The Effect of Concentration Acetic Acid in Extraction of Gelatin from Nila Fish (Oreochromis niloticus) to The Physical Characteristics

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Abstract. Gelatin is a natural material from animal which obtained from hydrolysis process of collagen. Generally, gelatin is extracted through an acidic process. This research to observe of concentration acetic acid in extraction of gelatin from nila fish (Oreochromis niloticus) skin to the physical characteristics. The treatment of concentration acetic acid (0,10 ; 0,15; 0,20 and 0,25 M ) for soaking time (1, 2, 3, 4 and 5 hours ) in demineralization process before extraction. The observation of physical characteristics is viscosity, ash content, pH and color. Viscosity of gelatin from 1 and 5 hour of soaking time at the same concentration treatment were significantly different. Ash content shows a significantly different from 5 hours of soaking time treatment compared others treatment, while pH was no significantly different. Analyze for color of gelatin based on L*, a*, b* space were describe the lightness. The value “L” and “b” color of gelatin sample from acetic acid concentration treatment were significantly different while from soaking time treatment for 1 - 4 hours were no significantly different. The value “a” from all of gelatin sample were significantly different.

Keywords: Gelatin, Nila, Physical, Characteristics

1. Introduction
Gelatin is a natural material from animals which obtained from hydrolysis process of collagen, Collagen is found in tissues of the skin, bones and muscles of animals. Collagen has a triple helix protein structure with a molecular weight of around 300 kD. In the production of gelatin could through an acid process (Type A) with an isoelectric point value at pH 7-9 or base process (type B gelatin) with an isoelectric point value at pH 4.7-5.4 [1]. The acid treatment in the production of gelatin is used for raw material for animal skin has a higher yield value than bone [2].

The treatment of acid solution could break down molecular chain bonds of triple helix molecule collagen. It causes the molecule hydrolyzed to obtain a gelatin molecule containing a molecular structure with a single chain α-helix. This breakdown of collagen molecules into gelatin can reduce the functional properties of gelatin [2]. Gelatin is soluble in water, so it can be used as a thickener, gel-forming, emulsion forming and stabilizer. It needed a large of gelatin in food industry used in confectionery, water jellies and desserts, dairy products [3].

In addition, gelatin is also very necessary in the pharmaceutical industry, for the manufacture of capsule shells. It causes the need for gelatin to increase every year, while so far gelatin is still an
imported product from various countries. Based on commodity import data from the Ministry of Industry, the import of gelatin is very large for the needs of the food industry (as food additives) and the pharmaceutical sector (as capsules). The import value of gelatin in powder form in Indonesia from 2012 to 2016 with ranging from 42.4-2,443.5 thousand US$ and 1,861.9-2,769.6 thousand US$ for gelatin capsules as pharmaceutical products [4].

Generally, imported gelatin is produced from raw materials of pig skin, which until now still a halal problem. Therefore gelatin research from other sources of raw materials is continuously carried out, one of which from fish. Nila fish (*Oreochromis niloticus*) is one of the aquaculture commodities that has high economic value. Fillet product from nila fish has been exported to many countries in the form of fresh frozen and obtained a large of waste from fish skin. From the raw material of nila skin gelatin can be obtained through the acetic acid process with yield $>20\%$ with the value of gel strength $>200$ g bloom [5]. The treatment of acid type and soaking time in demineralization before gelatin extraction effect on yield and gel strength characteristics [5] and affect color [6]. Other specific characteristics of gelatin are viscosity which determines to ability of the functional properties of gelatin results a thick liquid texture. Viscosity is one of the gelatin quality standards in addition to the gel strength which has a value ranging from 1.5-7.5 cP [1]. When gelatin granules are immersed in cold water, they hydrate into discrete, swollen particles. When it warmed, gelatin disperses into the water, resulting in a stable suspension [1] to form a thick liquid. Other characteristics of gelatin are ash content and pH [8] which can also determine the solubility of gelatin. Beside it, characteristic of color is an important quality factor directly related to the acceptability of food products.

In this study extracted gelatin from nila fish (*Oreochromis niloticus*) skin with the treatment of acetic acid concentrations (0.10; 0.15; 0.20 and 0.25) M for (1, 2, 3, 4 and 5) hours soaking time in demineralization process before extraction. The aim of this study was to determine the effect of acetic acid concentration and soaking time in the extraction process of gelatin from nila skin to its physical characteristics.

## 2. Materials And Methods

### 2.1. Material and Reagent

Raw material was nila fish (*Oreochromis niloticus*) skin obtained from an industrial factory of nila fish in Central Java, Indonesia. Other materials were acetic acid and reagents chemical in laboratory analysis. Equipment were used magnetic stirer, waterbath, vacuum oven, milling equipment, viscometer brookfield, Color Flex EZ HunterLab and pH meter.

### 2.2. Gelatin Extraction

Degreasing by soaking in water (60-70°C) for ±10 seconds and followed by reduction of skin size ± (4 x 4 cm). Demineralization process by soaking (0.10 ; 0.15; 0.20 and 0.25 M) acetic acid solution (1: 8 w/v) for 1, 2, 3, 4 and 5 hours before extraction. It was continued by washing until the pH close to neutral. Extraction with aquades at 80 °C for 3 hours at a ratio of 1 : 3 (w/v) and followed by filtration. Drying at temperature of 20 - 25 °C for 48 - 72 hours and milled to obtain gelatin powder [5].

### 2.3. Viscosity

Gelatin solution (6.67%) were measured viscosity by using viscometer brookfield with spindle no.2 at temperature $\pm 60$ °C [1].

### 2.4. Color

Gelatin color was analyzed by using Color Flex EZ HunterLab. The result of color with a value of the brightness ($L^*$), redness ($a^*$) and yellowness ($b^*$) [3].
2.5. pH
Gelatin solution (6.67%) was measured pH by using pH meter at room temperature (± 25 °C) [1].

2.6. Ash content
The ash content was analyzed based on SNI 01-2354.1-2006 [8]. The empty porcelain ash dish was put in furnace and burned at 550°C for overnight, then cooled in a desicator for 30 minutes and weighed by an empty porcelain ash dish (A). The sample was weighed until reach the constant weight. 2 grams (B) and placed inside the dish. The ash dish contains sample in the furnace at 550 ± 5°C overnight. When finished, the ash filled dish is cooled in the desiccator for 30 minutes and weighed (C). Ash content calculated by the formula :

\[ \text{Ash content (\%)} = \frac{C - A}{B} \times 100\% \]

Note :
A : The initial weight of empty dish (gr)
B : Weight of sample (gr)
C : The weight of dish and sample after burned (gr)

2.7. Data Analysis
Result of measurement viscosity, color, pH and ash content from three replicates treatment were statistically analysed using ANOVA (One-way Analysis of Variance) at 5% level of significance (p<0.05). Then, continued identified between samples with Tukey’s test.

3. Results and Discussion
Gelatin from nila fish skin (Oreochromis niloticus) were obtained from soaking acetic acid (0.10 ; 0.15; 0.20 and 0.25) M treatment for (1,2,3,4 and 5) hours are shown in Figure 1.

![Figure 1. Viscosity of gelatin from nila fish (Oreochromis niloticus) skin](image)

Viscosity is one of commercially important physical property of gelatin as well. Low viscosity gelatins give short, brittle gels, while high viscosity gelatins give tougher, extensible gels [2]. Figure 1 shows the viscosity of gelatin decreases with increasing concentration of acetic acid and soaking time. The viscosity for the samples was in the range of 2.26 – 3.81 cP and samples from concentration of acetic acid treatment at the same of soaking time were no significantly different (P > 0.05). Sample gelatin from 1 and 5 hour of soaking time at the same concentration treatment were significantly
different (p < 0.05). The greatest viscosity from the treatment of 0.10 M acetic acid for 1 hour was 3.81 cP and the lowest viscosity from the treatment was 0.25 M acetic acid for 5 hours was 2.26 cP. The viscosity values of for red and black tilapia were 3.2 cP and 7.12 cP [6]. Generally, gelatin from nila fish skin has viscosity values included in the standard of commercial gelatin viscosity with range from 2.0-7.0 cps [3].

The viscosity characteristic of gelatin is strongly related to molecular weight distribution. Low gelatin viscosity was caused by the content of many low molecular weight peptide chains produced from the over-hydrolysis process of collagen during the pre-extraction of gelatin. Gelatin solution with a low viscosity usually yields a short and little texture gel, while a high viscosity gelatin solution yields a tough and extensible gel that has higher commercial value [10]. In general application of gelatin in the food industry, more high viscosity gelatin is needed to provide other characteristics of gelatin according to its function in forming thick textures [3].

Ash content of gelatin from nila skin produced by acetic acid processes (0.10, 0, 15; 0.20 and 0.25 M) and soaking time for 1, 2, 3, 4 and 5 hours is presented in Figure 2. Figure 2 shows a significantly different (p < 0.05) from 5 hours of soaking time treatment compared others sample. The lowest ash content in the treatment concentration of 0.10 M acetic acid in soaking time for 5 hours was 0.35% and the highest in the treatment of 0.25 M acetic acid in soaking time for 1 hour was 1.08%. The difference in the ash content gelatin of nila skin is due to the many components of the collagen molecule that are hydrolyzed during acid process which could be wasted during the washing process. In the treatment of high concentrations of acetic acid and longer soaking times could release a lot of the carboxyl group and amide groups in the triple helix collagen molecule. It also cause remove of lipid components, minerals and other unwanted components during the washing process before extraction. [2]. However, the ash content from gelatin of nila skin was according to the requirement for gelatin quality standards based on the Gelatin Manufacturers Institution of America (GMIA) with range of 0.3-2% [1] and gelatin quality standards based on the Indonesian National Standard, which is a maximum value of 3.25% [7].

![Figure 2. Ash content of gelatin from nila fish (Oreochromis niloticus) skin](image)

Figure 3 shows pH from all of sample were no significantly different (P> 0.05). pH of gelatin nila fish skin ranged from 5.11 - 5.45 according to the requirement of gelatin quality standard GMIA (2012) [1] with range is 3.8 - 5.5. pH of gelatin is related to the ash content, where the solution at acidic pH can release the triple helix bonding structure of the collagen molecule in a longer soaking time. It cause ash content of gelatin sample from longer soaking time treatment were higher.
Figure 3. pH of gelatin from nila fish (*Oreochromis niloticus*) skin

Color measurements of the gelatins are shown in Figure 4. Color is an important quality factor directly related to the acceptability of food products and is an important physical property to report for extracted gelatin but does not influence the functional properties. The color of gelatin depends on the nature of the raw material used in its preparation and also whether the gelatin represents a first, second or final extraction [3].

Figure 4. pH of Gelatin nila fish (*Oreochromis niloticus*) skin

Figure 4 shows the treatment of acetic acid concentration and soaking time in the extraction process of gelatin from nila skin affected to color of lightness. Based on Figure 4, shows the value “L” and “b” color of gelatin sample from acetic acid concentration treatment were significantly different (P
< 0.05) while from soaking time treatment until 4 hours were no significantly different (P > 0.05). The value “a” from all of gelatin sample were significantly different (P < 0.05). The value “ L” and “b” color of gelatin first increased and then decreased with increasing concentration of acetic acid and longer in soaking time, while value “a” of gelatin first decreased and then increased with increasing concentration of acetic acid and longer in soaking time. The lightness and darkness color of gelatin is commonly caused by inorganic, proteinaceous and mucosubstance contaminants introduced or not removed during its extraction and the filtration process during gelatin extraction affected the degree of clarity of gelatin solution [9].

4. Conclusion

Treatment of concentrations (0.10; 0.15; 0.20 and 0.25) M for 1, 2, 3, 4, and 5 hours in soaking time before extraction affected to physical characteristics of gelatin from nila fish (Oreochromis niloticus) skin with viscosity, ash content, pH and color. The higher the concentration of acetic acid and longer in soaking time results in lower viscosity and pH, but ash content increases. The physical characteristics of the colors associated with the values "L" and "b" are lower, while the value of "a" increases with greater concentration and longer in soaking time.

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