The Effect Of Different Acetic Acid Concentration On The Quality Of Tuna Fish Skin Collagen

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Abstract: The purpose of this study was to determine the effect of the concentration of acetic acid on yield, moisture content, melting point, protein profile and microstructure of the best Tuna fish skin collagen and control. Observation and recording of water content, melting point, electrophoresis and SEM parameters were carried out after the analysis process was completed. This activity is carried out on all test parameters in each test, where the repetition is done four times. The research method used was descriptive, each with four treatments and four replications. From the results of the study several conclusions can be drawn: (1). In applying different concentrations of acetic acid it has a very significant effect on yield, significantly influences of water content, melting points and is supported by electrophoresis results; (2) Based on the Effectiveness Index, the best treatment was obtained at 0.7 M acetic acid concentration; and (3). Collagen fibers form at concentrations of 0.9 M which are thin and many holes. At a concentration of 1.7 M was thick and solid fibers are formed.

Index Terms: acetic acid, collagen, acetic acid, SEM, electrophoresis

1. INTRODUCTION

Fish skin is one of the wastes produced from the processing of fish fillets, for example skinless fillets from tuna [1]. Fish skin consists of three layers, namely the epidermis layer, corium layer (dermis) and the hypodermis layer (subcutis) [2]. According to Borgstorm [3], the dermis consists of several layers of collagen fibers arranged in parallel, holding back the growing scales. The basic membrane content is not much and the hypodermis is thin but grows well and contains loose connective tissue. Mendivil et al. [4], added that the dermis layer is a main part of the skin composed of binding tissue fibers such as collagen, elastin and reticule. Tuna has a skin that is rich in collagen [5]. Hashim et al. [6], state that the dermis layer of the skin is a thick binding tissue that contains a number of collagen fibers. Therefore, an alternative processing for tuna skin is to make collagen products that can be used as food additives in the form of gelatin making materials that are as emulsifiers and stabilizers, whereas in non-food products can also be used in the pharmaceutical, medical, cosmetics and other fields. The process of making collagen with skin sources and pig and cow bones as well as cones and cod has been carried out by researchers to determine the type and concentration of ingredients and for optimal deposition with this type of immersion / precipitation is an acid solution. Acid solutions are used such as acetic acid, nitric acid or hydrochloric and alkaline acids such as ammonium sulfate in the alkalization process. In this study acetic acid was used because according to Asghar and Hendrickson [7] and Liu et al [8], stated that the use of acetic acid solutions can develop collagen as much as 50% more than HCl.

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In previous studies, the results of the results are not yet known, whereas in preliminary studies that have been carried out using acetic acid with a concentration of 0.5 M made from raw shark skins yielding results of around + 12%. For higher concentrations of acetic acid, a clearer picture has not been obtained.

2. MATERIALS AND METHOD

The material used in this study was tuna skin waste from PT. "Kelola Mina Laut", Gresik regencies. Tuna skin was cleaned of meat that is still attached using clean water, then as much as 50 grams and cut into small pieces and put in a 500 ml beaker glass. Fish skin was replaced with 1.4 M NaCl 3 times, 10 minutes at 16 °C until it looked clear for each. Extracted with 400 ml of 0.5 M acetic acid for around 16 hours at 16 °C (1 gram of skin per 8 ml of acetic acid), then this is added with 1.5 M of NaCl as much as 400 ml, then cold centrifuged at 4 ° C with 5000 rpm for 15 minutes. Research using experimental methods [9]. Quality analysis of Tuna fish collagen consists of yield (%), water content (%), melting point (°C), electrophoresis, and SEM (Scanning Electron Microscopy), which are carried out at Universitas Brawijaya Malang. Different concentrations of acetic acid were used with concentrations of 0.3M; 0.5M; 0.7M; and 0.9M in this study.

Electrophoresis

Electrophoresis is the separation of molecules based on their charge which has the principle that the number of biological molecules depends on the pH and composition of the medium in which the biological molecules dissolve. When in the electric field, positively charged biological molecules migrate to negative electrodes and vice versa. The principle of electrophoresis is the attraction of particles or ions to electrodes with opposite charges in the electric field. Negatively charged anions will migrate to the anode (positive electrode), while positively charged cations migrate to the negatively charged cathode. In principle, electrophoresis is that charged molecules and particles will move towards electrodes that have opposite charges under the influence of an electric field. The rate of migration of charged molecules towards negatively charged electrodes is called Westermeier electromobility

[10].

Scanning Electron Microscopes

The principle of scanning electron microscope (SEM) is to place a membrane sample in a thin electron beam containing 1-25 kV kinetic energy. The electrons that hit the membrane are called the main electron (high energy), and the reflection is called the second electron (low energy). The second electron is not reflected but is released from the atoms in the air, electrons determine the shadow (image) seen on the micrograph screen [11].

4. RESULT

The results of water content analysis and melting point of Tuna fish skin collagen are listed in Table 1. SEM and electrophoresis analysis are shown in Figures 1, 2 and 3.

Table 1. *The average value of research results*

Treatment (Molar)	Yield (%)	Water content		elting point (ºC)
		Wb (%)	db (%)	
0,3	14,685	84,79293	539,578	32,75
0,5	20,4125	85,64096	584,1511	33,75
0,7	27,85	87,96469	710,9506	38
0,9	14,06	85,89632	596,5725	33

5. DISCUSSION Yield

From Table 1 above have shown that the lowest collagen yield at 14.06% of acetic acid concentration of 0.9 M, while the highest value was obtained from the treatment of acetic acid concentration of 0.7 M with a yield of 27.85%. The addition of acetic acid concentration tends to reduce the yield produced. The yield produced decreases dramatically when the concentration of acetic acid is added to 0.9 M. This is due to the amino acid peptide bonds of the collagen undergoing denaturation and degredation. The denaturation and degradation of the constituent components of collagen cause a number of collagen to dissolve and be wasted during precipitation, resulting in less. The result of dissolution of collagen is characterized by changes in thickness of the immersion solution. The higher the concentration of acid, the thicker thesoaking solution. Allegedly a thicker soaking solution means more collagen is dissolved and eventually wasted. According to Waheed [12], if the acid concentration or duration of demineralization is increased, many collagen fibrils are damaged so that the dissolved collagen is high enough during the immersion period. Decomposition of proteins due to breaking of hydrogen bonds and the structure of collagen coils by the higher concentrations causes the yield produced decreases. This is supported by Blanco et al. [13], where collagen is one of the binding tissue proteins, which are composed of amino acids that make up proteins. As a protein, collagen can be damaged by chemical, acidic or basic reactions and by heat, even in certain pH solutions the protein can be denatured and precipitated.

Water content

From Table 1 above has shown that the highest water content of 87.96469% in the 0.7 M of acetic acid, while the lowest water content of 84.79293% was obtained in the treatment of 0.3M. The optimal concentration at 0.7 M and tends to increase the water levels with increasing concentrations of acetic acid. When extracting with a concentration of 0.3 M acetic acid and continued to 0.7 M, there was an increase in the water content of the skin of tuna fish. This happens because soaking water (acetic acid) continues to fill part of the tuna skin until the volume expands until it reaches equilibrium between the soaking water and the water in the tuna skin. According to Civera and Parisi [14], the ability of meat and skin to bind water is caused by muscle protein and fibers, especially actomyosin, the main component of myofibril. Manjula,.Jayamanne, and Thushari [15] also confirmed that when the tuna skin is soaked the osmosis events will occur (osmotic pressure), where this process takes place due to different concentrations, from high concentrations to low concentrations. This is what causes the absorption of water into the skin when immersed. The addition of concentrations of acetic acid reach to 0.9 M, causes a decrease in water content even though in an insignificant amount. Increasing the concentration of acetic acid will cause accumulation of water in the tuna skin fibers due to continuous water penetration so that the free water in the skin of tuna fish more and more. This is supported by Martin et al. [16], that the hydrolysis of collagen is an event of the breakdown of amino acid covalent bonds with the addition of water by acid, base or enzyme catalysts. With the breaking of amino acid bonds by too high concentrations of acetic acid, the water content will decrease.

Melting Point

From Table 1 above have been shown that the lowest collagen melting point at 32.75 ºC at 0.3 M acetic acid concentration, and the highest at 0.7 M with a melting point value of 38ºC. The decrease in melting point after passing the optimum extraction concentration limit is probably caused by structural changes in collagen. This is thought to be caused by the lack of proline and hydroxyproline content of collagen produced, causing a decrease in melting point due to lack of hydrogen bonds of collagen in solution. Eberhardt [17] explained that hydrogen bonding is a stabilizer of polypeptide chains that bind to triple helix. Bond instability will reduce the melting

point of collagen.

Scanning Electron Microscopy

From the SEM photographs conducted can be seen that the differencies of collagen with low concentration and high concentration of acetic acid (Figure 1). In the photo can be seen the long and no string of fibers. This is because the use of low concentrations will cause the fibers from the collagen to elongate and no one breaks or clots. In contrast to the use of high concentrations or highest extraction (Figure 2). With high concentrations it is expected that the extracted collagen will be more numerous and the yield produced will be even more. But there is a consequence of using high concentrations, in which many collagen fibers will be broken and many will clot. Although there are still many elongated fibers [18].

Electrophoresis

The basic principle of electrophoresis is on the presence of proteins in solution. At pH above and below the isoelectric point in the electric field, the protein will move in the direction of the charge opposite the charge of the protein itself. Protein molecules of the same type will move at the same speed in solution. According to Lehninger [19] at a certain pH the protein mixture will contain several groups which are positively charged, negatively charged and some which are uncharged. If placed in an electric field the positively charged protein will move towards the negative electrode and the negatively charged protein will move towards the positiveelectrode, while the uncharged will remain. In addition, protein molecules with relatively high charge densities move towards the electrodes faster than proteins with low densities. Electrophoresis usually requires a buffer media as a place to migrate biological molecules. Polyacrylamide gels can be used for protein electrophoresis and peptides. Polyacrylamide is formed from polymerization of acrylamide monomer structure. Polyacrylamide gels form a 3-dimensional structure, and their concentration in solution will affect the gel's molecular size, elasticity and mechanical strength. Polyacrylamide can separate proteins with a molecular weight range of 500-250,000 Dalton or polynucleotides in the range of 5-2000 base pairs [20].

5. CONCLUSION

The conclusions that can be drawn from this study are: (1). the application of different concentrations of acetic acid it has a very real effect on yield, water content, melting point and is supported by electrophoresis results; (2). Based on the Effectiveness Index, the best treatment was obtained at 0.7 M acetic acid concentration with a yield value of 27.85%, water content of 87.96%, melting point of 38ºC.

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