The Production of Gelatin from Snapper Scales *(Lutjanus camphecanus Sp.)* through Enzymatic Pretreatment

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***Abstract*– Fish wastes such as fish bones and scales have a potential to be used as a gelatin source. It can be used as an alternative material to replace pork or bovine which ordinarily conflicted with religion issue. Gelatin from fish has different characteristics compared with mammalian source. It has several advantageous properties such as high amount of hydrophobic amino acids, less proline and hydroxy proline, lower gelling ability, and melting point. In this study, gelatin was produced from fish waste especially snapper scales using the bromelain enzyme at the pre-treatment stage to improve its characteristic. The snapper scales soaking in hot water at 80ºC for about 30 minutes to remove the fat which attached the scales. A pre-treatment has been conducted using bromelain enzyme solution at various concentrations of 1%, 2%, 3%, 4% and 5% for 6 hours to remove non-collagenous proteins and loosen the bonds of the fish scales. The next step is demineralization using a 15% concentration of citric acid solution for 3 days. The ossein formed in the demineralization stage was then hydrolyzed at 50ºC and 70ºC for 9 hours with stirring at 300 rpm. The best gelatin yield was obtained from the pre-treatment of bromelain enzyme solution with a concentration of 3% at a hydrolysis temperature of 70ºC with a yield of 9.13%, pH value of 3.86, water content of 8%, ash content of 0.80%. FTIR spectra shows the presence of functional groups correspond to gelatin formation, such as carbon, hydrogen, hydroxyl group (O-H), carbonyl group (C=O), amine group (N-H) and alkene group (C=C). The heavy metal content test showed that the gelatin contained no heavy metal content of lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As).**

***Keywords*– Bromelain enzyme; Gelatin; Hydrolysis; Snapper scales**

I. INTRODUCTION

Indonesia is a maritime country which has a great potential of marine resources, especially fish. It is widely used as the main menu of *seafood* restaurants in Indonesia. Therefore, it produces many byproducts of sea fish waste which not utilized optimally and cause environmental pollution. Fish scales consist of ash water content 39%, fat 5%, protein 30%, and carbohydrates 15% on the scales of dried sea fish [1]. Therefore, it highly potential as a raw material for gelatin [2].Gelatin is a denaturate protein derived from collagen through hydrolysis process. It is mostly utilized in photographic, pharmaceutical and food industry.

In 2002, Indonesia import gelatin around to 2,144 tons. The value increase in the next year by 6,233 tons. This showed that the gelatin consumption increases, and Indonesia has potential to produce gelatin independently due to the abundant of gelatin resource. Almost 90% of the imported gelatin is produced from pig skin, cowhide, and cow bone. However, gelatin from pig skin has conflicted with the religion issue. In recent years, several research have produced gelatin from halal and hygienic resources which acceptable to various religions in Indonesia. The resource is mostly from fish waste [3].

Gelatin extraction from collagen involves several steps, such as alkaline and/or acid pre-treatments for collagen hydrolysis, then followed by the main extraction in water at 318 K or 45ºC. There are two types of gelatins which are type A and type B. In gelatin type A soaking of raw materials is carried out in an acid solution otherwise known as an acidic process. While in type B, the salting of raw materials was conducted in an alkaline solution or known as alkaline process. Gelatin from fish waste is classified as gelatin type A. Acid processes are preferred over alkaline processes because they are considered more economical because immersion in acid processes is relatively shorter than alkaline processes [1].

The physical characteristics of gelatin are influenced by the source of gelatin and extraction methods. Gelatin extraction consists of two stages, namely pre-treatment and main extraction. The pre-treatment stage uses strong acids or strong bases solution. Extraction generally uses a mixture of alkaline acids and water. Today the use of citric acid and water as solvents is preferred in industrial process[4]. Gelatin from fish scales has different properties compared to mammalian gelatin. Gelatin which produces from coldwater fish has several good properties such as high in hydrophobic amino acids, low in proline and hydroxy proline, lower gelling ability, and lower melting point than mammalian gelatin. Therefore, the production of gelatin from by-products requires proper selection and optimization of pre-treatment and extraction to obtain good physical and chemical properties of gelatin. In this research, the novel pre-treatment treatment using the bromelain enzyme has been conducted in the production of gelatin from snapper scales. The use of bromelain enzymes in the pre-treatment process is useful for removing non-collagen proteins and removing interfering tissue before demineralization using chemical solutions.

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II. METHOD

The materials used in this study were dried snapper scales, aquadest, citric acid (Merck), and bromelain enzyme (Protease). All the chemicals were directly used without further purification.

Furthermore, the first stage is degreasing by adding 800 mL hot water at 80ºC into 50 grams snapper scales for 30 minutes. Then, Pre-Treatment Phase Immersion with Bromelain Enzyme, snapper scales soaking by 0%, 1%, 2%, 3%, 4% and 5% bromelain enzyme solution for 6 hours. At demineralization stage, snapper scales soaking by 6% citric acid solution. A snapper scale ratio to solvent is 1:6 (weight/volume). Snapper scales have been soaked into solvent for three days. Then ossein was washed with aquadest until neutral pH (pH 6-7). Ossein hydrolyzed with aquadest at 50ºC and 70ºC for 6 hours) with stirring speed of 300 rpm. Gelatin was dried at 55^0C for 24 hours so until solid formed.

Some analyses have been conducted to the gelatin product which are yield, pH, water content, ash content, heavy metal content, and Fourier Transform InfraRed (FTIR).

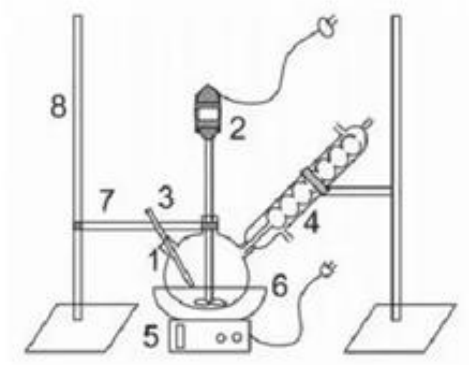

Figure 1. Scheme of hydrolysis instrument

1. Three neck rounded Flask
2. Stirrer
3. Thermometer
4. Condensor
5. Control of waterbath
6. Waterbath
7. Clamp
8. Stative

III. RESULT

Yield is one of the important parameters in the gelatin production to determine the effectiveness of the method. The efficient and effective level of the extraction process of raw materials used in the manufacture of gelatin can be seen from the yield. The greater the yield, the more efficient the treatment of the method used [5]. The results of the yield obtained in this study can be seen in Table 1 and Figure 1.

TABLE 1
YIELD OF GELATIN SCALES SNAPPER WITH BROMELAIN ENZYME IMMERSION

Concentration of Bromelin Enzymes (%)	Yield 50ºC (%)	Yield 70ºC (%)
0	3.08	7.85
1	3.7	9.54
2	2.85	9.26
3	5.06	9.13
4	2.8	7.46
5	3.1	5.28

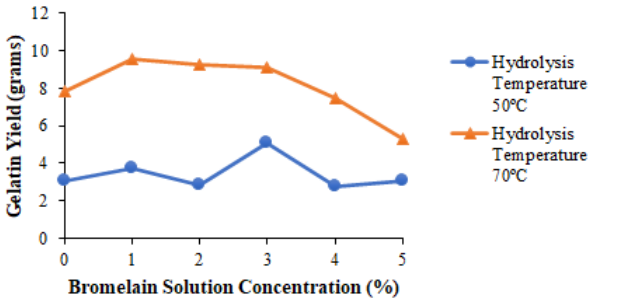

Figure 2. Effect of Bromelain Enzyme Concentration on The Yield of Gelatin produce from Snapper Scales

Based on Table 1 gelatin yield at various concentration of bromelain enzymes 0%, 1%, 2%, 3%, 4% and 5% at hydrolysis temperatures 50ºC are 3.08%, 3.7%, 2.85%, 5.06%, 2.8%, and 3.1% consecutively. At 70ºC, sequentially 7.85%, 9.54%, 9.26%, 9.13%, 7.46, and 5.28%. Figure 2 shows that the concentration of bromelain enzyme solution affects the yield, at hydrolysis temperatures 50ºC and 70º C have the same fluctuating value. At the concentration of bromelain enzymes 0% and 1% tend to increase but decreased at the 2% and experienced a rise back at the concentration of 3% and decreased at 4% and 5%.

The fluctuating yield correspond to the higher the concentration of enzymes affected the more peptide bonds are hydrolyzed. The yield resulting from the reaction between the substrate and the enzyme is affected by the enzyme concentration. In the existence of enzyme concentration increases while the concentration of the substrate remains constant, the concentration or number of enzyme molecules is lower than the number of substrate molecules to be catalyzed, so the resulting product will be proportional to the number of substrates converted by enzymes into products. When the number of enzymes increases then more substrates will be converted into products so that at some point the number of enzymes is excess, but the substrate runs out. As a result, the addition of enzymes cannot change the yield of reactions to enzyme concentration [5].

The difference amount of this yield could be due to the difference in the brand of citric acid used because it could be that the citric acid content in each brand is different. The above results show no constant increase or decrease in yield resulting in any enzyme concentration. So, it can be said that in these circumstances the substrate has been catalyzed so that the Figure 2 obtained does not show an increase in yield results. The highest yield obtained was 9.54% with the treatment of enzyme concentrations of 1% with a temperature of 70^0C.

A. pH Analysis

The degree of acidity (pH) is one aspect of the parameters applied in determining the quality standards of gelatin. pH value measurement is important because the pH of the solution affects other gelatin properties such as viscosity, gel strength and the application of gelatin [6].

The first pH testing step is 0,2 gram of gelatin powder dispersed in 20 mL aquadest at a temperature of 80ºC, then samples homogenized with magnetic stirrer, then measured the degree of acidity (pH) at room temperature with pH meter. The results of the pH test can be seen in Table 2.

TABLE 2
PH TEST RESULTS OF SNAPPER SCALES GELATIN WITH BROMELAIN ENZYME IMMERSION

Bromelain Enzyme Solution Concentration	pH		SNI	
	50ºC	70ºC	Min	Max
0	3.93	3.84	3	6
1	3.94	3.83	3	6
2	3.95	3.93	3	6
3	3.86	3.86	3	6
4	4.48	3.94	3	6
5	3.95	4.08	3	6

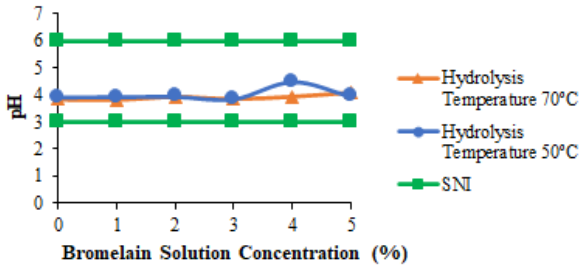

Figure 3. Effect of Bromelain Enzyme Concentration on pH of Gelatin

From Table 2 it can be seen that the pH value in gelatin with the concentration of bromelain enzymes 0%, 1%, 2%, 3%, 4% and 5% with hydrolysis temperatures of 50ºC is consecutive at 3.93; 3.94; 3.95; 3.86; 4.8; 3.95, and for hydrolysis temperatures 70ºC in a row is 3.84; 3.83; 3.93; 3.86; 3.94; and 4.08. The pH value tends to fluctuate, but all of them show an acidic range. This is due to the demineralization process of snapper scales using citric acid and the remains citric acid carried away during hydrolysis, thus affecting the pH of the gelatin produced [7].

The pH value of gelatin is also influenced by the type of extraction material. Extraction of gelatin from fish carried out in acidic condition will produce type A (acid), while type B (base) produce at the base condition or a mixture of acids and bases. Extraction using a neutral solution, or a weak acid can keep the polypeptide chain bonds from being damaged [8].

The pH value is one indicator that affects gelatin. Gelatin with neutral pH is applied to meat products, pharmaceuticals, chromatography, paints and so on. Gelatin with low pH is used for juice products, jelly, syrup and so on. The pH value of gelatin is strongly influenced by the type of soaking solution used to extract the gelatin [9].

B. Water Content Analysis

Moisture content is the water content of the material which can be expressed based on the wet and dry weight. Water content can affect the appearance such as texture, taste, and food ingredients. Water content of gelatin will affect the shelf life because it is closely related to metabolic activities that occur during storage of gelatin such as enzyme activity, microbial activity, and chemical activity, the occurrence of rancidity and reactions non-enzymatic, causing changes in organoleptic properties and quality values [10].

TABLE 3
RESULTS OF GELATIN WATER CONTENT TEST SNAPPER SCALES WITH BROMELAIN ENZYME IMMERSION

Bromelain Enzyme Solution Concentration	Water Content (%)		SNI
	50ºC	70ºC	Maximum (%)
0	10	12	16
1	10	12	16
2	12	8	16
3	8	8	16
4	10	10	16
5	10	12	16

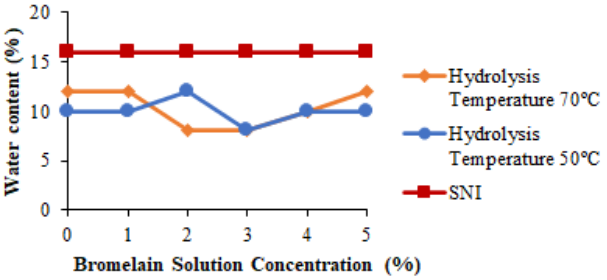

Figure 4. Effect of Bromelain Enzyme Concentration on Water Content of Gelatin

Based on Table 3 gelatin water content at temperature 50ºC bromelain enzyme concentrations 0%, 1%, 2%, 3%, 4% and 5% are consecutively 10%; 10%; 12%; 8%; 10%; and 10% and at temperature 70ºC concentrations of bromelain enzymes 0%, 1%, 2%, 3%, 4% and 5% are respectively 12%; 12%; 8%; 8%; 10%; and 12%.

The water content decreased as the concentration of the bromelain enzyme solution increased in the pre-treatment stage. The hydrolysis process requires water so that it can affect the water content in a material because of the binding between the enzyme and the substrate which is strongly influenced by the presence of hydrogen bonds.

Based on Table 3, it shows that the results of the analysis of the water content in the gelatin of snapper scales are in accordance with the Indonesian National Standard (SNI) 01-3735-1995, which is a maximum of 16%. The water content contained in gelatin will affect the resistance of gelatin to microbial attack [11].

The water content of gelatin is not affected by temperature, but is influenced by several factors, including the type of solution, the source of gelatin, and the drying and storage process prior to analysis [12].

C. Ash Content Analysis

Ash is an inorganic substance left over from the combustion of an organic material in food. Ash content will determine the purity of a material. The high or low ash content of a material is caused by the mineral content present in the raw material [6].

TABLE 4

TEST RESULTS OF ASH GELATIN SCALES SNAPPER WITH BROMELAIN ENZYME IMMERSION

Bromelain Enzyme Solution Concentration	Ash Content (%)		SNI
	50ºC	70ºC	Maximum (%)
0	2.80	1.00	3.25
1	2.45	0.90	3.25
2	3.20	0.90	3.25
3	3.03	0.80	3.25
4	3.16	1.20	3.25
5	3.14	1.40	3.25

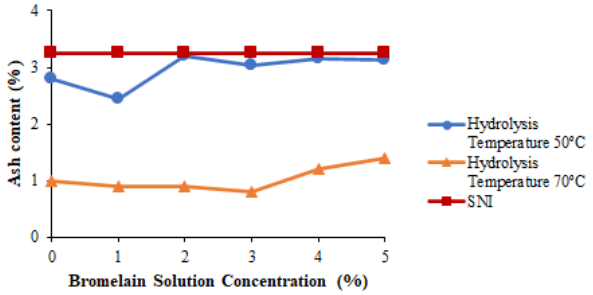

Figure 5. Effect of Bromelain Enzyme Concentration on Ash Content of Gelatine in Snapper Scales

From Table 4 it can be seen that the ash content value of gelatin with the treatment of bromelain enzyme concentrations of 0%,1%, 2%, 3%, 4% and 5% at hydrolysis temperatures of 50ºC in a row is 2.80%,2.45%, 3.2%, 3.03%, 3.16%, and 3.14% respectively, and for hydrolysis temperature treatment 70ºC were 1%, 0.90%, 0.90%, 0.80%, 1.20%, and 1.40%.

The ash content of gelatin is due to the presence of mineral components bound to collagen that have not been released during the demineralization and washing processes, so that they are extracted and carried to the produce gelatin. The ash content in gelatin is indicated by the presence of calcium minerals and other inorganic minerals. Even so, the gelatin ash content of snapper scales produced has met the requirements of SNI No. 06-3735 (1995) which is a maximum of 3.25%.

Ash content is one of the parameters used to see the quality and success rate of the extraction process on gelatin. The stages of the gelatin pre-treatment process carried out to remove minerals in snapper scales, so as to produce ossein which is then extracted into gelatin. This study shows that the pre-treatment process went quite well because it was stirred using a shaker during the pre-treatment, so that not a lot of minerals were carried when filtering gelatin [6].

D. Heavy Metal Content Analysis

One of the food safety requirements for gelatin products is that the heavy metal content should not exceed the permissible limit (Said, 2011). Metal content test on gelatin has been conducting using XRF method which shows at Table 5.

TABLE 5

HEAVY METAL CONTENT IN GELATIN SCALES SNAPPER VARIABLE CONCENTRATION ENZYME BROMELAIN 3% AND WITH A TEMPERATURE OF 50ºC

Metal Type	Gelatin Snapper Scales	SNI
	mg/Kg	Maximum (mg/Kg)
Mercury (Hg)	Not Detected	50
Lead (Pb)	Not Detected	50
Cadmium (Cd)	Not Detected	50
Arsenic (As)	Not Detected	2

Table 5 shows the levels of heavy metals contained in the gelatin of snapper scales. Heavy metal analysis in gelatin was carried out to determine the safety of gelatin in consumer products. Heavy metals lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) are contaminants that are harmful to humans if they exceed the specified limits. Based on the results of the heavy metal test, the gelatin of snapper scales showed that the heavy metal content of lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) was not detected in the gelatin. These results meet the gelatin quality standards set by SNI No 06-3735 (1995).

E. FTIR Analysis

Characteristics of the molecular structure is one of the important parameters in the isolation of gelatin. To find out whether the extraction process has been running optimally. The analysis used to determine the characteristics of the typical molecular structure of gelatin is FTIR. This FTIR analysis was carried out to determine whether the isolation results were really in the form of gelatin.

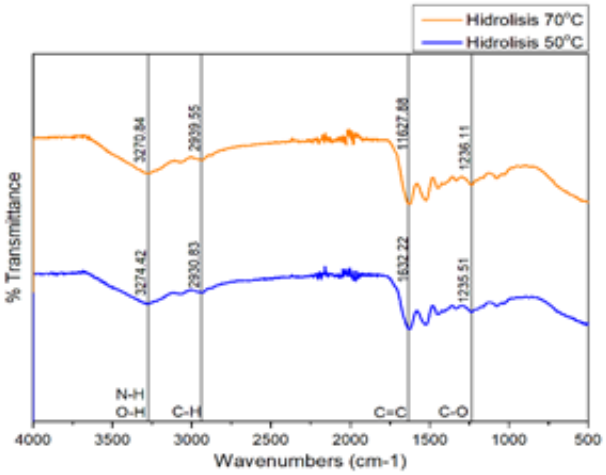

Figure 6. FTIR Analyze of Gelatin with 3% bromelain enzyme concentration

In the results of the FTIR test of snapper gelatin with a hydrolysis variable of 9 hours and a concentration of 3% bromelain solution, it was found that the amide A absorption area contained N-H, O-H functional groups in the 3000-3750 cm^{-1}, and C-H in the 2850-2970 cm^{-1}. At 50ºC hydrolysis temperature, the functional group N-H, O-H was indicated by a peak of 3274,2 cm^{-1}, at 70ºC hydrolysis was indicated by a peak of 3270,84 cm^{-1}. C-H functional group in gelatin hydrolysis at 50ºC was indicated by a peak of 2930,83 cm^{-1}, and hydrolysis of 70ºC was indicated by a peak of 2939,55 cm^{-1}.

The amide I absorption region is found in the C=C and C-O functional groups, C=C has a wave region of 1610-1680 cm^{-1} and C-O has a wave region of 1000-1300 cm^{-1}. At 50ºC hydrolysis of gelatin the functional group C=C was indicated by a peak of 1632,22 cm^{-1}, on hydrolysis of

70ºC it was indicated by a peak of 1627,88 cm^{-1}. The C-O functional group on gelatin hydrolysis at 50ºC was indicated by a peak of 1235.51 cm^{-1}, and the hydrolysis of 70ºC was indicated by a peak of 1236.11 cm^{-1}.

The amide II absorption region is found in the C-N functional group with a wave region of 1180-1360 cm^{-1}. The C-N functional group at a temperature of 50ºC is shown at the wave number 1334.86 cm^{-1}, and at a temperature of 70ºC it is shown at the wave number 1332.64 cm^{-1}.

This shows that snapper gelatin with 9 hours hydrolysis and 3% bromelain enzyme solution concentration has a similar structure with gelatin, because the FTIR test results show a typical gelatin curve. However, it can be seen that the peak intensity for each specific wave number is relatively different. This may also affect the physicochemical properties between the two such as viscosity and gel strength values where the proportions of collagen and gelatin are also relatively different.

IV. CONCLUSION

Gelatin from snapper scales waste has been produced by using bromelain enzymatic pre-treatment for 6 hours and citric acid as solvents. The process was carried out in two stages namely the manufacture of gelatin with various concentration of bromelain enzymes 0%, 1%, 2%, 3%, 4% and 5% with a temperature of 50ºC and 70ºC.

Snapper scales can be processed into gelatin as evidenced by the results of the FTIR test on the functional groups that make up gelatin, such as carbon, hydrogen, hydroxyl group (O-H), carbonyl group (C=O), amine group (N-H) and alkene group (C =C).

The yield of the snapper scales gelatin is influenced by the concentration of enzymes, but the greater the concentration of enzymes does not affect the amount of yield produced due to the absence of constant decline or increase. The highest yield of extraction obtained was 5.06% with the treatment of enzyme concentrations of 3% with a temperature of 50ºC and 9.54% with the treatment of enzyme concentrations of 5% with a temperature of 70ºC.

The characteristics of gelatin produced such as pH, moisture content, ash content, and metal content that meets the Indonesian National Standard (SNI) 06-3735 (1995).

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Industrial Chemical Engineering for funding this research.

REFERENCES

[1] L. I. La Ifa, A. Artiningsih, J. Julniar, and S. Suhaldin, "PEMBUATAN KITOSAN DARI SISIK IKAN KAKAP MERAH," *Journal Of Chemical Process Engineering*, 2018, doi: 10.33536/jcpe.v3i1.194.

[2] H. Faradiella, P. D. F. Ningsih, and W. E. Triastuti, "The acid solvent experimental studies on gelatin producing by utilizing snapper fishes scales waste (Lutjanus camphecanus sp.)," in *AIP Conference Proceedings*, 2017, vol. 1823. doi: 10.1063/1.4978115.

[3] A. C. N. Talumepa, P. Suptijah, S. Wullur, and I. F. M. Rumengan, "Kandungan Kimia dari Sisik Beberapa Jenis Ikan Laut," *Jurnal LPPM Bidang Sains dan Teknologi*, 2016.

[4] R. Kusumawati, T. Tazwir, and A. Wawasto, "Pengaruh Perendaman dalam Asam Klorida Terhadap Kualitas Gelatin Tulang Kakap Merah (Lutjanus sp.)," *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan*, 2008, doi: 10.15578/jpbkp.v3i1.10.

[5] D. Haryati, L. Nadhira, H. Hera, and N. Abdullah, "EKSTRAKSI DAN KARAKTERISASI GELATIN KULIT IKAN BARONANG (Siganus canaliculatus) DENGAN METODE ENZIMATIS MENGGUNAKAN ENZIM BROMELIN," *Canrea Journal: Food Technology, Nutritions, and Culinary Journal*, 2019, doi: 10.20956/canrea.v2i1.177.

[6] M. Pertiwi, Y. Atma, A. Mustopa, and R. Maisarah, "Karakteristik Fisik dan Kimia Gelatin dari Tulang Ikan Patin dengan Pre-Treatment Asam Sitrat," *Jurnal Aplikasi Teknologi Pangan*, vol. 7, no. 2, 2018, doi: 10.17728/jatp.2470.

[7] E. Cahyono, R. Rahmatu, S. Ndobe, and A. Mantung, "Ekstraksi dan Karakterisasi Gelatin Tulang Tuna Pada Berbagai Konsentrasi Enzim Papain," *Jurnal FishtecH*, vol. 7, no. 2, 2018, doi: 10.36706/fishtech.v7i2.6594.

[8] M. Nurilmala, A. M. Jacoeb, and R. A. Dzaky, "Karakteristik Gelatin Kulit Ikan Tuna Sirip Kuning," *J Pengolah Has Perikan Indones*, vol. 20, no. 2, 2017.

[9] A. T. AGUSTIN, "Kajian gelatin kulit ikan tuna (Thunnus albacares) yang diproses menggunakan asam asetat," Aug. 2015. doi: 10.13057/psnmbi/m010539.

[10] G. E. Hema, "Scholars Research Library Annals of Biological Research," 2013.

[11] C. Santoso and S. Titi, "Perbedaan penggunaan konsentrasi larutan asam sitrat dalam pembuatan gelatin tulang rawan ikan pari mondol (Himantura gerrardi)," *Jurnal Pengolahan dan Bioteknologi Hasil Perikanan* , vol. 4, no. 2, pp. 106–114, 2015.

[12] M. Wahyuningtyas, N. Jadid, P. Burhan, and L. Atmaja, "Physical and Chemical Properties of Gelatin from Red Snapper Scales:Temperature Effect," *Jurnal Teknik ITS*, vol. 8, no. 2, pp. F95–F101, 2019.