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The Effect of Variation of Acetic Acid Concentration on Characteristics of Gelatin from Milkfishskin (Chanoschanos)

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Abstract: *Gelatin is a hydrocolloid product obtained by hydrolyzing the protein collagen found in the skin, bones and connective tissue. Gelatin is obtained by heat denaturation from collagen. Gelatin has been applied in food as a gelling agent, thickener, emulsifier, pharmaceutical, health, cosmetics and photography industry. gelatin sourced from fish is still small. The purpose of this study was to determine the characteristics of milkfish skin gelatin with acetic acid 4%, 6% and 8%. The results showed that gelatin with the highest percentage of glycine. the best research results at a concentration of 8%, namely gel strength 98.07 g / bloom, Viscosity 6 cP, yield 19.4%. Use of high concentrations of acetic acid can decide which amino acids have been formed so that the amino acid chain of the damaged gelatin causes the gel strength to decrease. While the use of acid solutions with low concentrations can produce small gel strength because collagen is converted into less gelatin.It is also stated that amino acids in the skin of fish contain amino acids, such as Alanine, Arginine, Aspartate Acid, Cysteine, Glutamine, Glysin, Histidine Hydroxyprolin, Isoleucine and explained that the highest amino acid is Glycine. The quality of gelatin is influenced by the stages of the gelatin making process, namely swelling (extraction), extraction, and drying.*

Keywords: *Gel Strenght, Milfish Skin, Gelatin*

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I. Introduction

Milkfish in Indonesia is a commodity with a high level of consumer demand, in addition to its high nutritional content (Vatria, 2010). On the other hand milkfish is also very easy to cultivate in Indonesian waters which have a tropical climate. From year to year the demand for milkfish has always experienced an increase both in the domestic and export sectors. Fish is one food that has a high protein content and is therefore very beneficial for humans (Firlianty et al., 2014). Generally, parts of fish that cannot be eaten can reach 35%. Waste from fisheries processing, in the form of skin, fish bones, fish head, and internal organs is usually left wasted so that it can pollute the environment (Koli et al., 2012). Even though there are skin and bones from the waste that contains collagen which if processed further can produce gelatin.

Gelatin is a hydrocolloid product obtained by hydrolyzing the protein collagen found in the skin, bones and connective tissue. Gelatin is a type of protein extracted from animal collagen tissue. In animals, collagen is found in bones, cartilage, skin, and connective tissue. Gelatin is obtained by heat denaturation from collagen. Sobral (2001) explains that gelatin is a denaturation protein derived from collagen and is an important function of biopolymers that has very wide application in various fields of industry. Gelatin has been applied in food as a gelling agent, thickener, emulsifier, pharmaceutical, health, cosmetics and photography industry. Until now the raw material that is widely used for the production of the gelatin industry is cow bone, cow leather and pork skin (Nurilmala et al., 2006). The use of gelatin from these mammals still faces many obstacles including the beliefs held by consumers where Hindus are prohibited from consuming cows. Some people are also worried about consuming cow waste because of mad cow disease, mouth and nail disease (foot and mouth), and Bovine Spongiform Encephalopathy (BSE) (Nurilmala et al., 2017). In addition, ingredients derived from pigs must not be consumed by Muslims. Now, we have started switching raw materials for gelatin from fish, which come from waste or byproducts from the production of wasted fish processing, including skin, bones and scales.

Gelatin is rich in amino acids 26% glycine (Gly) (almost one third of total amino acids), 16% proline (Pro), and 14% hydroxyproline (Hyd), the content depends on the raw material. It is also stated that amino acids in the skin of fish contain amino acids, such as Alanine, Arginine, Aspartate Acid, Cysteine, Glutamine, Glysin, Histidine Hydroxyprolin, Isoleucine and explained that the highest amino acid is Glycine (Agustin, 2013). The content of Hydroxyproline influences the strength of the gelatin gel, where the higher the amino acid, the better the strength of the gel. The amino acid composition of gelatin depends on the source of collagen, animal species, and the type of collagen. Can also decrease the composition of amino acids depending on the method of manufacture. Alkaline processing generally contains more hydroxyproline and less tyrosine than acidic processes. The quality of gelatin is influenced by the stages of the gelatin making process, namely swelling (extraction), extraction, and drying.

This research, considering the potential of hydrolysis of collagen protein to gelatin is a potential product that is highly determined by the constituent amino acids. The aim of this study was to make milkfish skin gelatin with acetic acid 4%, 6% and 8%, looking for the best characteristics of gelatin from the difference in concentration.

II. Materials And Methods

2.1 Materials

The research material used consisted of raw materials and materials for the manufacturing process. The raw material used in this study is milkfish skin which is obtained from the remaining processed products in Poklahsar, Sumber sari, Sidorejo Village, Pagelaran District, Malang Regency. Other materials or materials for the manufacturing process used are aquades, acetic acid (CH3COOH), label paper and bland fabrics.

In this study, it was divided into two stages, namely preliminary research and main research. In the preliminary study obtained the best results with a concentration of 6% acetic acid. Then the process of making gelatin from milkfish skin in the main study, the skin was cleaned with clean water. After that, immersion was carried out using acetic acid 4%, 6%, and 8% (b / v) for 48 hours. After the immersion process is complete, the skin is washed to a neutral pH.in the process of soaking gelatin with the addition of acid aims to hydrolyze collagen in fish skin. and to dissolve minerals in bone and fish skin which will affect the physical characteristics and amino acid gelatin.Then enter the extraction stage, with a temperature of 65-75oC for 4 hours. The processed material is mixed with distilled water in a ratio of 1: 3 (w / v). The extracted material is filtered through a double-layered cheese cloth. The filtrate is dried at 60 ° C in a hot air oven for 48 hours earlier. After that the gelatin in the form of the sheet will be mashed into powder. All gelatin samples were weighed, calculated for extraction results, amino acid analysis, gel strength analysis and viscosity analysis

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 110oC 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.

2.3 Gel Strength Analyze

Gel strength is very important in the process of improving gelatin extraction because one of the important properties of gelatin is being able to convert liquids into solids or turn soles into reversible gels. For gel strength testing, what was done was preparation of gelatin with a concentration of 6.67% (b / b) prepared with distilled water (7.5 grams of gelatin plus 105 ml of distilled water). The solution is stirred using a magnetic stirrer until it is homogeneous then heated to a temperature of 80^{0}C for 15 minutes. The solution was poured in Standard Bloom Jars (bottles with a diameter of 58-60 mm, height 85 mm), closed and left for 2 minutes. Then incubated at 10 ° C for 17 ± 2 hours.

2.4 Viscosity Analyze

The amount of viscosity is influenced by the substance dissolved in the solution. If more substances are dissolved and the solution gets thicker then the value of the resulting viscosity will be higher. To determine the viscosity value, what needs to be done is a gelatin solution with a concentration of 6.67% (b / b) prepared with distilled water (7 g gelatin plus 105 ml of distilled water) then the solution is measured by using a Brookfield Syncro-Lectric Viscometer. Measurements were carried out at 60ºC with a shear rate of 60 rpm using a spindle. The measurement results are multiplied by the conversion factor.

2.8 Rendemen

The yield is one of the important parameters in making gelatin. The yield is the percentage between the final weight of the process and the initial weight before the process. According to AOAC (1995), the final weight of the process used is the weight of the gelatin powder produced, while the initial weight used is the weight of the fresh material (skin that has been washed clean). The yield is determined by weighing the initial weight and final weight.

III. Results And Discussion

3.1 Amino Acid Composition

The amino acid composition of gelatin depends on the source of collagen, animal species, and the type of collagen. Can also decrease the composition of amino acids depending on the method of manufacture. Alkaline processing generally contains more hydroxyproline and less tyrosine than acidic processesAmino gelatin acid milkfish skin (Chanoschanos) based on the best treatment obtained amino acid test results from the treatment of acetic acid variations can be seen in Tabel 1.

Table 1. Amino acid composition of different types of gelatins

No	Amino Acids	Gelatin of Milkfish skin (%)*	Gelatin of Mackerel Fish skin (%)**
1	L-Tirosin	0,53	0,4
2	L-Leusin	2,19	1,91
3	L-Prolin	12,00	9,13
4	L-Histidin	0.72	0,48
5	L-Theorin	2,53	2,46
6	L-AsamAspartat	3,30	4,69
7	L-Lisin	2,27	3,7
8	L-Glisin	25,15	18,4
9	L-Arginin	10,31	6,53
10	L-Alanin	7,24	8,87
11	L-Valin	1.60	1,6
12	L-Isoleusin	1,18	1,06
13	L-Fenialanin	2,92	1,72
14	L-AsamGlutamat	6,40	8,39
15	L-Serin	3,99	2,32

**Gunawan*et al.*, (2017)

Amino test results on gelatin milkfish skin can be calculated based on the value obtained at L-Glycine higher, namely 25.15% and L-Proline 12.00%. According to Yuniarti et al. (2013), the high amino acid glycine because of the content of collagen obtained from fish skin which is still attached to meat. Protein as a whole does not contain much glycine. The exception is collagen which is two-thirds of the total amino acid is glycine. The largest amino acid content in gelatin is the amino acid glycine. Another large amino acid content after glycine is proline. Another large amino acid content after glycine is proline (Santoso et al., 2013). The stability of gelatin is very dependent on the amino acid content of proline, because these amino acids will form a strong helical structure and maintain the resistance of the triple helix structure.The triple helix is the basic structural unit of collagen called tropocolagen. The breakdown of the structure of tropocolagen into a random twist uses this acid solution called gelatin. Conversion of collagen to gelatin can cause changes in amino acid composition, this is related to the method used. Extraction of gelatin with an acidic process generally contains more glycine and less tyrosine. The type and concentration of acid solution can affect the characteristics of the gelatin produced.

According to research by Adiningsih and Tatik (2015), the high glycine content in gelatin can be used to dissolve gelatin in the air and be able to make emulsions. This is because glycine is an amino acid that has hydrophilic properties. Gelatin with high amino acid glycine and proline content will also have high gel strength values.Specific characteristics of gelatin are the amino acid proline composition. Proline amino acids play a role in the triple helix molecular structure through hydrogen bonds between free water molecules.

3.2 Gel Strenght

Gelatin The average gel strength obtained is 94.55 - 98.78 g / Bloom. the highest average gel strength was obtained by immersion training using acetic acid with a concentration of 8% ie 98.07 and collected at a concentration of 14% at 94.55. The results of this study indicate that the higher the acetic acid given, the higher the gel strength in the gelatin product produced. This is related to the amount of protein each time the acetic acid concentration is given.

Gel strength is influenced by hydrogen bonds between water molecules with hydroxyl groups from amino acid groups, short lengths of protein chains, concentration and molecular weight distribution (Arnesen and Gildberg, 2002). The use of concentrations of acidic and basic solutions that are high in the immersion process can cause gel strength to increase and decrease (Ockerman and Hansen, 2000).The low concentration of gelatin and the high non-gelatin component such as ash content can reduce the quality of gelatin. In addition, molecular weight distribution, solution concentration and salt content in gelatin can affect the results of testing the strength of gelatin gel and viscosity (Karim and Batt, 2008).

3.3 Viscosity

Viscosity (thickness) of gelatin is one of the important properties of gelatin. Viscosity testing aims to determine the level of viscosity of gelatin as a solution at certain concentrations and temperatures (Rusli, 2004).The average viscosity of gelatin obtained ranged from 4 cP to 6 cP. The highest average viscosity value was obtained by immersion treatment using acetic acid with a concentration of 8% that is equal to 6 cP and the lowest value was obtained at a treatment of 4% concentration of 4 cP. This value has met the SNI No. standard. 3735 in 1995 for gelatin products, namely 1.5 - 7.0 cP, besides that the viscosity of this treatment had met the standard B type gelatin which was 2.0 - 7.5 cP.

The higher the concentration of acetic acid given in the immersion process will cause a high viscosity value in the gelatin product produced. This is due to the occurrence of bonds between polypeptide chains so that the molecular weight becomes larger, thus the value of viscosity also increases. Collagen values may be varied, collagen which binds to mineral particles causes the bonding of collagen molecules with solution to be less so that the distribution of gelatin molecules gets faster and the viscosity value also decreases. The smaller the molecular weight of collagen can cause the distribution of collagen molecules in solution to be faster, resulting in low viscosity values (Avena-Bustillos et al., 2006).

3.4 Rendemen

The yield is one of the important values in making gelatin. The greater the number of yields produced, the more efficient the treatment with the note that it does not rule out other physical or chemical properties. The yield is the result of comparison of the dry weight of gelatin produced from the weight of the skin used as raw material (Yuniarifin*et al*., 2006).The average yield of gelatin obtained ranged from 16.9 to 19.4%. The highest average renement value was obtained by immersion treatment using 8% acetic acid concentration which was equal to 19.4% and the lowest was obtained at 4% acetic acid treatment which was 16.9%. the results of this study indicate that the higher the concentration of acetic acid solution used it produces a high yield. This is because acetic acid functions to hydrolyze collagen in fish skin so that the structure of gelatin is more easily broken down during the extraction process (Lombu*et al.*, 2015).

Acetic acid in milkfish skin gelatin serves to hydrolyze collagen on fish skin so that the structure of gelatin is more easily broken down during the extraction process. According Zhou and Joe (2005), The higher the concentration of acetic acid given the greater the yield value produced. This is due to the influence of H + ions on acetic acid which hydrolyzes collagen from the triplehelixs chain into a single chain. this tendency reaches its limit if excessive H + ions hydrolyze collagen further so that physical and chemical changes occur

IV. Conclusion

The results of this study obtained the best results at 8% treatment with Gel strength 98.07 g / bloom, Viscosity 6 cP, yield 19.4%. Use of high concentrations of acetic acid can decide which amino acids have been formed so that the amino acid chain of the damaged gelatin causes the gel strength to decrease

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