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Ekstraksi Kolagen Sisik Ikan Kakap Merah (*Lutjanus malabaricus*) Berbasis Hidroekstraksi: Sediaan Pengembangan Nanokolagen

Collagen Extraction from Red Snapper (*Lutjanus malabaricus*) Scales via Hydroextraction: Toward Nanocollagen Development

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Abstrak

Umumnya kolagen diisolasi menggunakan metode hidrolisis asam, basa dan enzimatik. Namun hal ini memiliki kendala lamanya waktu saat pelepasan prokolagen hingga menghasilkan kolagen. Hidrolisis asam asetat yang dikombinasikan dengan metode hidroekstraksi merupakan salah satu alternatif untuk menghasilkan kolagen yang berkualitas. Penggunaan asam asetat dalam ekstraksi sisik ikan kakap merah (*Lutjanus malabaricus*) akan menghasilkan ion H^+ untuk melepaskan prokolagen dalam bahan baku. Penelitian bertujuan untuk melihat potensi sisik kakap merah (*L. malabaricus*) sebagai sumber kolagen dengan metode hidroekstraksi sebagai sediaan pengembangan nanokolagen. Penelitian ini menggunakan rancangan acak lengkap (RAL). Isolasi kolagen sisik ikan kakap merah menggunakan konsentrasi pelarut asam asetat sebesar 1 M; 1,5 M dan 2 M dengan lama waktu hidrolisis 2 jam dan hidroekstraksi selama 2 jam dengan tiga kali pengulangan. Parameter yang diamati pada penelitian ini adalah prosimat bahan baku kolagen, rendemen kolagen, dan asam amino kolagen. Analisa data hasil diuji statistik menggunakan *Analysis of Variance* (ANOVA) yang dilanjutkan dengan uji lanjut Tukey. Hasil terbaik dari isolat kolagen sisik ikan kakap merah (*L. malabaricus*) yaitu hidrolisis asam asetat 2 M yang dikombinasikan dengan hidroekstraksi. Hasil penelitian ini menunjukan bahwa kombinasi konsentrasi asam asetat berbeda yang dikombinasikan dengan hidroekstraksi berpengaruh nyata ($P<0.05$) pada parameter rendemen kolagen. Kandungan asam amino glisin sebesar 22,37 % dan prolin sebesar 12,45 %. Hal ini menujukan bahwa sisik ikan kakap merah (*L. malabaricus*) berpotensi untuk dikembakan sebagai bahan sediaan nanokolagen.

Kata kunci: ikan kakap, kolagen, perikanan, rendemen, sisik

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Abstract

Generally, collagen is isolated using acid, base and enzymatic hydrolysis methods. However, this has the constraint of the length of time for the release of procollagen to produce collagen. Acetic acid hydrolysis combined with the hydroextraction method is one alternative to produce quality collagen. The use of acetic acid in the extraction of red snapper scales (*Lutjanus malabaricus*) will produce H+ ions to release procollagen in the raw material. The study aims to evaluate the potential of red snapper (*L. malabaricus*) scales as a collagen source through the hydroextraction method for the development of nanocollagen formulations. This study used a completely randomized design (CRD). Isolation of red snapper scales collagen using acetic acid solvent concentrations of 1 M; 1.5 M and 2 M with a hydrolysis time of 2 hours and hydroextraction for 2 hours with three repetitions. The parameters observed in this study were the proximate of collagen raw materials, collagen yield, and collagen amino acids. The data analysis results were tested statistically using Analysis of Variance (ANOVA) followed by Tukey's further test. The best result of collagen isolation from red snapper (*L. malabaricus*) scales was achieved using 2 M acetic acid hydrolysis combined with hydroextraction. This study demonstrated that the combination of different acetic acid concentrations with hydroextraction had a significant effect ($P<0.05$) on collagen yield parameters. The amino acid content consisted of 22.37% glycine and 12.45% proline. These findings indicate that red snapper (*L. malabaricus*) scales have the potential to be developed as a raw material for nanocollagen developments.

Keywords: scales, fiheries, collagen, snapper, yield

1. Introduction

The fishing industry often produces waste that can pollute the environment if not managed properly. One type of waste produced is fish scales. Red snapper (*Lutjanus malabaricus*) is a fishery commodity that is widely needed by people in Indonesia. The amount of red snapper fish scale waste produced from processing is around 41,002 tons/year or 10-13% of the total body weight of the fish (Pratiwi *et al.,* 2019). Fish scales generally consist of organic components, such as collagen, which is a valuable raw material in the health, cosmetics, and food industries. In addition, fish scales also contain minerals and other bioactive compounds that can have health benefits (Cutajar *et al.,* 2022).

Collagen is a type of structural protein, around 30% of which is found in almost all animal tissues, including fish. Research conducted by Hsueh *et al.* (2019) shows that red snapper fish scales contain collagen types I and III, as well as glycosaminoglycans. Bioactive compounds such as polysaccharides, amino acids and phenolic compounds are also contained in red snapper scales. The importance of waste management and sustainable utilization of resources is an effort to process fish scale waste into value-added products. Collagen extraction is carried out to obtain collagen from natural sources, such as fish scales or other tissues. So that this waste can be converted into useful raw materials and can be used in various applications. One method used in collagen extraction is acid hydrolysis involving the breakdown of chemical bonds in collagen using acid as a hydrolysis agent (Li *et al.,* 2016).

The commonly used organic solvent is acetic acid, because it has the ability to break chemical bonds in collagen (Blanco *et al.,* 2019). The use of acetic acid has a major influence on the extraction results (Mardina *et al.,* 2014). Different concentrations of acetic acid can affect the rate of hydrolysis, collagen extraction yield, and the quality and characteristics of the collagen produced (Adnan *et al.,* 2019). Extraction of red snapper (L. *russellii*) scale collagen using 0.5 M acetic acid for 72 hours produces type I collagen which is characterized by the presence of Amide A, B, I, II, and III functional groups (Subagja *et al.,* 2022). Acetic acid concentration of 1.5 M for 12 hours at 45ºC on red snapper (*Lutjanus* sp.) scale collagen produces a yield of 7.91% (Rismayanti *et al.,* 2019). Collagen exposed to acetic acid environment for a

long time, hydrogen bonds and electrostatic interactions in the collagen structure can be disrupted or broken (Li *et al.,* 2016; Li *et al.,* 2017; Bai *et al.,* 2019; Laohakunjit and Kerdchoechuen, 2019; Yang *et al.,* 2019). This causes structural denaturation and results in changes in shape, helix damage, and loss of functional properties of collagen.

In addition to acid hydrolysis, the hydroextraction method is also used in the extraction process. Hydroextraction is an extraction method that involves the use of water or hydrophilic solvents with a boiling point of 40ºC high temperature short time (HTST) as a heat transfer medium which aims to avoid further denaturation of collagen into gelatin and is able to produce collagen in a short time. This process allows dissolved compounds to come out of the raw material and enter the solution. The combination of acetic acid hydrolysis with various concentrations combined with hydroextraction is an interesting approach. The combination of a concentration of 1.5 M acetic acid and a hydroextraction temperature of 120ºC for 5 hours in the extraction of tilapia fish scale collagen produced a yield of 23.78% (Chen *et al.,* 2018). Isolation of tilapia fish scale collagen extracted using 2 M acetic acid concentration and hydroextraction for 2 hours resulted in a yield and protein content of 15.91% and 91.94% respectively compared to using acetic acid maceration alone of 9.17% and 87.48%.

The combination of these two methods is expected to increase the yield of collagen extraction, optimize the use of red snapper fish scale raw materials, and obtain collagen with desired properties. This study aims to see the potential of red snapper scales (L. *malabaricus*) as a source of collagen with the hydroextraction method as a preparation for developing nanocollagen.

2. Material and Methods

Isolation of red snapper (*L. malabaricus*) scale collagen was carried out using acetic acid hydrolysis of different concentrations combined with hydroextraction carried out at the Chemical Analysis Laboratory, Faculty of Fisheries and Marine Sciences, Ainglangga University, Surabaya. Amino acid analysis using HPLC was carried out at SIG Surabaya. The equipment used in this study were Bench top shaker incubators (TOU-120 N, MRC Labortaory), Hettich centrifuge (Brand Rotanta 460), freeze dryer (Brand Buchi Lyovapor L-200), analytical balance (Brand PIONEER PX224/E), Erlenmeyer flask (Iwaki, Germany), measuring cylinder (Brand Herma), Measuring flask (Brand Iwaki), hot plate stirrer (Brand Stirrer Thermo Scientific Cimarec), moisture analyzer (BEL i-Thermo 163L, India), soxhlet extractor apparatus 250 ml (Brand Duran), oven (Brand thermo scientific) micropipette (Brand thermo scientific), amino acids (Acquity UPLC), refrigerator (Brand Gea type expo-350/phar), and freezer (Brand Modena).

The materials used in this study were red snapper scales (Lutjanus malabaricus), distilled water (H2O), sodium hydroxide (NaOH), acetic acid (CH3COOH), boric acid (H3BO3), alcohol, hexane (C6H14), hydrochloric acid (HCl), sulfuric acid (H2SO4), Kjeldahl tablets, methanol (CH3OH), Sodium Dodecyl Sulfate (SDS) 10%, Tetramethyl ethylene diamine (TEMED), Ammonium Persulphate (APS) 10%, comassie brilliant blue R-250, running buffer (tri-base, glycine, SDS), sample buffer (0.5 M Tris-HCl pH 6.8; glycerol; 1% Bromphenol blue; β-mercaptoethanol; SDS 10%), and protein marker (GangNam-STAIN Prestained Protein Ladder). Paper sleeve, cotton, plastic wrap, filter paper, 45 µ milipore filter paper.

The research design for the isolation of red snapper (L. *malabaricus*) scale collagen used acetic acid hydrolysis at different concentrations combined with hydroextraction using a Completely Randomized Design (CRD). There were three research treatments, each with three replications. The research treatments were as follows:

P1: Hydrolysis of acetic acid concentration 1 M

P2: Hydrolysis of acetic acid concentration 1.5 M

P3: Hydrolysis of acetic acid concentration

2 M

The selection of these concentrations was based on previous research with the results proving that the optimal concentration of acetic acid of 0.5 M for 24 hours gave the highest collagen yields of 12.2% and 7.96%, respectively. However, when the experiment was conducted in the field, the concentration of 0.5 M for 2 hours carried out on red snapper scales had a very low yield of 0.22%. Other studies use acetic acid concentrations of 0.5-2 M as extraction agents on tilapia scales. The results showed that the concentration of acetic acid 1.5 M for 24 hours gave the highest collagen yield of 8.66%. However, higher concentrations of acetic acid tend to produce coarser and less soluble collagen. Concentrations of acetic acid 1M and 2M were used because based on the experiment there were collagen fibers and collagen protein profiles.

Preparation of Raw Materials

The red snapper fish scale samples were first washed using distilled water five times, and washed using distilled water three times (Xu *et al.,* 2017) to remove dirt that was attached, then the meat that was still attached to the fish scales was cleaned. then drained and dried in a tray at a chiller temperature of >10ºC to avoid denaturation of the material. After the dry material was stored in a polyethylene bag in the freezer until the sample was to be reused. The washed red snapper fish scale samples were then weighed first before entering the deproteinization process stage.

Deproteinase/Non-Collagen Protein Separation

The washed red snapper fish scale samples were then weighed before entering the deproteinization process stage. The deproteinization process is carried out by soaking the weighed fish scales in an Erlenmeyer flask and then placing them on a hot plate using a magnetic stirrer. The fish scales are soaked in sodium hydroxide (NaOH) solution which aims to remove non-collagen proteins. The ratio between the scales and the NaOH solution is 1:10 (w/v) with a soaking time of 3 hours and a new NaOH solution is replaced every 1 hour. The best deproteinization process snapper fish scale sample with a NaOH concentration of 0.1 M was washed with distilled water until it reached a neutral pH so that it could be continued to the second stage, namely the collagen hydrolysis process.

Preparation of Water-Soluble Collagen Extraction with Hydroextraction Combination

Samples of red snapper fish scales that have gone through the washing process until they reach a neutral pH are soaked in an Erlenmeyer flask using acetic acid solvent (CH3COOH). The collagen hydrolysis stage in this study used three different concentrations of acetic acid, namely 1 M; 1.5 M and 2 M with a soaking time of 2 hours. The ratio between the red snapper fish scale sample and the acetic acid solution is 1:10 (w/v). After soaking with acetic acid, the snapper fish scale sample was washed using distilled water until it reached a neutral pH, then the sample that had reached a neutral pH was stored in the freezer until the sample would be reused for the third stage, namely the collagen hydroextraction process using an incubator shaker. Samples of red snapper fish scales from the results of acetic acid hydrolysis were washed using distilled water first until they reached a neutral pH before the hydroextraction process was carried out. The hydroextraction process of water-soluble collagen was carried out at a temperature of 40ºC for 2 hours using an incubator shaker with a speed of 150 rpm. The ratio between fish scales and distilled water is 1:2 (w/v). The extraction results are in the form of water-soluble collagen which is filtered using a sieve to obtain the collagen filtrate. Then the collagen filtrate is dried using freeze drying to obtain collagen isolate in the form of a sponge and chemical characteristic analysis is carried out on the red snapper fish scale collagen isolate.

Collagen Yield of Snapper Fish Scales

The yield of collagen is the percentage of collagen that can be produced from raw materials before processing into collagen. The calculation aims to determine the economic value and effectiveness of a material (Girsang *et al.,* 2020). The collagen yield is calculated based on the wet weight of the collagen sample itself. The yield value is the percentage of the dry weight of collagen produced against the raw material of red snapper fish scales in dry conditions (Romadhon *et al.,* 2019). The first testing procedure in this study was the calculation of the yield produced from red snapper fish scales using the hydroextraction method. The yield calculation formula is as follows:

$$\text{Yield (\%)} = \frac{\text{initial weight of snapper scales } (g)}{\text{final weight of collagen extraction results } (g)} \times 100$$

Proximate Analysis of Raw Materials

The proximate test aims to analyze the characteristic composition of a material. The proximate test of snapper fish scales raw materials consists of testing water content, fat content, protein content, and ash content by referring to the AOAC proximate testing guidelines (2005). The following is the proximate analysis procedure.

a. Water content test

The water content test uses the HB43-S Moisture Analyzer which combines weighing and halogen heating. The average test time is 3-15 minutes per sample, depending on the type of sample, and the analysis results can be seen directly on the screen or printed. All processes, from weighing, drying, to calculating the results, are carried out in one tool, minimizing human error and increasing accuracy. The procedure begins by weighing a 0.5 gram sample of snapper fish scales, pressing the F2 button, and waiting 3 minutes until finished at a temperature of 100°C. The test is carried out 3 times for each sample.

b. Fat content test

Fat content testing is carried out using the soxhlet extraction method according to AOAC 2005, which separates components in solids using certain solvents through repeated filtration. The material being tested must be dry, because moisture can affect the extraction process. The process is considered complete when the solvent becomes clear, indicating that the fat has been extracted. After that, heating is used to separate the solvent and fat, because the boiling point of fat is higher, and the weight of the fat can be measured (Nurhidayah *et al.,* 2019).

The fat test procedure begins by preparing the materials: 0.5 gram sample, 250 ml n-hexane solvent, baker glass, filter paper, wool rope, ice cubes, hand gloves, cups, and ovens. The filter paper is cut 5 cm, soaked in n-hexane for 10 minutes, then ovened at a temperature of 60-70 ° C for 30 minutes until it does not smell of n-hexane. The filter paper is then put into a desiccator for 10 minutes and weighed. A 0.5 gram collagen sample was placed on filter paper, then heated at 90ºC for five reflux cycles. After extraction, the sample was oven-dried at 105-110ºC for 24 hours, cooled in a desiccator for 30 minutes, and weighed. Fat content was calculated using the AOAC formula (2005).

Fat Content (%) = (W3 – W1) : W2 x 100

Description:
W1 = Weight of empty fat flask (grams)
W2 = Weight of sample (grams)
W3 = Weight of fat flask with fat weight (grams)

c. Protein content test

Testing the protein content of snapper fish scale collagen using the Kjeldahl method (AOAC, 2005) consists of

three stages: destruction, distillation, and titration. In the destruction stage, a 0.5 gram sample is put into a Kjeldahl flask with HgO, K2SO4, and H2SO4, then heated at a temperature of 430ºC for 1-1.5 hours until the solution is clear. After that, the solution is cooled and diluted with distilled water. The distillation stage begins by transferring the destruction solution to the distillation apparatus, adding NaOH-Na2S2O3 solution and boric acid (H3BO3). Distillation is carried out until about 15 ml of distillate is collected. The titration stage is carried out by adding 0.02 N HCl until the color of the solution turns pink. The volume of HCl used was recorded, and the protein content was calculated using the AOAC formula (2005).

$$N\ (\%) = (A - B) \times N\ HCL \times 14 \times 100 : mg\ snapper\ fish\ scale\ sample$$

$$Protein\ content = \%N \times factor\ conversion$$

Description:
A = ml titration sample
B = ml titration blank
FK = 6.25
Mr N = 14

d. Ash content test

The purpose of the ash content test is to determine the amount of minerals in the material. The process begins by cleaning and drying the porcelain ash cup at a temperature of 105ºC for 2 hours, then cooling it in a desiccator for 30 minutes. A 0.25 gram sample of snapper fish scale collagen was put into the cup and burned in an electric ash furnace at a temperature of 600ºC for 3 hours until it turned into ash. After that, the cup was cooled and weighed. The ash content was calculated using the AOAC formula (2005).

$$Ash\ Content\ (\%) = (B - A) \times 100 : C$$

Description:
A = Weight of empty ash dish (grams)
B = Weight of ash dish + sample after drying (grams)
C = Weight of sample (grams)

Amino acid test

Amino acid analysis refers to the Saraswanti Indo Genetech (SIG) laboratory method in 2013, namely using the Ultra Performance Liquid Chromatography (UPLC) method). Amino acid analysis testing is taken based on the highest protein content from collagen proximate analysis. Amino acid analysis consists of 4 stages. The first stage is the preparation of protein hydrolysate, soluble red snapper fish scale acid collagen is weighed as much as 0.1 grams then ground and put into a closed test tube. Furthermore, red snapper fish scale acid soluble collagen is hydrolyzed using 5-10 mL of 6 N HCl, then heated in an oven at 110ºC for 22 hours and cooled at room temperature and transferred to a 500 mL measuring flask, then distilled water is added to the maximum limit mark. Red snapper fish scale acid soluble collagen was filtered using a 45 µl millipore. The second stage, the filtered results were pipetted as much as 10 µL and added 70 µL of AccQ Fluor Borate reagent and then vortexed, after which 20 µL of Flour Adan reagent was added and vortexed again. The sample was left for 1 minute and incubated for 10 minutes at a temperature of 55ºC. The third stage of the red snapper fish scale acid soluble collagen sample that had been added with reagent and incubated was injected into UPLC as much as 1 µL, with chromatography using an ACCQ - Tag Ultra C18 column, at a temperature of 49ºC, a gradient composition system mobile phase, PDA detector, gradient pump system, flow rate 0.7 µL / min and a wavelength of 260 nm. The calculation of amino acid concentration uses a comparison (ratio) of the analyte area to the internal standard, with the following formula (AOAC, 2012):

Standard sample ratio = Area of amino acid analyte : Internal area of standard

Amino acid content mg/kg = ((Standard sample ratio : Standard ratio) x (C std : 1000000) x BM x Va x Fp) : W sample or V sample

Description
C std = Concentration of standard amino acid solution (pmol / µL)
Fp = Dilution factor
Va = Final sample volume (mL)
BM = Molecular weight of each amino acid (g / ml)
W sample = Sample weight (g)
V sample = Sample volume (mL)

Statistical Analysis

The research analysis aims to determine the optimization of snapper fish scale collagen extraction using the hydroextraction method and its nanocollagen form. This analysis was carried out with Microsoft Excel using a two-factor Analysis of Variance (ANOVA). The research design can be used to determine the effect of several independent variables on the dependent variable, as well as to determine the interaction of a combination of several variables, with the main objective of determining the strongest interaction in influencing the research results.

3. Results and Discussions

Results

a. Proximate of red snapper scales (Lutjanus malabaricus)

The proximate analysis of the raw material of red snapper scales (*L. malabaricus*) is as follows. Protein content 43.61%, fat content 5.41%, water content 18.88% and ash content 2.86%. It can be seen that the proximate value of the raw material of red snapper scales (*L. malabaricus*) has chemical characteristics that are suitable as a raw material for making collagen (Table 1).

Table 1. Proximate analysis of red snapper scales (*L. malabaricus*)

Parameter	Percentage (%)
Protein Content	43,61
Fat Content	5,41
Water content	18,88
Ash Content	2,86

Description: Proximate Analysis Research Data

b. Red snapper (Lutjanus malabaricus) scale collagen yield

Isolation of red snapper (*L. malabaricus*) scale collagen using acetic acid hydrolysis of different concentrations combined with hydroextraction had a wet yield of 52.30 - 66.95% and a dry yield of 1.05 - 2.60% in (Table 2). The ANOVA results showed that red snapper (*L. malabaricus*) scale collagen isolated by the hydroextraction method using several different concentrations of acetic acid solvents had a significant effect on the wet yield and dry yield of the collagen produced ($P < 0.05$).

The Tukey test results showed that the highest wet yield and dry yield were produced from the isolation of red snapper (L. malabaricus) scale collagen using the hydroextraction method using 2 M acetic acid of 66.95% and 2.60%. The lowest wet and dry yield values were produced from collagen isolation using the hydroextraction method using 1 M acetic acid at 52.30% and 1.05%.

Table 2. Wet yield and dry yield of red snapper fish scale collagen (*Lutjanus malabaricus*)

Characteristics	Percentage (%)		
	P1	**P2**	**P3**
Wet Yield (wy)	$52,30^{a} \pm 3,05$	$58,63^{b} \pm 1,15$	$66,95^{c} \pm 6,64$
Dry Yield (dy)	$1,05^{a} \pm 0,02$	$1,81^{b} \pm 0,03$	$2,60^{c} \pm 0,67$

Description: P1: Hydrolysis of 1 M acetic acid, P2: Hydrolysis of 1.5 M acetic acid and P3: Hydrolysis of 2 M acetic acid. The sign (±) is the standard deviation of triplicate repetitions. Different superscript letters (a, b and c) in the row indicate that there is a very significant difference in each treatment ($p<0.05$).

c. Amino acid profile of red snapper (Lutjanus malabaricus) scale collagen

Isolation of red snapper (*L. malabaricus*) scale collagen using 2 M acetic acid hydrolysis combined with hydroextraction has the following amino acid test results (Table 3). Glycine compound 22.37%, Proline 12.45%, Arginine 11.30% and Glutamic acid 10.31% which are amino acid components in collagen.

Table 3. Amino acid analysis of red snapper fish scale collagen *(Lutjanus malabaricus*)

Amino Acid Name	Result (%)
Glysine	22,37
Proline	12,45
Arginine	11,30
Alanine	8,49
Glutamic acid	10,31
Serine	4,93
Aspartic acid	5,74
Threonine	4,02
Lysine	8,68
Leucine	2,19
Phenylalanine	6,30
Valine	1,18
Tyrosine	0,49
Isoleucine	0,80
Histidine	0,75
Total	**100**

Discussion

Isolation of red snapper (*L. malabaricus*) scale collagen using acetic acid hydrolysis of different concentrations combined with hydroextraction significantly affected the yield and amino acid profile. The content of procollagen in red snapper (*L. malabaricus*) scales has an important role in the production of

collagen. Generally, collagen is isolated using acid, base and enzymatic hydrolysis methods. However, this has the constraint of the length of time for the release of procollagen to produce collagen. Acetic acid hydrolysis combined with the hydroextraction method is one alternative to produce quality collagen. The use of acetic acid in extraction will produce H+ ions to release procollagen in fish scale raw materials. Then during the hydroextraction stage, hydrogen bonds are broken which causes changes in the structure of procollagen to collagen so that it affects protein content, yield and water content.

The yield of red snapper (*L. malabaricus*) scale collagen isolated using 2 M acetic acid hydrolysis combined with hydroextraction for 2 hours produced the highest yield of 66.95 ± 6.64% (bb) or 2.60 ± 0.67% (bk), respectively. Collagen yield can provide an indication of the effectiveness of raw materials in producing collagen and is an important parameter for determining the economic value of a product. The higher the yield value of a treatment, the higher the level of effectiveness of the treatment (Samosir *et al.,* 2018). This shows that 2 M acetic acid hydrolysis combined with hydroextraction for 2 hours can convert procollagen into collagen. The highest collagen yield value was followed by a high protein content of 76.30 ± 0.09%, because the largest component of collagen is protein content (Kim *et al.,* 2012; Jiang *et al.,* 2016; Zou *et al.,* 2017; Akram and Zhang, 2020).

Different concentrations of acetic acid in the hydrolysis process can affect the ability of acetic acid to dissolve collagen from the raw material matrix. Higher concentrations of acetic acid tend to have better dissolving ability, because they can increase the acidity of the environment and improve the efficiency of collagen extraction (Thitipramote and Thongthai, 2017; Chen *et al.,* 2018). In this study, 2 M acetic acid hydrolysis was the optimal concentration in producing the highest yield in the isolation of red snapper (*L. malabaricus*) scale collagen after being combined with hydroextraction.

Acetic acid has acidic properties that can affect hydrogen bonds and electrostatic interactions in the collagen structure. When collagen is exposed to an acetic acid environment for a long time, hydrogen bonds and electrostatic interactions in the collagen structure can be disrupted or broken (Li *et al.,* 2016; Li *et al.,* 2017; Bai *et al.,* 2019; Laohakunjit and Kerdchoechuen, 2019; Yang *et al.,* 2019) which causes structural denaturation and results in changes in shape, helix damage, and loss of functional properties of collagen. Acetic acid hydrolysis for 2 hours on the isolation of red snapper (*L. malabaricus*) scale collagen can reduce the occurrence of collagen denaturation produced after combined hydroextraction. The high or low concentration of acetic acid and the length of time during hydrolysis affect the percentage of collagen yield after the hydroextraction process.

Hydroextraction helps acetic acid to remove procollagen complex molecules using water or other hydrophilic solvents. The process of isolating red snapper scale collagen using acetic acid hydrolysis combined with hydroextraction can increase the rate of chemical reactions and mass transfer between collagen and solvents (Palma *et al.,* 2018). Therefore, the combination of these two methods works synergistically to isolate collagen quickly and precisely in breaking covalent bonds in red snapper scale raw materials (Kim *et al.,* 2012; Tetti, 2014; Schmidt *et al.,* 2016; Jiang *et al.,* 2016; Zou *et al.,* 2017; Akram and Zhang, 2020; Shaik *et al.,* 2021). The use of optimal temperature and time during hydroextraction can help expand the pores of the collagen matrix and reduce damage to collagen during the extraction process (Chen *et al,* 2018). Collagen isolation using acetic acid hydrolysis and hydroextraction combination produces higher yield compared to acetic acid hydrolysis method alone. In addition, the collagen produced has better quality in terms of viscosity and purity (Santoso et al., 2017).

Isolation of red snapper (*L. malabaricus*) scale collagen using acetic acid hydrolysis combined with

hydroextraction for 2 hours contains 15 amino acids dominated by glycine and proline. Red snapper (*L. malabaricus*) scale collagen contains glycine and proline amino acids amounting to 34% of the total amino acids (Table 3). This is in accordance with the fact that collagen amino acids are characterized by tripeptide repeats consisting of glycine (Gly) proline (X) and hydroxyproline (Y) which are the main components of the triple helix chain formation (Vijayan *et al.,* 2018; Asaduzzaman *et al.,* 2020; Girsang *et al.,* 2020).

Glycine amino acids affect the formation of collagen triple helix chains. This is because glycine has a smaller molecular size than other amino acids, so glycine amino acids make collagen chains bend more easily and form a stable triple helix structure. Glycine amino acids are found in the center of the collagen triple helix chain, the presence of glycine amino acids in the triple helix chain can provide space for proline and hydroxyproline to form strong cross-links between collagen structures (Gorres and Raines, 2010; Chen *et al.,* 2016; Liu *et al.,* 2017; Fabella *et al.,* 2018; Atef *et al.,* 2020).

Proline amino acids affect the thermal resistance of collagen, because proline amino acids have pyrrolidine rings that can form hydrogen bonds with water molecules. These hydrogen bonds help maintain the stability of the collagen structure at high or low temperatures (Jalan *et al.,* 2017; Vijayan *et al.,* 2018; Asaduzzaman *et al.,* 2020; Atef *et al.,* 2020; Ghosh *et al.,* 2021). The levels of glycine and proline amino acids in red snapper scale collagen in previous studies produced glycine amino acids of 9.70% and proline amino acids of 9.30%. This indicates that red snapper scale collagen is a type I collagen (Atef *et al.,* 2020).

The amino acid content of arginine in red snapper scale collagen is 11.30%. Arginine amino acids affect the thermal stability of collagen, because arginine amino acids contain guanidinium groups that have a positive charge at pH. This positive charge can form salt bridges with negatively charged residues in triple helix collagen so that it can stabilize the structure. Overall, the chemical properties of arginine make it an important contributor to the thermal stability of collagen, the presence of arginine amino acids can have a significant effect on the structure and function of collagen fibers (Kadler *et al.,* 2007; Srichana *et al.,* 2013; Huang *et al.,* 2018).

The amino acid content of histidine in collagen is 0.75%. The presence of histidine in red snapper scale collagen indicates metal in collagen, so it can be interpreted that low levels of histidine amino acids indicate the presence of metal in collagen is low, this is in line with the ash content of the collagen produced. The low histidine in collagen is because the pre-treatment process has worked optimally (Song *et al.,* 2021).

4. Conclusion

The use of acetic acid with different concentrations significantly affected the wet and dry yield of red snapper (L. malabaricus) scale collagen. The optimal acetic acid concentration was found in the treatment of 2 M acetic acid hydrolysis for 2 hours combined with hydroextraction for 2 hours with a wet yield of 66.95% and a dry yield of 2.60%, protein content of 76.30%. The red snapper (L. malabaricus) scale collagen produced has the characteristics of type I collagen with a triple helix structure. Type I collagen has the characteristics of glycine amino acids of 22.37% and proline of 12.45%.

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Author Contributions

All authors have contributed to the final manuscript. Contributions of all authors: Cholivia Mayangsari and Gunanti Mahasri: conceptualization, methodology, format analysis, original drafting, writing review and editing. Ahmad Shofy Mubarak: writing review and editing. All authors have read and approved the

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Conflict of Interest

The authors declare no financial or commercial conflict of interest.

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