

Effect Of Demineralization With Sulfuric Acid On Yield, Gel Strength, Viscosity, And Amino Acids Of Gelatin Extracted From Fish Bones Lencam (*Lethrinus lentjan*)

Janet Bela Nor Malita *, Eddy Suprayitno **

* Department of Fisheries Technology
Faculty of Fisheries and marine science
Brawijaya university
Indonesia
janetbela014@gmail.com

** Department of Fisheries Technology
Faculty of Fisheries and marine science
Brawijaya university
Indonesia
eddysuprayitno@ub.ac.id

DOI: 10.29322/IJSRP.9.06.2019.p9032
<http://dx.doi.org/10.29322/IJSRP.9.06.2019.p9032>

Abstract- Gelatin is a protein that is hydrolyzed from collagen. Gelatin is an easily digestible protein, containing all essential amino acids except tryptophan. Gelatin can be obtained from the skin and bones of animals. The highest production of gelatin is sourced from cows and pigs. Because some religions prohibit the consumption of foods that containing cows or pigs. For this reason, it is necessary to have an alternative source of gelatin which is lawful and can be consumed by all people. One alternative source of halal gelatin is lencam fish bone waste (*Lethrinus lentjan*). Making the gelatin from this fish bone can be done by soaking (demineralization) using acid. One of the acid solution that can be used is sulfuric acid (H₂SO₄). Soaking with acid solution can convert the triple helix structure into a single helix on the tropocollagen. Demineralization in this study using 5% sulfuric acid for 12, 24, and 36 hours. Gelatin extracted from *Lethrinus lentjan* bones yielded higher 14, 6%, gel strenght hinger 368 bloom, and viscosity higher 6,2cP. The higher amino acid in this study is Gly (Glycine) 18,29%. Generally there are 15 kinds of amino acids contained in gelatin. Amino acids composition determined by HPLC (*High Performance Liquid Chromatography*).

Keyword: *Gelatin, demineralization, yield, gel strenght, viscosity, amino acids*

I. INTRODUCTION

Gelatin has the molecular formula C₁₀₂H₁₅₁N₃₁O₃₉. Gelatin is a protein composed of several amino acids, at least there are 18 amino acids making up gelatin, including: glycine, alanine, phenylalanine, isoleusine, methyonine, and others¹. Gelatin is a natural product because it can be obtained through partial hydrolysis of collagen from the skin and bones of animals. Gelatin is a derivative of protein derived from collagen fibers found in cartilage². Gelatin is a type of protein derivative from collagen fibers that can be obtained from extraction from bone. The character of gelatin is very unique as it has the ability to turn the shape of the sol into a gel, is amphoteric and maintain colloidal properties. Gelatin is usually used for food processing, microbiological media, and cosmetics³.

The process making gelatin consist of 3 steps, degreasing, demineralization, and extraction. Demineralization is one of most important process in making gelatin. The demineralization process is the process of soaking in an acid solution to continue the swelling of the bones so collagen in the bones easily comes out. Demineralization aims to remove calcium and other salts to obtain ossein (bone that has undergone demineralization, namely calcium removal)⁴. The quality of gelatin can be seen from yield, gel strength, viscosity and amino acid profile⁵.

Yield is one of the most important parameters to determine the effectiveness process of the gelatin making. Gel strength is one parameter to determine the physical quality of a gelatin product⁶. Viscosity is one of the requirements in determining the feasibility of using gelatin for industrial use. Viscosity is one of the important rheological properties in food products. Viscosity is needed to test the quality, control quality and control process as long as needed⁷. The quality or physical chemistry of gelatin is basically determined by

amino acids making up the gelatin molecule⁸. The chemical quality of gelatin is a protein composed of amino acids proline, glycine and hydroxyproline⁹

II. MATERIALS AND METHOD

Materials needed are lencam bones (*lethrinus lenjan*) that was obtained from PT. Alam Jaya Surabaya, sulfuric acid (H₂SO₄), aquadest, aluminum foil and calico fabric.

In this study using an experimental method. The treatments are used in this study is variation of the times demineralization with sulfuric acid solution (H₂SO₄). The experimental design used in this study was a completely randomized design with 3 treatments and 5 replications. The variation of the times demineralization used were 12, 24, and 36 hours. The process of making gelatin have a three steps, degreasing, demineralization, and extraction.

Degreasing process is done by boiling fish bones in boiling water at 100°C for 15 minutes. Then meat on the fish bones is cleaned. And then the bone is cut about 2-4 cm. **Demineralization** carried out by soaked fish bones in a 5% sulfuric acid solution for 12, 24 and 36 hours. After demineralization, the bone will increase swelling called ossein. This ossein then washed in running water to neutralize the pH. Neutral ossein is added distilled water and extracted. **Extraction** gelatin doing in waterbath at 70°C for 6 hours.

III. RESULT

Results of the study showed that the longer demineralization caused the yield, gel strength, and viscosity increase. The highest yield was 14,6% , the highest gel strength was 368 Bloom or 9,2 N and the highest viscosity was 6.2 cP at 36 hours demineralization. While the lowest yield was 11,2%, the lowest gel strength was 256 Bloom or 6,4 N and the lowest viscosity was 2.0 cP at demineralization 12 hours. The results of the amino acid profile test showed that the highest amino acid of lencam bones gelatin was Glycine at 18.29% and the lowest was L-Tyrosine at 0.48%. The result of yield, gel strength, and viscosity presented in Figure 1. The result of amino acids can seen in Table 1.

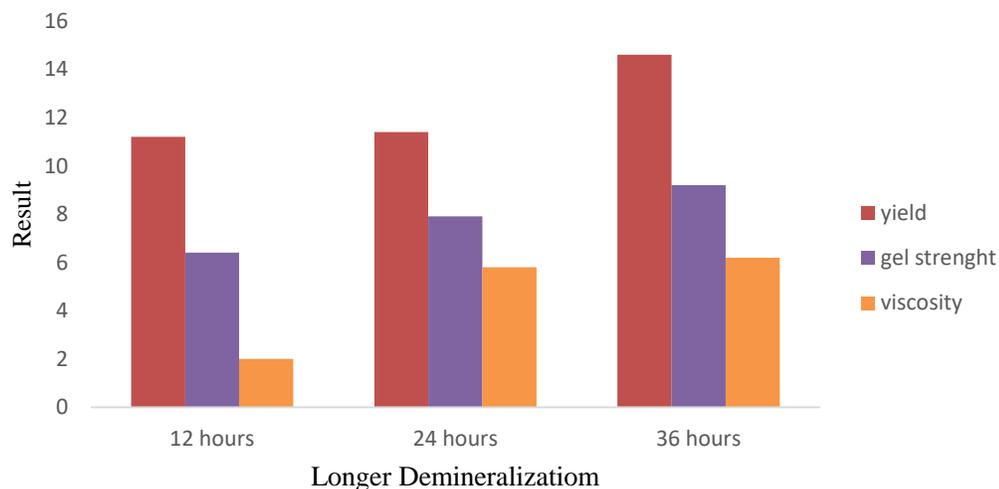


Figure 1. The Result of Yield, Gel Strength, and Viscosity

Table 1. Amino Acids Profile

No	Parameter	Unit	Result
1	L-Serine	%	2,86
2	L-Glutamic Acid	%	6,70
3	L-Phenylalanine	%	1,94
4	L-Isoleucine	%	0,85
5	L-Valine	%	1,61
6	L-Alanin	%	6,99
7	L-Arginine	%	6,25
8	Glysin	%	18,29
9	L-Lysin	%	2,52
10	L-Aspartic Acid	%	3,46

11	L-Leusin	%	1,90
12	L-Tyrosin	%	0,48
13	L-Proline	%	8,31
14	L-Threonin	%	1,99
15	L-Histidine	%	0,67

IV. DISCUSSION

Yield is the one of the important parameters and properties in making gelatin. The resulting yield value determines the efficiency and effectiveness of the raw material extraction process in gelatine making. The gelatin yield was calculated based on the weight of the gelatin per weight of the cleaned fish bone¹⁰. In this study, yield showed differences in each times demineralization. At 12 hours demineralization showed the yield was 11,2%, at 24 hours the yield was 11,4%, and 36 hours the yield was 14,6%. The highest yield was obtained at 36 hours demineralization. The yield if compared with comercial gelatin is higher, it was 8,68%¹¹.

Yield of gelatin extracted from fish bones *Lethrinus lentjan* increased with the lenght of the demineralization. Sulfuric acid is a strong acid so that it can increase the yield of gelatin compared to using weak acids. Selected acid solution for demineralization process can affect the yield obtained. Acid solutionn can affect swelling of cartilage determines the presence of protons from acids that enter the cartilage structure that replaces minerals or the presence of empty space in the tropocollagen. The H+ ion from the acid will involve the carboxyl gropu so that it can disrupt the bond between molecular molecules that can affect the yield rate produced¹².

Gel strenght is one of the aspects needed to determine the quality of gelatin. Gelatin can be assessed from various aspects, including gel strenght and viscosity. The higher gel strenght and gelatin eviscosity can increasing the gelatin quality¹³. Gel strenght in this study showed differences in each time demineralization. At 12 hours demineralization showed the gel strenght was 6,4N or 256 Bloom, at 24 hours the gel strenght was 7,9N or 316 Bloom, and at 36 hours the gel strenght was 9,2N or 368 Bloom. The highest gel strenght was obtained at 36 hours demineralization. The result of gel strenght in this study is according to the standards *Gelatin Manufacturing Institue of America* (GMIA), 2012¹⁴.

Gel strenght of gelatin extracted from fish boned *Lenthrinus lentjan* increased with the lenght of the demineralization. The acid treatment is able to degrade collagen molecules to asimple level, being able to transform the structure of the triple helix protein into single chain. Gel strenght depends on the amino acid chain lenght. If the condition of the collagen has been hydrolyzed to a simple level, the strenght of the gel can increase. Hydrolyzed collagen can produce a long polypeptide chain¹⁵.

Viscosity of gelatin using the acid method has a higher value. The right extraction method will affect the short lenght of the formed α -helix amino acid chain. If the α -helix amino acid chain is long can cause the molecular weight of the gelatin increase, then the flow rate will obstructed. If the flow rate is increasingly inhibited, the value of the gelatine viscosity gets bigger¹⁶. Viscosity in this study showed differences in each lenght demineralization. At 12 hours demineralization showed the viscosity was 2 cP, at 24 hours the viscosity was 5,8 cP, and at 36 hours viscosity was 6,2 cP. The highest viscosity was obtained at 36 hours demineralization. The result of viscosity in this study is according to the standards *Gelatin Manufacturing Institue of America* (GMIA), 2012¹⁴

Viscosity in this study increased with the lenght of demineralization. The difference value of viscosity can be caused by the extraction process and the composition of the raw materials used where each material has a different level of cross-linking strenght of the tropocollagen as well age, genetic, and environmental factors. The weak cross link causes collagen to be easily hydrolyzed, this hydrolysis can reduce the molecular weight of gelatin. This is causes reduce the viscosity of the gelatin¹⁷.

Amino acid profile analysis can provide important information about the composition of essential and non essential amino acid as well as to show the overall amino acid composition which ca affect the flavor characteristics of the samples analyzed¹⁸. The highest amino acids in this study is glycine, obtained 12,89%. And the lowers amino acids is L-Tyrosine was 0,48%. The most important amino acid in gelatin is glycine, proline, and hydroxyproline.

The main amino acids that obtained in gelatin are glycine, proline. And hydroxyproline. Glycine and proline are the main amino acids making up gelatin. Gelatin contains 9 of the 10 essential amino acids needed by the body, one essential amino acid which is almost not contained in gelatin is triptopan. The amino acid composition causes gelatin as a multi-using material in various industries¹³.

V. CONCLUSION

The best gelatin extracted from fish bones *Lethrinus lentjan* obtained at 36 hours demineralization. The result is yield was 14,6% , the gel strenght was 368 Bloom or 9,2 N and the viscosity was 6.2 cP. For the amino acids in this study, the highest amino acids is glycine was 18,29%.

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AUTHORS

First Author – Janet Bela Nor Malita, Department Of Fisheries Technology, Faculty Of Fisheries And Marine Science, Brawijaya University, janetbela014@gmail.com .

Second Author – Eddy Suprayitno, Department Of Fisheries Technology, Faculty Of Fisheries And Marine Science, Brawijaya University, eddysuprayitno@ub.ac.id.