



Gelatin Extraction Optimization from Skin of Sub Adult and Adult *Pangasius Hypophthalmus*

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Abstract

Fish skin is the potential gelatin source since the increase of the demand for the halal food due to the religious consideration. The yield of gelatin obtained from connective tissue of animal skin greatly affected by the extraction process and age of the animals that used as raw material. Research about the potential of the *Pangasius hypophthalmus* (striped catfish) skin as halal gelatin source have been developing. However, there was no information about the correlation between the length of striped catfish that representing age with the gelatin content. Here we optimize the gelatin extraction method from striped catfish skin with different length body size (sub adult and adult size) using statistical analysis of Placket Burman Design. We performed preliminary study to determine the variables used in the main study. We used 9 variables in the extraction process and analyzed the p-value of each variable. According to this analysis, three variables with the lowest p-values were selected: temperature ($p=0.000$); soaking time in alkali solution ($p=0.055$) and soaking time in acid solution ($p=0.244$). Range value of selected variables were determined according to the related previous studies. Results from our study showed that the maximum yield of sub adult and adult were obtained when we used 70°C as temperature of extraction, i.e. 37.42% and 30.31%, respectively. In general, sub adult striped catfish have higher yield than the adult one at the temperature of 40 and 70°C. However, the gelatin yielded from extraction process temperature of $\geq 70^\circ\text{C}$ exhibited dark pigmentation, while the gelatin that obtained with temperature treatment of $\leq 55^\circ\text{C}$ showed less pigmentation. The gelatin yielded from



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sub adult striped catfish showed more pigmentation than the adult one. We suggested for process of extraction both size of striped catfish should be at 55°C, since it showed higher yield extraction with less pigmentation.

Introduction

Gelatin is a natural product obtained from hydrolysis collagen with many uses.¹ Besides being used in food products, gelatin is also used in products like capsules, photographic films and in various cosmetic and medical applications.^{1,2} Most of the gelatin is produced from raw materials of pork and cattle skin.³ In Indonesia, a country with the largest Muslim population in the world, it is very selective towards products made from cattle or pigs because they are related to halal status. Related to this, the raw material to produce gelatin from other sources continues to be studied.

Currently, gelatin from fish is an alternative for producing gelatin because fish is a halal food although it is not slaughtered. One of the fish that has the potential for gelatin production is catfish,³ such as striped catfish (*Pangasius hypophthalmus*). Recently, the demand of *Pangasius* Catfish has been increasing in Indonesia. Based on data from the Ministry of Maritime Affairs and Fisheries there was an increase in catfish production from 2015 (604,700 tons) to 2016 (725,600 tons), and it is predicted that catfish production will continue to increase until 2019, which is to become 1,149,400 tons.⁴ The raw material for fish gelatin can be obtained from the skin, bones, and fins of fish. In the fillet industry, fish skin is a waste product that has a high quantity and has a low economic value. In general, vertebrate animal skin, including fish consists of several layers, with two main layers, namely, the outer layer is called the epidermis layer and the inner layer is called the dermis layer. In the dermis layer, there are blood vessels, nerves, and connective tissue. Connective tissue has a major component in the form of fibrous protein which is also referred to as collagen.⁵

Fish Gelatin is made by pre-treatment hydrolysis of collagen fibers. Based on the previous study, production of fish gelatin can be produced by soaking with acidic conditions, alkaline or boiled with high pressure.^{6,7,8} The yield of gelatin that obtained from the connective tissue of fish skin greatly affected by the extraction process. According to Karayannakidis

and Zotos,⁹ pretreatment and extraction conditions such as soaking time, chemical concentration, temperature and extraction time can affect the length of the polypeptide chain and the functional properties of gelatin. In addition, another influential factor is the age of the animals used as raw materials.^{10,11} Research on the potential of *Pangasius* skin as halal gelatin source have been developed, however, the correlation between *Pangasius* length body size that represents age has no significant information.

Here we show that the process of extraction for both size of striped catfish should be performed at 55°C as optimal temperature, with the highest yield of extraction combined with lowest degree of pigmentation.

Materials and Methods

Raw Material Preparation

Skin of striped catfish were obtained from the waste of a filleting industry in Pasuruan, East Java, Indonesia. Fish skin from different size from sub small size (total body length body size 38.9 ± 0.5 cm with average weight of 454.6 ± 17.1 grams) and the big size (has a total length of 48.6 ± 1.9 cm with an average weight of $1,194 \pm 27.0$ grams). The smaller group is considered to represent catfish at the age of 6 months because of their range of length of 35–40 cm. This is in accordance with Khairuman and Suhenda.¹² Furthermore, the large size group is average. Mean while, the larger size groups in this study are considered to represent the size of 12 months old *Pangasius* catfish which is based on data from the Directorate, BPR and MSME of Bank Indonesia¹³ which study reported that *Pangasius* catfish culture using the fence system with a stocking time of 12 months, the average weight of catfish harvested up to 1000 grams. The by-product of *Pangasius* catfish filleting was transported to the laboratory on ice. In the laboratory, the *Pangasius* catfish skin were separated from other waste material that can contaminate gelatin product, such as the fat and attached flesh. The contaminant of skin was removed by scraping with a knife and

washing in tap water. The fish skin was stored in a freezer(-20°C). Acetic acid and alkaline solution

used were analytical grade and the concentrations used were determined by placket-burman design.

Table 1: Design of Experiment (DOE) Placket-Burman Methods

No	Variable	Unit	Range	
			Minimum	Maximum
1	Acid concentration	M	0.05	0.12
2	Soak time in acid	minute	40	60
3	Ratio of acid	m/v	6	9
4	Alkali concentration	M	0.1	0.2
5	Soak time in alkali	minute	40	60
6	Ratio of alkali	m/v	6	9
7	Temperature extraction	°C	40	70
8	Extraction time	minute	30	60
9	Water ratio extraction	ml	3	9

Design of Experiment (DOE) Placket-Burman Methods

Gelatin extraction was performed in two steps, the first step being pre-treatment and second main extraction.¹⁴ During the pre-treatment step, the skin soaked and dripped in acid and alkaline solvent by certain concentration and duration of soaking time as variable in this study. There are 9 variables being observed in this study as shown in Table 1.

The cleaned skin was prepared in the same weigh for each sample, fish skin soaked in acid with varying concentration (0.05 and 0.12 mol) and varying ratio (1:6 and 1:9 w/v) at room temperature with varying time periods (40 and 60 minute) for demineralization. The leached skin were neutralized by washing it with distilled water until reaching pH 7. The pre-treatment solvent continued using alkaline varying concentration (0.1 and 0.2 mol) and varying ratio (1:6 and 1:9 w/v) at room temperature with varying time periods (40 and 60 minute). For second time, swelled skin neutralized by washing it with distilled water until reaching pH 7.

The neutralized skin transferred to an Erlenmeyer flask, and then distilled water was added at varying ratio of 1:3 and 1:9 skin/water (w/v) for the main extraction step. The main extraction was carried out for various time (30 and 60 min) at various temperatures (40 and 70°C). Finally, the gelatin

extracted was filtered with a filter paper and refrigerated at 10°C. The resulting extracted gelatin was dried by using freeze dry for 4 day. The gelatin yield was calculated as the ratio of weight wet fish skin gelatin to the total weight of dried gelatin 15 using the formula of Yield of fish skin gelatin (%) below:

$$\text{Weight of gelatin (g) / Weight of fish skin (g) } \times 100\%$$

Results

Variable Screening in the Pre-treatment using Placket-Burman Analysis

The method of extracting gelatin from striped catfish skin in this study includes two stages: pre-treatment and extraction. Pre-treatment process began with acid soaking and continued with alkali soaking, while the extraction process was by heating the catfish skin using distilled water at a temperature of distilled water being constant. In this study, the test of collagen response skin against physical-chemical properties were measured during pre-treatment as well as the extraction process to get gelatin. Based on statistical tests using Placket-Burman against twenty samples with nine extraction variables (Table 2) were screened three variables that determined based on the smallest of p-value included: treatment of temperature (p=0.000); soaking time in alkali solution (p=0.055) and soaking time in acid solution (p=0.244).

Table 2: Statistical Test Results Using the Plackett-Burman response to skin collagen

Analysis of Variance		
No of variable	Source	P-Value
	Model	0.002
	Linear	0.002
1	Acid concentration	0.588
2	Soak time in acid	0.244
3	Ratio of acid	0.681
4	Alkali concentration	0.593
5	Soak time in alkali	0.055
6	Ratio of alkali	0.578
7	Temperature extraction	0.000
8	Extraction time	0.714
9	Water ratio extraction	0.979

Extraction Process

We determined the variation of the selected variables (Table 3) as determined according to the related previous studies, such as temperature was in range of 40 – 70°C.^{16, 17} Extraction was done with the parameters of acid soaking time, alkaline soaking time and treatment of temperature. Acid soaking causes swelling of the skin due to the ingress of water into collagen fibers. The entry of

water into collagen fibers is due to the occurrence of electrostatic forces between collagen polar groups and H⁺ from acids, or the formation of hydrogen bonds between collagen non-polar groups and H⁺ from acids.¹⁸ This swelling can support damage to the structure of collagen fibers, through disruption of non-covalent bonds and ultimately facilitate extraction and increase collagen solubility.¹⁹

Table 3: Codes and combination numeric

Numeric codes	Combination numeric		
	-1	0	1
X1	40 minutes	50 minutes	60 minutes
X2	40 minutes	50 minutes	60 minutes
X3	40 °C	55 °C	70 °C

Notes X1: Acid soaking time
X2: Alkaline soaking time
X3: Treatment of temperature

From the variation listed in Table 3, we calculated the variation for the extraction step using Plackett-Burman statistical simulation and we got 20 different variations in total. We used these 20 different variations in the extraction process of striped catfish with different length which represents the age of the striped catfish (adult or sub adult). The

variations shown in Table 4 which are not in the range (Table 3) are used as controls and were resulted from the simulation using Plackett-Burman method. We obtained the yield of gelatin from the sub adult were in the range of 0.25 - 37.42% (w/w) while the yields from the adult fish range from 0.07 to 30.31%. The maximum yield of gelatin extraction from the sub

adult striped catfish was 37.42% and was obtained from extraction -variables variations of 60 minutes soaking in acid; 40 minutes soaking in alkaline and the temperature of extraction was 70°C. Meanwhile for the maximum yield of gelatin extraction from

the adult stripe catfish was 30.31% which was obtained from extraction variations of 40 minutes soaking in acid; 40 minutes soaking in alkaline and the temperature of extraction was 70°C (Table 4).

Table 4. Yield obtained from different variations and different length

Sample Code	X1	X2	X3	Yield (%)	
				Sub adult	Adult
1	50	50	55	0.25	25.80
2	60	60	70	33.50	28.53
3	50	50	55	21.54	22.33
4	60	60	40	16.42	10.93
5	50	50	55	25.67	25.93
6	40	60	70	37.33	26.93
7	50	66	55	22.62	21.63
8	50	33	55	20.31	21.38
9	66	50	55	17.77	27.86
10	60	40	70	37.42	26.73
11	40	60	40	12.08	0.47
12	50	50	80	0.38	18.00
13	50	50	29	0.62	0.07
14	50	50	55	11.54	23.87
15	40	40	40	11.93	0.33
16	33	50	55	31.67	17.50
17	60	40	40	13.79	0.38
18	50	50	55	11.85	14.54
19	40	40	70	33.85	30.31
20	50	50	55	15.92	18.38

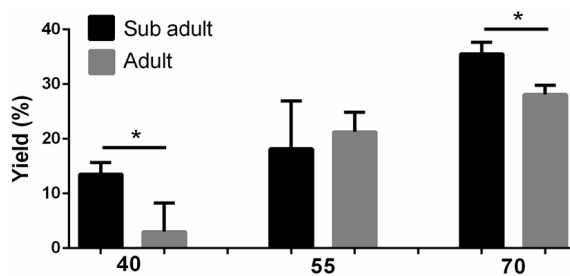


Fig.1: The Yield of Gelatin Extraction in different temperature. We statistically compared the data of the yield extracted at different temperatures from sub adult and adult using Student’s t test with 95% confidence. The gelatin extracted from sub adult showed significantly higher yield compared to the yield from the adult one (p<0.05)

Discussion









































We observed the yield obtained significant different at certain extraction temperature from different sizes.

The yield from sub-adult groups which were extracted at 40°C (yield range=11.9-16.4 %) and 70°C (yield range=33.5%-37.4%) were significantly higher than

the adult groups (yield range in 40°C=0.3 -10.9% and yield range in 70°C=26.7-30.3%). Whereas the extraction at 55°C, we observed no significant different yield obtained from sub adult and adult group. Similar results as in Table 2, our analyses using Pearson correlation resulted that the yield obtained from both from sub adult and adult showed

significant positive correlation ($p < 0.05$) with the temperature (i.e. 0.467 and 0.786, as coefficient of correlation, respectively). This is acceptable since the higher temperature also provides more energy for the collagen denaturation process, which is an important step in gelatin extraction 20.

Table 5: The color of gelatin extracted from striped catfish

Sample Code	X1	X2	X3	Gelatin from sub adult		Gelatin from sub adult	
				color	category	color	category
1	50	50	55		3		4
2	60	60	70		4		4
3	50	50	55		3		3
4	60	60	40		1		1
5	50	50	55		3		3
6	40	60	70		5		3
7	50	66	55		2		3
8	50	33	55		1		2
9	66	50	55		3		4
10	60	40	70		5		2
11	40	60	40		3		2
12	50	50	80		5		2
13	50	50	29				0
14	50	50	55		1		1
15	40	40	40				
16	33	50	55		3		3
17	60	40	40				
18	50	50	55		1		1
19	40	40	70		5		
20	50	50	55		1		2

Quality of Yield Based on Color of Gelatin

We then examined the quality of the gelatin based on the color of the extracted gelatin. We categorized the color based on the level of dark pigmentation in the gelatin. We obtained the results that shows gelatin yielded from sub adult striped catfish tends to have darker color than gelatin yielded from the adult one (Table 5). This dark pigment is possibly occurred because in the young pangasius catfish (the juveniles and sub-adults) has dark coloration due to higher content of melanin compared to the adult fish.²¹ As we see in the Table 5 that extraction with temperature 70°C and 80°C showed darker pigmentation than gelatin extracted at temperature ≤ 55°C. We then analyzed the color category from

each sample against the temperature of extraction using Pearson correlation. It resulted that in gelatin extraction using sub adult skin of striped catfish has positive correlation (correlation coefficient: 0.732) to the temperature used in the extraction process ($p < 0.05$). The higher temperature showed higher dark pigmentation in the extracted gelatin.

The thermal treatment is an important step in gelatin processing, since it results in the irreversible kinetic process of collagen denaturation for obtaining the random coiled polymeric chains, namely gelatin.²⁰ However, the increase of temperature in the collagen extraction may lead to the peptide chains degradation.²² Accordingly, we suggested

that high yield gelatin extraction and pigmentation in temperature of $\geq 70^{\circ}\text{C}$ possibly occurred due to the adversely effect of thermal treatment towards peptide chain disruption. The gelatin extraction at high temperature ($\geq 70^{\circ}\text{C}$) may disrupt the melanocytes in the skin which lead to the melanin released and cause dark pigmentation in the gelatin

Our study revealed that temperature of extraction has positive correlation with the gelatin yielded from sub adult and adult striped catfish. The higher temperature used in the extraction, the higher yield was obtained. Unfortunately, the temperature also has positive correlation with the dark pigmentation in the extracted gelatin. The higher temperature used, the extracted gelatin showed more dark pigmentation. Then, the optimal temperature for gelatin extraction from sub adult and adult of striped catfish is at 55°C , since we could get relatively higher yield with less pigmentation.

Gelatins from alternative sources²³ including from fish²⁴ have been studied extensively. Future research will include the investigation of the quality of the

obtained extraction products. This will involve both its performance in some applications as well as characterization with spectroscopic techniques like circular dichroism^{25, 26} and rheological techniques.^{27, 28}

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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