerel fish skin gelatin (a. based on yield response; b. based on gel strength response)

The three dimensions plot (Figure 1) shows that the yield of fish skin gelatin increased on the extraction temperature beyond 60 °C and decreases at temperatures above 70 °C and 55 °C below. So also with longer extraction time will affect the yield value. The results showed that the extraction time for 4 to 6 hours will increase the value of the yield of gelatin. Based on the response of gel strength (Figure 2.), the extraction of gelatin increased as temperatures between 50 °C to 70 °C. While based on the length of time of extraction, gel strength will increase with an extraction time of about 4 hours. The longer the extraction time, the resulting gel strength will be lower.

3.3. Physicochemical properties of gelatin

The physicochemical properties are shown in Table 2. The moisture content of Spanish mackerel dried skin gelatin was lower than the commercial gelatin. Indonesian National Standard [14] regulates the maximum level of gelatin moisture is 16%, while the maximum moisture content according to [15] is 14%. Therefore, the moisture content of these gelatin had fulfilled the standard regulations.

Table 2. The chemical compositions of Spanish mackerel dried skins gelatin

Properties	Gelatin	
	Spanish mackerel dried skins	Bovine commercial
Moisture (% DB)	10.81 ± 0.08	13.24 ± 0.03
Ash (% DB)	1.44 ± 0.03	1.51 ± 0.01
Protein (% DB)	89.93 ± 0.15	92.57 ± 0.02
Fat (% DB)	1.78 ± 0.01	0.11 ± 0.01
Gel strength (Bloom)	291.33 ± 6.98	216.98 ± 21.58
Viscosity (CPS)	8.07 ± 0.17	8.23 ± 0.13
Colour :		
L	52.15 ± 0.95	64.32 ± 0.19
a	4.90 ± 0.26	1.74 ± 0.16
b	21.77 ± 1.01	17.49 ± 0.14

Data presented as means standard deviation of triplicate determinations

Ash content of Spanish mackerel dried skin gelatin showed a low value when compared with the ash content of commercial gelatin. Indonesian National Standard [14] regulates the maximum level of gelatin moisture is below 3.25%. Gelatin with ash content of less than 0.5% indicates a high-quality gelatin, while according to the FAO recommended levels of ash in the food less than 3%.

The low ash content is important in determining the quality of gelatin. Ash content of gelatin may be contributed by the residual of chemicals after processing, come from raw material, or also the possibility of mixing with the other ingredients [13]. Ash content also showed the efficiency of demineralization of raw material pre-treatment [16-17].

The protein content of the commercial gelatin gave higher protein content (92.57%) compared to Spanish mackerel dried skins gelatin(89.93%). SNI does not provide a minimum limit for protein content. High levels of protein showed a good extraction process. Levels of protein in gelatin is also influenced by the process of soaking the skin. The soaking process resulted in the termination reaction of hydrogen bonds and opening of the coil structure of collagen, therefore, contributes to the amount of protein extracted.

Fat content of Spanish mackerel dried skin gelatin is higher than the commercial gelatin. SNI does not provide rules about fat content. Commercial gelatine showed the fat content is 0.11%. Generally, commercial gelatin contains almost no fat (<0.5%). The differences in fat content might be due to the differences in fat content in differences raw materials.

Gel strength is one of the most important functional properties of gelatin. The gel strengths of commercial gelatins are expressed using Bloom values and the value is the weight in grams that is required for a specified plunger to depress the surface of a standard. The average molecular weight of gelatin is largely responsible for its gelling behavior. After overnight maturation (18 hour, 7-8°C), gelatin extracted from Spanish mackerel dried skin showed higher gel strength (291 Bloom) than that of commercial bovine (217 Bloom).

The gelling strength of commercial gelatin ranges from 100 to 300, but gelatin with Bloom values of 250–260 are the most desirable. Gel strength is one physical properties and most important function of gelatin and it is governed by molecular weight, as well as by complex interactions determined by the amino acid composition. The large range of Bloom values found for the various gelatin arises from differences in proline and hydroxyproline content in collagens of different species and is also associated with the temperature of the habitat of the animals. Hydrophobic amino acids (Ala, Val, Leu, Ile, Pro, Phe, and Met) could also contribute to the high Bloom value of tilapia fish gelatin. This study shows that the gel strength of Spanish mackerel dried skins was higher than the commercial bovine gelatin [18].

Viscosity is the resistance to the flow of a fluid which is defined as the ratio between the shear stress on the rate of shear rate. The more viscous fluid, the greater the force required for fluid to flow with a certain rate[19]. Viscosity is an important physical property of the gelatine after the gel strength [20]. The viscosity values (CP) for mackerel gelatine were lower than commercial gelatin. SNI does not give the terms of the viscosity of gelatin. The viscosity characteristics displayed by a given gelatin grade are primarily related to the molecular weight distribution of the gelatin molecules [21]. Higher molecular weight subunits give an increase in viscosity. The longer the chain of amino acids, the high molecular weight of gelatin, so that the higher viscosity [6].

Colour of L shows the lightness, a shows redness and b shows yellowness. Gelatin extracted from mackerel skin was darker compared to commercial gelatin. In general, satisfactory gelatin should have little color. The color of gelatin depends on the raw material. However, it does not influence other functional properties. The light color is preferred and considered as a positive attribute since it is easier to incorporate these light colored gelatin into any food system without imparting any strong color attribute to the product [13].

3.4. Amino acids composition

The amino acids composition of the gelatin from mackerel skin gelatin compared to commercial gelatin are shown in Table 3. The amino acids (proline and hydroxyproline) and glycine contents of the Spanish mackerel dried skins gelatin were 8.27% and 6.10%, respectively. The content of amino acid and glycine are important for gel strength. The mammalian gelatine have a high composition of these amino acids, especially proline and hydroxyproline, which are related to gelling property a low content of amino acids indicates poor gelling power [22]. Although proline is important,

hydroxyproline is believed to play a singular role in the stabilization of the triple-stranded collagen helix due to its hydrogen bonding ability through its hydroxyl group. Besides the amino acids, lysine also stabilizes gelatin structure by forming crosslinking structure between chains [13].

Table 3. Amino acids composition of mackerel gelatin and commercial bovine gelatin

Amino acids	% amino acids	
	Spanish mackerel dried skins	Commercial Bovine
Aspartic acid (Asp)	5.21	5.48
Glutamic acid (Glu)	10.86	9.87
Serine (Ser)	1.86	2.32
Glycine (Gly)	6.10	8.92
Histidine (His)	2.08	3.38
Arginine (Arg)	2.79	3.74
Threonine (Thr)	1.06	1.98
Alanine (Ala)	1.29	2.12
Tyrosine (Tyr)	1.12	2.58
Valine (Val)	2.03	2.23
Methionine (Met)	2.01	2.61
Cystine (Cys)	1.71	2.35
Isoleucine (Ile)	1.13	1.76
Leucine (Leu)	1.07	1.78
Phenylalanine (Phe)	1.72	3.37
Lysine (Lys)	5.89	4.49
Hydroxyproline (Hyp)	2.89	2.68
Proline (Pro)	5.38	10.49

4. Conclusion

This study suggests that Spanish mackerel dried skins can be used in gelatin production. The optimal solution for multiple responses extraction yield and gel strength showed that an extraction temperature of 59.71°C and extraction time of 4.25 hour gave the best result. This study shows that the gelatin gel strength extracted from Spanish mackerel dried skin was higher than that of commercial gelatin. However, the gelatin extracted from Spanish mackerel dried skin gave lower viscosity, moisture, ash, protein and amino acid content compared to commercial gelatin.

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