

The Influence of Acid Type and Extraction Temperature on Amino Acid Profiles and Chemical Physical Characteristics of Gelatin Snapper Fish Bone

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Abstract: *Red snapper bone is a waste produced by fisheries processing which has not been fully utilized. Red snapper bones can be used as a substitute for gelatin, which generally comes from the skin and bones of cattle and pigs. The type of acid and extraction temperature are used to determine how optimal some types of acid and temperature variations are to the characteristics of gelatin. This study was conducted to determine the effect of acid type and extraction temperature on the characteristics and amino acid profile of gelatin red snapper bone. The method used in this study is the experimental method. The experimental design used in this study was factorial Complete Complete Design (RAL) using acid type and extraction temperature with 9 treatments and 3 replications. Characteristic analysis parameters include 2 types, namely physical and chemical. Physical characteristics such as yield, viscosity and gel strength. While the chemical characteristics such as fat, protein, and amino acid profile. Data were analyzed using ANOVA, and for further analysis significantly using the Tukey test. To determine the best treatment using the De Garmo method. This study resulted in the best treatment for phosphoric acid with an extraction temperature of 78 °C with physical characteristics including yields of 11.60%, viscosity of 12.02 Cp, and gel strength of 311.33 g / bloom. As well as chemical characteristics including fat of 0.54%, protein of 88.46%, amino acid profile of glycine at 24.11%, proline of 10.12%, and arginine of 9.11 % and Alanine at 7.98%.*

Keywords: *Gelatin red snapper bone, acid type, extraction temperature*

Date of Submission: 09-05-2019

Date of acceptance: 25-05-2019

I. Introduction

Gelatin comes from the Latin "gelatus" which has the meaning of freezing. Gelatin itself is a protein produced by the partial hydrolysis of collagen derived from the skin, white connective tissue, and animal bones and polypeptides with a molecular weight of about 20,000 g / mol to 250,000 g / mol. Gelatin itself is composed by 18 amino acids that are mutually bound, which are composed of glutamate acid, serine, valine, aspartic acid, lysine, arginine, tyrosine, threonine, histidine, glycine, hydroxyproline, hydroxylysine, leucine, isoleucine, proline, alanine, phenylalanine, and methionine (Suryaniet al., 2009). The benefits of gelatin are for stabilizers, gelling agents, binders, thickeners, emulsifiers, and as food wrappers. In addition, the food industry is generally used for the ice cream, candy and jelly industries (Wijaya et al., 2015).

Amino acids are constituents of proteins, proteins are composed by 23 or more simple amino acid units. If a protein is hydrolyzed using acid it can produce a mixture of amino acids. Amino acids themselves are composed of carboxyl groups (COOH), amino groups (NH₂), hydrogen atoms (H), and R groups that are bound to a C atom or also called carbon α (Winarno, 2004). Amino acids are classified into two, namely essential amino acids (indispensable) and non-essential amino acids (dispensable). Essential amino acids are amino acids that cannot be synthesized by the bodies of animals or humans and are not made in the body. Whereas for non-essential amino acids is an amino acid that is sufficiently synthesized by the body (Suprayitno and Sulistiyati, 2017).

Fish bones are fishery wastes that have not been utilized optimally. Making gelatin from fish bones can reduce environmental pollution and increase the added value of the fish bone waste. Generally gelatin raw materials are made from cows or pigs such as skin and bones. In Indonesia the majority of the population embraces Islam, so the selection of raw materials is very important. Pigs themselves are raw materials which are included as non-halal, and sometimes diseases from cow animals such as mad cow are a problem if raw materials are used. Hence fish bones are used as alternative ingredients that are safe and guaranteed halal (Istiqlaal, 2018).

Several factors that can affect the characteristics of gelatin itself are solutions used in the process of immersion, stirring, temperature and time. The immersion process is the process of denaturing the amino acids that make up collagen. The immersion process using acid solvents can produce a higher amount of yield and a shorter processing time. The temperature used in extraction can affect the gelatin itself. The use of extraction temperatures above 95 °C results in decreased properties of gelatin (Siregaret al., 2015). The extraction temperature can also

affect the quality of the gelatin itself. Generally in the gelatin industry, the temperature used is around 55-90 °C (Wulandari *et al.*, 2013).

II. Materials And Methods

2.1 Materials

The research material used in this study consisted of 2 parts such as raw materials and other ingredients to make gelatin for red snapper bones. The raw material used for making gelatin red snapper is red snapper bone from the rest of the filleting process of PT. Alam Jaya Seafood Surabaya, East Java. While the other materials used are distilled water, acetic acid, HCL, phosphoric acid, label paper, litmus paper, filter paper, and transparent fabrics and silica gel. The equipment used for the manufacture of gelatin for red snapper is plastic basin, knife, plate, baking sheet, waterbath, spatula, hot plate, oven, magnetic stirrer, 1000 ml beaker glass, 500 ml beaker glass, 100 ml measuring cup, analytic scale, scales digital, 10 ml volume pipette, serological pipette, suction ball, and crushable tang.

The process of making red snapper bone gelatin consists of 2 stages such as preparation of raw materials and making gelatin. preparation of raw materials, first the red snapper's bones are washed and cleaned of dirt and remaining meat attached by soaking the bones of snapper in boiling water for 30 minutes. then wash again until clean from the remaining meat and dirt. Then drained and reduced in size to ± 2 cm, then dried. The second stage is the manufacture of gelatin, which is carried out first in the manufacture of gelatin bone red snapper is the process of demineralization which is as much as 100 g of red snapper bone. Then soaked with acid solvents (acetic acid, phosphoric acid, HCL) in a 500 ml beaker glass with a bone ratio: 1: 4 (b / v) acid solution for 2 days to produce ossein. Then ossein is split until a neutral pH is obtained. The next process is extraction, the neutral ossein is placed into Erlenmeyer and added aquadest with a ratio of 1: 3 (b: v). erlenmeyer which contains ossein extracted in waterbath with a temperature of 78 ° C, 80 ° C, and 82 ° C for 6 hours. Then the gelatin extract was obtained, then pour it into a baking sheet and dry it using an oven at 55 ° C for 48 hours. After drying, then mashed.

2.2 Yield Analyze

The yield analysis can be determined using the method according to Ananda *et al.*, (2018). The yield is the amount of dry gelatin obtained from raw materials with clean conditions after passing the extraction stage compared to the initial amount of raw material. Calculation of yield can be calculated using the formula:

$$(\%) \text{ Yield} = \frac{\text{Dry gelatin}}{\text{Gelatin raw material}} \times 100$$

2.3 Viscosity Analyze

Viscosity analysis can be determined using Brookfield Viscometer according to Pertiwi *et al.*, (2018), that is, prepare a gelatin solution with a concentration of 6.67% (b / b) by making 7 g gelatin dissolved in 105 ml of distilled water. Then the 6.67% gelatin solution measured the viscosity using a Brookfield Viscometer at a temperature of 60°C with a shear speed of 60 rpm using a spindle. The results obtained from measurements are multiplied by conversion factors. The unit of viscosity used is centipoise (cP).

2.4 Gel Strenght Analyze

Analysis of gel strength can be determined by the method used according to Rahmawati and Hasdar (2016), ie gelatin solution with a concentration of 6.67% (b / v) was prepared first. Then stir until homogeneous using a magnetic stirrer, then heat for 15 minutes with temperatures reaching 60 ° C. Pour the solution into a standard bloom jars, then cover and let stand for 2 minutes. After that it was incubated for 17 ± 2 hours with a temperature of 10 ° C. then measured by means of the TA-XT Plus Texture Analyzer with 4 mm in depth and probe speed of 0.05 mm / s and the results are in the form of g Bloom units.

2.5 Fat Analyze

Fat content analysis can be determined by the Soxhlet method according to Prihatiningsih, *et al.* (2014), is to first weigh a sample of about 2 g and puree, after that wrap it in the form of a timble and put it into Soxhlet. Then add the n-hexane solvent to Soxhlet to taste. Then extract for 4 hours until the solvent drops back to the clear colored flask, then the solvent in the fat flask is distilled and the solvent is collected again. Then the fat in the fat flask extracted is heated with an oven temperature of 105 ° C constant constant. Then the fat flask is cooled in the desiccator. Furthermore, the weight of the residue in the bottle is weighed and expressed as fat weight.

2.6 Protein Analyze

Analysis of protein levels can be determined using the method according to Sukesu and Mulyani (2010), which is by the mikrokjeldahl method. This method consists of three stages, namely destruction, distillation, and titration. At first the sample snippet is inserted into the kjeldahl flask (can also use a test tube). Then add 1 gram of CuSO₄ and add 2.5 ml of concentrated H₂SO₄. Then sample samples were destroyed for 2 hours at 100 ° C. After the destruction results are cooled, then put into a round flask that has been given boiling stone and added with 50 ml of aqua DM and 15 ml of NaOH 50% w / v and distillation. Distillate was covered in liquid containing 10 ml HCl 0.20 N; 4 drops of methyl red and 4 drops of methylene blue to a total volume of 40 ml. Then the erlemeyer solution is titrated with a standardized NaOH solution with 0.2C H₂C₂O₄ solution. The end point of the titration is marked by a change in color from purple to green. The volume of NaOH used for titration is recorded. Replication for each snippet 5 times.

2.7 Amino Acid Profile Analyze

Amino Acid Analysis can be determined using the UPLC method according to Suryanti *et al.*, (2017). the first step is to prepare 0.1 g of the sample to be tested. Then add as much as 5 ml of 6N HCL and mix it using the vortex until it is evenly distributed and then hydrolyzed for 23 hours at a temperature of 110 °C. then filtration, 10 µl of a solution consisting of a sample filtrate, AABA (Alpha Amino Butyric Acid) and aquabides was added with 70 µl AccQ-Flour Borate and 20 µl of reagent flour A (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) then homogenized with use vortex after that let stand for 1 minute. Then incubate at 55 °C for 10 minutes, then inject into the UPLC. Prepare a standard solution of 40 µl amino acid standards and 40 µl of internal AABA (Alpha Amino Butyric Acid) standards and 920 µl of aquabides then homogenized. Then 10 µl of the standard solution was added 70 µl AccQ-Flour Borate and 20 µl reagent flour A. Then incubate at a temperature of 55 °C for 10 minutes and the standard solution was injected into the UPLC. Analysis of amino acid profiles using the UPLC H-Class with the C18 Ultra ACCQ-Tag column (2.1 × 100 mm), with a temperature of 49°C, mobile phase with a gradient composition system (acetonitrile and aquades), a flow rate of 0.7 ml per minute, PDA detectors, wavelength 260 nm and injection volume 1 µl.

III. Results And Discussion

3.1 Yield

The results of the study showed that the yield of red gelatin of red snapper bone around 1.60% -14.33%. and obtained yield with the best treatment of 11.60%. The use of an acid that is too strong makes collagen composed of amino acid peptides which are the basic structure of further denatured collagen. The use of acetic acid produces a low yield caused by the ability of acetic acid to break collagen peptide bonds that are less than optimal so that the yield is low (Siregaret *et al.*, 2015). The high yield due to high extraction temperatures results in more conversion of collagen to gelatin (Suryanti *et al.*, 2006). A high extraction temperature will increase yield due to the open collagen structure caused by the release of several bonds in protein molecules (Islam *et al.*, 2018).

3.2 Viscosity

The results showed that the gelatin viscosity value of red snapper bones was around 9.70 cP-12.02 cP and viscosity with the best treatment was 12.02 cP. The use of relatively high temperatures can lead to further hydrolysis of collagen which has become a gelatin molecule resulting in a broken bonding process between amino acids and can produce low viscosity values (Saputra *et al.*, 2015). Viscosity is strongly related to the molecular weight of gelatin and the distribution of molecules. The molecular weight of gelatin is also related to the amino acid chain, the longer the amino acid chain, the higher the viscosity value, and vice versa. Different types of acids and concentrations also influence the molecular weight of gelatin produced (Hidayat *et al.*, 2016). The type of acid also affects the viscosity value, due to the ability of each type of acid to break the bonds between molecules. The low viscosity caused by the short chain of amino acids contained in it and the weak cross-linking will cause collagen molecules to be easily hydrolyzed, resulting in decreased molecular weight of gelatin (Ridhayat *et al.*, 2016).

3.3 Gel Strength

The results of the study showed that the strength of gelatin gel strength of red snapper bone was around between 267 g / bloom - 311.3 g / bloom and the gel strength with the best treatment was 311.3 g / bloom. According to Saputra *et al.*, (2015), the decrease in gel strength due to extraction temperature due to high extraction temperatures causes further termination of gelatin molecules so that the amino acid chain gets shorter and the shorter the amino acid chain results in lower molecular weight and gel strength low. Gel strength depends on the amino acid chain length. If the conditions of collagen are completely hydrolyzed, the gel strength will increase. This occurs because hydrolyzed collagen can produce long polypeptide chains (Huda *et al.*, 2013). The type of acid has an effect on the value of gel strength due to the different ability of acids to break bonds between molecules and can influence the composition of their constituent amino acids and molecular weight distribution (Ridhayat *et al.*, 2015).

3.4 Fat

The results of the study showed that the value of gelatin fat content of red snapper bones is around 0.35% - 1.03%. and fat content was obtained with the best treatment of 0.54%. Fat content in gelatin generally depends on the treatment during the gelatin making process, starting from the cleaning or degreasing stage until the filtration process results from extraction. Good treatment can reduce the amount and content of fat contained in the raw material so that gelatin has a low fat content (Yentiet *al.*, 2015). In general, the use of acids can accelerate the hydrolysis process of fat into glycerol and free fatty acids, so the stronger the acid, the higher the fat that will decompose, thereby reducing the fat content of gelatin. Heating with high temperatures can cause levels of degraded fat. The higher the temperature used, the fat damage will increase and can reduce fat levels. High heat or temperature can also accelerate oxidation reactions (Winarno, 2004).

3.6 Protein

The results of the study showed that the value of the gelatin protein content of red snapper bones was around 86.44% - 88.49% and the results of the best treatment were 88.46%. The use of HCL in the immersion process produces lower levels of protein than phosphoric acid. According to Siregaret *al.*, (2015) the stronger the acid used, the acid molecule will bind to the calcium phosphate molecule contained in the bone and the bigger it causes damage to the protein structure. As well as the ability of the type of acid to affect protein because proteins will experience changes in the structure of the constituent proteins and can damage the structure of collagen proteins. Whereas in the immersion process using acetic acid has a low protein content, because the breaking of hydrogen collagen bonds using acetic acid is not maximal or optimal, according to Astawan and Aviana (2003), protein content can be affected by immersion or curing processes, due to immersion or curing occurs the process of breaking the hydrogen bond and opening the collagen structure optimally so that the protein is extracted and released from the gelatin. The result can reduce levels of gelatin protein.

3.7 Amino Acid Profile

Factors that can affect the amino acid content of gelatin are sources of collagen and animal species. sometimes the method of making gelatin can also reduce the amino acid content of gelatin, such as using the acid method, acid type, and extraction temperature. Based on the best treatment obtained from the analysis of amino acid profiles of the acid type treatment and extraction temperature of the gelatin bone red snapper bone can be seen in Table 1 and table 2.

Table 1. Results of Analysis of Essential Amino Acid Gelatin Red Snapper Bone

No	Amino Acid type	Level (%)	Pangas catfish gelatin**
1	Histidine	1,01	0,60%
2	Threonine	2,78	2,24%
3	Arginine	9,11	7,87%
4	Valine	1,84	2,10%
5	Phenylalanine	2,96	2,39%
6	Isoleucine	0,79	1,34%
7	Leusin	2,31	2,55%
8	Lysine	2,20	4,00%

**Nasution *et al.*, 2018

Table 2. Results of Analysis of Non Essential Amino Acid Gelatin Red Snapper Bone

No	Amino Acid type	Level (%)	Pangas catfish gelatin**
1	Aspartic acid	3,34	4,16%
2	Glutamic acid	6,70	9,10%
3	Serin	3,22	2,08%
4	Gycine	24,11	16,90%
5	Alanine	7,98	7,82%
6	Tyrosine	0,67	0,64%
7	Proline	10,12	11,08%

**Nasution *et al.*, 2018

In this study the results of the amino acids found in the gelatin of red snapper bone were glycine at 24.11%, proline at 10.12%, and arginine at 9.11% and Alanine at 7.98%. When compared with amino acid results according to Nasution *et al.*, (2018). Levels of vali, isoleucine, leucine, lysine, aspartic acid, glutamic acid and proline results in lower red snapper bone amino acids.

The most amino acid content in gelatin is Mino glycine acid. The polypeptide chain at geltin is composed of repeating the amino acids glycine-proline-proline or glycine-hydroxyproline-proline. Gelatin which has high amino acid glycine and proline, also has high gel strength (Santoso *et al.*, 2015). The amino acid glycine is present in every third collagen triple helical amino acid arrangement (Gly-X-Y). glycine has a role to reduce steric

resistance and can trigger hydrogen bond interactions in helical chains (Alhana et al., 2015). Proline amino acids have a function to maintain the integrity of the collagen structure and to hold the superhelical structure in collagen (Djailani et al., 2016).

IV. Conclusion

So it can be concluded that in this study the red snapper bone gelatin obtained with the best treatment was obtained 78°C phosphoric acid including rendement 11.60 g, viscosity 12.02 Cp, and gel strength 311.33 g / bloom, fat 0,54%, protein 88,46% and glycine amino acid profile of 24.11%, Proline by 10.12%, and Arginine by 9.11% and Alanine at 7.98%

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Manish Kumar. " The Influence of Acid Type and Extraction Temperature on Amino Acid Profiles and Chemical Physical Characteristics of Gelatin Snapper Fish Bone. "IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 12.5 (2019): PP- 57-61.