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The Effect of the Long Time of NAOH Seeding In the Loss Process Fat to the Quality of Gelatin Tiger Grouper Fish Bone (*Epinephelusfuscoguttatus*)

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Abstract: *Gelatin is a protein obtained from the partial hydrolysis of proteins of collagen fibers which are abundant in the skin, bones, and connective tissue of animals. Gelatin can be used as a multipurpose product and is widely used in the food and non-food industries. NaOH is a type of metallic base that has the character of dissolving fat tissue.Proteins are classified into 2, namely essential amino acids and non-essential amino acids. Essential amino acids are those that the body cannot synthesize. Whereas non-essential amino acids are the oppositeThe purpose of this study was to determine the effect of the long time of naoh seeding in the loss process fat to the quality of gelatin tiger grouper fish bonefor 4.5 days, 5 days, and 5.5 days. The best grouper bone gelatin was obtained by soaking naoh for 5.5 days of quality value of fat content of 0.02%; gel strength 108.04%; viscosity of 8.90 (cP); and yield of 13.28%; in addition, the highest profile of the essential amino acids in arginine was 8.89% and histidine, isoleucine was 0.93%. Whereas the highest non-essential amino acid profile at glycine was 23.49% and the lowest was tyrosine 0, 62%.*

Keywords*: gelatine, amino acid, fat*

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I. Introduction

Marine wealth includes various types and species of fish. One type of fish that has high economic value is tiger grouper fish (Epinephelusfusccogattus). In the international world, tiger grouper is known as the carped cod. These coral dwellers are usually caught with fishing rods, fish traps, and gill nets (Murtidjo, 2002)

The development of the fish processing industry in Indonesia is currently increasing, such as the frozen fish fillet industry which produces waste processing in the form of fish bones. Usually fish bones are only used as animal feed, so it only increases economic value. However, fish bones are often thrown away. Based on this, efforts need to be made to use fish bones to be more useful. One of them is processing fish bone waste into gelatin (Wijaya*et al*., 2015).

Gelatin is a protein obtained from the partial hydrolysis of proteins of collagen fibers which are abundant in the skin, bones, and connective tissue of animals. Gelatin can be used as a multipurpose product and is widely used in the food and non-food industries. Gelatin food products can be used as stabilizers, gelling agents, binders, thickener, emulsifiers, adhesives, whipping agents, and edible food wrappers (Puspawati*et al*., 2012). Proteins are classified into 2, namely essential amino acids and non-essential amino acids. Essential amino acids are those that the body cannot synthesize. Whereas non-essential amino acids are the opposite (Suprayitno and Sulistiyati, 2017).

A protein contains all the essential amino acids the amount needed by the body is called complete protein. Whereas if there is a protein that lacks one of its essential amino acids, the protein is classified as incomplete protein. Protein compounds obtained from cytoplasmic compounds in all living cells both animal and plant. Protein molecules are very large and consist of long chains of amino acids that are chemically bound. There are 26 amino acids found in proteins, 20 of which are often found in food (Suprayitno, 2017).NaOH is a type of metallic base that has the character of dissolving fat tissue. This is because the base has a heat that can erode fat, which is called the fat hydrolysis process (Tazwir*et al*., 2009).

II. Materials And Methods

2.1 Materials

The research material used consisted of the main ingredients and additional ingredients. The main material used in making this gelatin is tiger grouper fish bone obtained from PT. Alam Jaya Surabaya East Java Province. While the additional material used is sodium hydroxide (NaOH) which is in the form of thin slices and distilled water. These chemicals are purchased at the Panadia chemical shop in Malang City.

The first step in the process of making gelatin, which is weighing grouper bones weighing 100 g. Then the 0.6% NaOH solution was soaked for 4.5 days, 5 days and 5.5 days. After that, the bones are washed to a neutral pH. After neutral, the soaking was done again in 5% HCl solution for 2 days with bone comparison: HCl 1: 4. The bones are washed back to neutral pH. Then the bone is extracted with bone ratio: aquadest 1: 3 at 85° C for 6 hours. The final process is drying gelatin using an oven at 55° C for ± 2 days to dry. The analysis tested included analysis of fat content, gel strength, viscosity, yield, and amino acids.

2.2 Amino Acid Analyze

Amino acid analysis can be done using the UPLC method. Amino acid analysis according to Fawzya (2016), was carried out using ultra performance liquid chromatography (UPLC). The sample hydrolysis was carried out using 6N HCl by heating 110^{o}C for 22 hours. As an internal standard it is used α-amino butyric acid (AABA), and as a reagent for derivatization of amino acids used AccQ-Fluorine reagent kit. Powder gelatin is dissolved in 10 ml distilled water. Then take about 1 µL and inject it into the column with injection volume of 1 µL. After that, it was tested using an UPLC device with ACCQ-Tag Ultra C18 column testing conditions, column temperature 49ºC, mobile phase: gradient composition system, drift phase flow rate: 0.7 mL / minute, PDA detector, 260 nm wavelength.

Heating can result in damage to amino acids whose protein resistance is strongly associated with amino acids making up the protein, so this is the cause of protein levels decreasing by increasing temperatures (Yuniarti*et al*., 2013). This is also in line with the opinion of Suprayitno and Sulistiyati (2017), protein damage can be caused by heating with temperatures of 55-75^{o}C.

2.3 Fat Analyze

Fat analysis used based on the opinion of Angelia (2016), analysis of fat content by the Soxhlet method can be carried out by weighing 1-2 g of sample and then inserted in a paper sleeve coated with cotton. The sleeve is stuffed with cotton, then dried in an oven with a temperature below 80^{o} C ± 1 hour. After that, put it in the Soxhlet tool that has been linked to a fat pumpkin containing boiled stone that has been dried and weighed. Then extracted using hexane solvents or other fat solvents for ± 6 hours. The hexane contained in the material is distilled and the fat extract is dried in a drying oven at 105^{o}C. After drying, the sample is cooled and weighed. Drying can be repeated until a fixed sample weight is obtained. Calculation of percent fat content is as follows :

$$\% \text{ Lemak} = \frac{W - W1}{W2} \text{ x } 100\%$$

Information :
W = sample weight (g)
W1 =fat weight before extraction (g)
W2 = weight of fat pumpkin after extraction (g)

2.4 Gel Strength Analyze

Analyze gel strength based on research Santoso*et al*., 2013, strength of gelatin gel was measured by making a gelatin solution with a concentration of 6.67%. A total of 15 mL of the solution is then conditioned at 10 ° C for 16 hours, so that a gel is formed. The gel produced was then analyzed for the strength of the gel with Farrnel's LFRA Texture Analyzer using the TA 10 cylinder probe. The probe speed was set to 0.5 mm / s and a distance of 4 mm. Gel strength is the weight of the load recorded when the gel breaks to a depth of 4 mm, which is expressed in Bloom units

2.5 Viscosity Analyze

Testing of viscosity based on the opinion of Marine colloid (1984), measurement of viscosity of gelatin was carried out by making a concentration of 1.5% (b / b) gelatin solution heated by continuous stirring until the temperature reached 80° C then cooled to 76-77^{o} C. An example is placed in the measuring cylinder container at Viscometer Brookfield, using number 1 spindle at a speed of 100 rpm. The scale on the tool is read after several rounds and the number shown on the tool is stable.

2.6 Rendemen

The calculation of the yield of gelatin refers to AOAC (1995), which is by comparing the dry weight of gelatin with the wet weight of the grouper bone raw material before extracting gelatin.The yield was obtained by comparing the dry weight of gelatin produced with the weight of the extracted dried fish bone.

$$\text{Rendemen} = \frac{\text{dry weight of gelatin}}{\textit{bone weig ht}} \text{ x } 100\%$$

III. Results And Discussion

3.1 Amino Acid Composition

Amino acids are the smallest unit forming proteins. Amino acid composition is very important in the characteristics of gelatin properties. Gelatin contains 9 of the 10 essential amino acids the body needs. One essential amino acid that is almost not contained in gelatin is tryptophan (Hastuti and Sumpe, 2007). The main amino acids that make up gelatin are glycine, proline and hydroxyproline (Gimenez*et al*., 2005). The glycine and proline amino acids have an important role in the physical characteristics of gelatin. Glycine content in gelatin plays an important role in binding water (Pranoto*et al*., 2011). Determination of amino acids was carried out by the Ultra Performance LyquidChrtography (UPLC) technique. The amino acid test results can be seen in Tables 1 and 2.

Table 1. Essential Amino Acids

NO	Essential Amino Acid	Composition (%)
1	Histidine	0,93
2	Threonine	2,82
3	Arginine	8,89
4	Valine	1,84
5	Phenylalanine	2,34
6	Isoleusine	0,93
7	Leusine	2,01
8	Lisine	2,24

Table 2. Non Essential Amino Acid

NO	Non Essential Amino Acid	Composition (%)
1	Apartate Acid	3,31
2	Glutamate Acid	6,61
3	Serine	3,66
4	Glisine	23,49
5	Alanine	7,45
6	Tirosine	0,62
7	Proline	10,28

The stability of gelatin is strongly influenced by the proline amino acid content, because these amino acids will form a strong helical structure and maintain the stability of the triple helical structure. Therefore, the higher the amino acid proline content, the gel that is formed will be more stable towards heating so that the melting point is higher (Poppe, 1992).Amino acids can produce histamine by decarbolasehistidine which results in these compounds being toxic (Agustiana*et al*., 2014). Protein profile and amino acid composition are indicators of nutritional value to determine the quality of food substances that are useful for human health (Firlianty*et al*., 2014). According to Nur (2009), proteins are broken down by proteolytic enzymes autolysis into carboxylic acids, hydrogen sulfide, ammonia and other acids.

3.2 Fat

The results of the fat content test on soaking NaOH alkaline solution for 4.5 days showed fat content of 0.05%; for 5 days at 0.03%; and for 5.5 days at 0.02%. The fat content in the gelatin of grouper bones has decreased with increasing length of immersion time with 0.6% NaOH base. As a comparison, the research conducted by Wijaya*et al*., (2015), the average value of fat content in tilapia bone gelatin from each treatment ranged from 0.64% - 2.073%. Whereas according to Sari (2012), the value of fat content of Red Snapper gelatin was 2.55%.

NaOH is proven to be able to maximize the degreasing process, namely the process of eroding fat in raw materials. The higher concentration and soaking time of NaOH, the smaller the value of fat content. According to Tazwir*et al*., (2007), soda soda in the chemistry called NaOH (Sodium hydroxide) is a type of metal base strong. In the medical world, soda soda is known as an ingredient that dissolves fat tissue. Sodium hydroxide is able to erode the remaining fat in fish bones, this is because sodium hydroxide dissolved in water will have heat so that it can erode fat. Pure sodium hydroxide is solid white and is available in the form of pellets, flakes, granules or 50% saturated solutions. It is moist liquid and spontaneously absorbs carbon dioxide from free air. It is very soluble in water and releases heat when dissolved. Sodium hydroxide solutions will cause chemical burns, permanent injuries or scars and blindness if direct contact with the body.

Fat content according to Setiawan*et al*., (2013), fat content is inversely proportional to water content. Low fat levels can be caused due to stirring that is less prone to kneading. This results in a non-homogeneous mixture. Fat is a triglyceride that binds to three fatty acid molecules and has one glycerol molecule. However, the average triglyceride is two or three different fatty acids and therefore can contain a variety of fatty acids (Suprayitno, 2017).The increase in temperature when processing will result in increased damage to fat and

essential fatty acids isomerized when in contact with light and oxygen. The biological function of fat will be inactive when fat undergoes hydrolosis and oxidation. The amount of fat content decreases when boiled and increases when fried (Sundari*et al*., 2015).

3.3 Gel Strength

The strength of the grouper gelatin gel showed differences in each treatment. In the treatment of soaking NaOH base solution for 4.5 days showed gel strength of 106.79%; for 5 days at 107.50%; and for 5.5 days it was 108.04%. The gel strength in the gelatin of grouper bone has increased with increasing immersion time with 0.6% NaOH base.

According to Wijaya*et al*., (2015), the raw material, handling, processing and added soaking materials can affect the quality of the gelatin to be produced, from the results of gel strength tests at different immersion times. The process of degreasing that is less than optimal causes HCl work in the demineralization process to be less than optimal. HCl erodes fat before removing minerals in the bone. According to Sari (2012), because the fat that comes out is not maximal during the degreasing process. The fat content will be released during the immersion process with HCl and during extraction

3.4 Viscosity

The gelatin viscosity of grouper bone showed differences in each treatment. In the treatment of soaking NaOH base solution for 4.5 days showed a viscosity of 7.66 cP; for 5 days at 8.42 cP; and for 5.5 days 8.90 cP. The viscosity in the gelatin of grouper bones has increased with increasing length of immersion time with 0.6% NaOH.The longer the immersion time, the value of gelatin viscosity increases. This is due to the increasing duration of contact between the acid and bone so that the chance of decomposition of amino acid chains increases as well.

Decreasing the value of the gelatin viscosity of grouper bones from different immersion periods is caused by the long influence of soaking NaOH solution which causes the formation of collagen too quickly so that collagen formed at this stage will be wasted in the extraction process. The longer the immersion process, the weight of the gelatin obtained also decreases. These results explain that if immersion is carried out too long, the trophagenagen does not only experience swelling (increase in volume or weight of a material when in contact with liquids, gases or vapors) but the chain of the tropochagen has decomposed into gelatin which dissolves in the curing solution thereby reducing yield of gelatin extract (Puspawati, 2005).

3.5 Rendemen

The yield of the grouper bone gelatin showed differences in each treatment. In the treatment of soaking NaOH base solution for 4.5 days showed a yield of 10.45%; for 5 days at 11.48%; and for 5.5 days 13.27%. The increase in the gelatin of grouper bone has increased with increasing length of immersion time with 0.6% NaOH.

In a study conducted by Saputra*et al*., (2015), regarding extraction of gelatin from catfish, the yield of gelatin was 11.06%. This result is lower than the results of this study. The longer the extraction time, the yield value will increase. This is presumably due to the greater number of H ion ions that hydrolyze collagen, while the longer extraction time causes more collagen to break down into gelatin, whereas at 65^0C, the yield of gelatin tends to be lower with high extraction time (Tazwir*et al*., 2007) .

According to Haris (2008), the amount of yield value is influenced by the concentration of acid solution used in soaking. The higher concentration of acid solution used will cause the acidic solution to become more acidic, so that H + ions that hydrolyze collagen from the triple helix chain into more single chains. High concentrations and long immersion times are thought to reduce the amount of yield of gelatin produced. This is because in the treatment the resulting ossein becomes very soft and destroyed, causing a lot of ossein to be lost during the neutralization process.

IV. Conclusion

The best grouper bone gelatin is obtained by soaking NaOH for 5.5 days from the value of 0.02% fat content; gel strength 108.04%; viscosity 8.90 (cP); and results of 13.28%. In addition, the profile of the highest essential amino acids in Arginine is 8.89% and Histidine, isoleucine is 0.93%. While the highest non-essential amino acid profile in Glycine is 23.49% and the lowest is tyrosine 0, 62%. The longer the gelatin is soaked using NaOH base, the lower the fat content it contains

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