Characterization and application of gelatin from red snapper skin for making wound healing membranes

Latif Sahubawa[1,*], *Anne* Handrini Dewi[2], *Irene* Raynitha Murdik[1], *Rio* Ari Setiyawan[1], and *Afida* Sabilil Hikma[1]

[1]Department of Fisheries Science, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia
[2]Department of Biomedical, Faculty of Dental & Oral Medicine, Universitas Gadjah Mada, Indonesia

Abstract. This study used the electrospinning method to process and characterize gelatin from red snapper skin for use as a raw material in biomedical membrane preparations. The research design used treatments involving different "ratios of fish gelatin to pullulan": 50% bovine gelatin: 50% pullulan (p0/control), 25% fish gelatin: 75% pullulan (p1), 50% fish gelatin: 50% pullulan (p2), and 75% fish gelatin: 25% pullulan (p3). The profile of gelatin from red snapper skin includes yield (12.27±3.37%), pH (5.05±0.06), moisture (3.45±0.20%), ash (1.46±0.44%), protein (53.40±3.15%), viscosity (9.24cP), and gel strength (99.37 bloom). Based on light microscopy observations, the percentage of nanofiber membrane threads formed (from the largest size/highest quality to the smallest size/lowest quality) was produced by treatments p0, p1, p2, and p3. SEM observations revealed that the size and shape of the threads in treatments p1 and p0 were relatively similar (<1 μm). FTIR analysis indicated that p0 and p1 have the same four functional groups (aliphatic phosphate, aliphatic secondary amine, monosubstituted alkyl, and aliphatic hydrocarbons). The p0 sample was superior because it also had one additional group detected: phenol. In the solubility test, p1 dissolved more slowly, taking 9.33±3.21 seconds, whereas p0 dissolved in 4.67±0.58 seconds.

1 Introduction

Red snapper is an economically important fishery commodity with an increasing trend of world market demand in the last 10 years (2013 - 2022). The total national production of red snapper in 2022 reached 324,803.69 tons with a production value of 11,890.80 billion rupiah [1]. Snappers are marketed in various products according to market demand, especially in the form of filets [2]. The production of fish filets produces a large amount of waste (by-products) in the form of skin, bones, heads, viscera, scales, and fins but have not been optimally utilized for commercial derivative product processing [3]. According to

*Corresponding Author: lsahubawa@ugm.ac.id

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Sumariono [4], the skin produced in the fish filet processing industry reaches 30-35% of the total by-products. Fish skin has a protein content of 29.72% and has potential as a raw material for gelatin in the processing of various food and health products [5].

Fish skin can be processed into gelatin through partial hydrolysis of collagen [6]. Gelatin has purer components than collagen so it has the potential to be developed in the food, medical (health, pharmaceutical, beauty), photography and other fields. In the medical field, gelatin can be used as a drug delivery, body tissue builder, bone scaffold, capsule, biomaterial development, wound healing, and anticancer [7]. Gelatin is used in the form of nanofiber membranes, skin-like sheets, and hydrogels [8]. Gelatin membranes are materials that have bioactive properties and can be applied in biomedical tissue engineering [9]. Membranes in the form of nanofibers have advantages in water vapor transmission and high hydrostatic pressure with optimal mechanical properties [10]. Nanofiber membranes produced by electrospinning method are used as bone scaffolds and wound healers.

Gelatin from red snapper skin has a moisture content of 10%, minerals/ash 0.40%, protein 88.88% and pH 5.45 [11]. The production of biomedical membranes from fish gelatin added with pullulan has potential as a tissue scaffold that can compete with membranes from other basic materials [12]. Membranes from red snapper skin gelatin are expected to have the same and or better quality compared to commercial membranes based on nonfish skin gelatin so that they can be widely marketed to consumers. Gelatin can also be used as drug delivery for several types of diseases, such as tuberculosis and has potential as a carrier for anticancer substances and biomaterial development [7].

Pullulan has a white profile that is stable, water-soluble, tasteless, and odorless [13]. Pullulan can be utilized in various fields such as food and medical fields. Pullulan can be used in the food sector as a thickening agent, coating agent, and binding agent. Pullulan can be used in the medical field as connective tissue in several parts of the body such as in bones and muscles, drug delivery, and gene and wound healing [14]. Pullulan has biodegradable, noncarcinogenic, nonimmunogenic, nontoxic, hydrophilic, and hemocompatible properties so that its utilization is wide [15]. This study aims to analyze gelatin profiles as well as biomedical membranes from red snapper skin compared to pullulan-based commercial membranes.

2 Research methods

2.1 Tools and materials

The tools used in this study were knives, plastic packaging, pH meter, centrifuge, beaker, filter cloth, desiccator, analytical balance, muffle furnace, porcelain chair, electric stove, baking sheet, silicone pan, oven, electrospinning, magnetic stirrer, vortex, measuring cup, stove, porcelain cup, watch glass, ruler, centrifugation, and digestion unit buchi. The materials used in this study were red snapper skin obtained from CV Bee Jay Seafood Tbk, NaOH, CH_3COOH, NaCl, distilled water, HNO_3, pullulan, bovine, kjeldahl tablet, boric acid, BCGMR, HCl, and simulated body fluid, obtained from the Integrated Research and Testing Laboratory, Gadjah Mada University Yogyakarta, 2024.

2.2 Raw material preparation

Raw materials for snapper skin were obtained from PT Beejay Seafood located in Probolinggo, East Java. The skin was packaged, frozen and shipped frozen. The snapper

skin was then cleaned of the remaining meat and cut into small pieces of approximately 2 cm. Fish skin was packaged and stored in a freezer until use.

2.3 Gelatin extraction [16]

Gelatin extraction was performed using the method of [16], [11], and the modified method of Islami [17]. The fish skin was cut, washed with running water repeatedly until it was clean and the initial weight of the skin was measured. The fish skin was soaked in acetic acid (CH_3 COOH) solution of 1% concentration (0.13 M) in a ratio of 1:4 (b/v) for 12 hours. The fish skin was washed with running water until it reached neutral pH (6-7), extracted with distilled water solution in a ratio of 1:3 (b/v) in a waterbath shaker at 80^{o}C for 3 hours. The filtrate from the skin bath was filtered using and dried in an oven at 55^{o} C for 48 hours. The dried gelatin sheets were pulverised with a blender.

2.4 Yield calculation [18]

The yield is the ratio between the extract obtained and the initial simplisia, with the aim of determining how much extract is obtained from the fresh simplisia used. The calculation of material yield uses the following formula.

$$Yield\ (\%) = \frac{Gelatin\ weight\ (g)}{Raw\ material\ weight\ (g)}\ x100 \qquad (1)$$

2.5 pH analysis [19]

A gram gelatin sample was dissolved in distilled water to a volume of 10 ml. The mixture was heated on a magnetic stirrer at 45^{o}C and while stirring at 200 rpm. The gelatin solution was cooled to a temperature of 25^{o}C and measured with a calibrated pH meter.

2.6 Moisture content analysis [20]

The water content test uses a moisture analyzer, which is based on the tool's manual book. The test steps are the tool turned on and opened the top. The aluminium pan is placed on the tool and confirmed to be in the correct position. The tool were closed and the tare button is pressed, the top of the tool is opened again and a gelatin sample was inserted with a weight of ±500 mg until the instrument indicator shows green color. The top of the device is closed and left until the sample heating process ends and the results are recorded.

2.7 Ash [18]

The ash content test was carried out with a porcelain chair that was fired in a muffle furnace. The porcelain chair was placed in an oven at 105^{o}C until the temperature decreased. The porcelain was put into a desiccator for ± 30 minutes until the temperature is the same as room temperature. The porcelain chair was weighed, 2 g of sample was put into the porcelain chair, HNO_3 was added as much as 1 mL, the sample was nestled with an electric stove. Porcelain cursor is put into a muffle furnace with the lid of the porcelain cursor opened slightly to accelerate the ignition process at a temperature of 600^{o}C for ± 3.5 hours. The porcelain was placed in an oven at 105^{o}C until the temperature decreased and placed in

a desiccator for 30 minutes. The porcelain cursor was weighed and the ash content was calculated using the following formula.

$$Ash\ content = \frac{Final\ weight - Initial\ weight}{Sample\ weight}\ x\ 100\% \qquad (2)$$

2.8 Protein [18]

The stages of the protein content test are weighing the sample as much as 0.15 - 0.2 g according to the estimated protein of the type of material to be tested. The sample is put into the deconstruction tube, added H_2SO_4 concentrated 2 mL and 1 g kjeldahl tablet. In the blank sample, 2 mL of concentrated H_2SO_4 and 1 g of kjeldahl tablet were added without adding the sample. The deconstruction tube is placed on the shelf in the buchi digestion unit. The tool is set at a temperature of 420ºC and the deconstruction process is carried out until the solution is clear green. The rack was removed and left until the temperature of the deconstruction tube was low and the smoke from the combustion disappeared. The test tube was mounted on the previously heated buchi distillation unit. Erlenmeyer tubes containing 5 mL boric acid and 3 - 4 drops of BCG-MR were prepared and attached to the sample receiver. The distillation device was turned on and waited for the process to end. Erlenmeyer was taken and continued with titration using 0.02 N HCl until the liquid contained in the erlenmeyer turned pink. The blank was titrated with the same solution. Protein content was calculated by the formula.

$$Protein\ content\% = \frac{(VA - VB)\ x\ N\ HCl\ x\ 14.007\ x\ 6.25}{W\ x\ 1000}\ x\ 100\% \qquad (3)$$

Description:
VA = ml HCl titration sample
VB = ml HCl titration blank
N HCl = Normality of HCl used
14,007 = Atomic weight nitrogen
6,25 = Fish protein conversion factor
W = Sample weight

2.9 Viscosity [18]

6.67 g of gelatin was put in a beaker, the added distilled water to 100 ml. The gelatin solution was heated and stirred with a magnetic stirrer at 60°C until the gelatin was completely dissolved. The gelatin solution was cooled to a temperature of 25°C, then the viscosity was measured using a Brookfield Metek DV-1 Viscometer at a speed of 100 rpm. The viscosity value is expressed in centipoise (cP).

$$Gel\ strength\ (bloom) = Maximum\ force\ \left(\frac{g}{mm^2}\right)\ x\ plunger\ surface\ area$$

Where: plunger serface area = 126,612 mm^2

2.10 Gel Strength [21]

Put 6.67 grams of gelatin into a beaker and add distilled water to 100 ml. The gelatin solution was heated and stirred with a magnetic stirrer at 60°C until the gelatin was completely dissolved. The gelatin solution is cooled to a temperature of 25°C then put into a cup and stored in the refrigerator at a temperature of 4°C for approximately 18 hours. Gel strength was measured using a Universal Testing Machine (UTM). The gel strength value can be calculated using the following formula.

2.11 Membrane manufacturing [12]

The gelatin membrane was prepared by a modified electrospinning [12]. Gelatin was dissolved in PA distilled water and then stirred using a stirrer until well mixed. The concentration of the resulting solution was 25% (b/v_{H2O}). The gelatin solution was added to the film solution made from pullulan with a concentration of 25% (b/v_{H2O}) in the ratio of 20/80, 30/70, 40/60 and 50/50. The solution was electrospinning at room temperature. Electrospinning was performed using a syringe connected to a steel plate, then connected to a power source with DC current with a current strength range of 0-50 kV. The pumping speed was set at 0.5 mL/hour with an electric voltage of 20 kV. The resulting membrane yarn was collected on the prepared plate.

2.12 Quality analysis of gelatin membrane [12]

A gram gelatin sample was dissolved with distilled water to a volume of 10 ml. The mixture was heated on a magnetic stirrer at 45ºC and while stirring at 200 rpm. The gelatin solution was cooled to a temperature of 25ºC and measured with a calibrated pH meter.

2.12.1 Scanning electron microscopy (SEM) test [21]

The SEM test was conducted based on [21] with modifications. The membrane sample was made in the form of a *coating* using gold at high pressure. The sample was observed with a distance between the sensor and the sample of about 10 mm. The sensor is directed at the point to be tested to see the threads formed. The magnifications used for this observation are 500; 1,000; 5,000; and 10,000.

2.12.2 Fourier transform infrared (FTIR) test [21]

The membrane was analyzed by Fourier Transform Infrared Spectroscopy (FTIR) observed in the 4000-400 cm^{-1} spectrum for 64 scans at a spectral resolution of 4 cm^{-1}.

2.10.3 Simulated body fluid test [22]

The test is carried out by inserting the gelatin membrane into the prepared simulated body fluid, recording the solubility time.

2.13 Experimental design

This study used the treatment of the ratio of gelatin to pullulan solution, which consisted of 4 (four) treatments (including control) with three replications. Observation data were

analyzed using a completely randomized design, with the following treatment types: p0 = ratio of bovine and pullulan (50% : 50%, control treatment); p1 = ratio of red snapper gelatin and pullulan (25% : 75%); p2 = ratio of red snapper gelatin and pullulan (50% : 50%); and p3 = ratio of red snapper gelatin and pullulan (75% : 25%).

2.14 Data analysis

Data were analyzed with SPSS and Microsoft Excel 2016. Observation data were tested for normality, and for parametric data, one-way ANOVA test was conducted to determine its significance. If the data showed significant results ($p<0.05$), DMRT further test was conducted. Parameters that are nonparametric, will be tested with the Kruskal-Wallis method, followed by the Mann Whitney test. FTIR analysis results using OMNIC-IR software at LPPT UGM.

3 Results and discussion

3.1 Characteristics of gelatin

The characteristic analysis (yield, pH, moisture content, protein content, ash content) of red snapper skin gelatin is described below with the observed data as shown in Table 1.

3.1.1 Yield

The mean gelatin yield value of red snapper skin was 12.27 ± 3.37%. Previous research results shown varying yield values depending on the type of material and method used. The research results of [23-25], produced high yields because they used materials with low nongelatin content. [23] used gelatin from red tilapia skin by acid method, [24] extracted gelatin from a mixture of chicken feet and heads by the acid method, and [25] utilized gelatin from camel skin by the acid-base method. The results of these studies showed higher yield values than the study of [17] at 5.51% and lower than the research of [26] of 31.11 ± 0.15%. [27] reported that the difference in the research results was caused by differences in the chemical profile of the gelatin produced. The chemical profile of the gelatin produced is influenced by pH, extraction time, and soaking the skin with acid. The optimal condition for gelatin extraction is with 5% CH_3 COOH, carried out at a temperature of 60ºC for 7 hours [26] Research by [29-31] has produced lower yields because the materials used had lower protein levels. According to Chua, gelatin is extracted from black soldier fly larvae by the acid method. [30] used chicken skin by the acid-base method [29] and [31] utilized peking duck feet by the acid method.

3.1.2 Acidity level (pH)

The mean pH value of gelatin from red snapper skin was 5.05 ±0.06, meeting SNI 8622:2018 and GMIA standards (3.8 - 7.5). The resulting pH value is relatively higher than the research results of [24, 29, 23], but lower than those of [30, 31], resulting in a higher gelatin pH than the results obtained by the researchers. The research of [30-31] which used the acid-base method to produce a pH close to neutral. The results of this study showed a lower pH value compared to previous research using red snapper skin raw materials. Research by [32] produced a pH of 6.10, and [33] amounted to 6.3. The lower the concentration of acid used, the higher the pH of the gelatin produced [34]. According to [35], post-acid immersion

washing, which is less than optimal, causes acid residue to remain on the gelatin, so that the pH of the gelatin is even lower.

3.1.3 Moisture

The average moisture content of red snapper skin gelatin was 3.45 ± 0.20%, in accordance with the SNI 8622:2018 standard (<12%). The results of this research are lower than the results of [23-25, 29-31, 36] with a moisture content of 9.63±1.99%. Research by [37], who extracted gelatin from red snapper skin, showed lower moisture content (2.57%). The low moisture content indicates that the drying process was optimised. According to [26], gelatin moisture content is influenced by several factors, namely the type of fish skin, length of drying time, and the type and water content of raw materials.

3.1.4 Ash

The mean ash of gelatin from red snapper skin was 1.46 ± 0.44%, in accordance with SNI 8622:2018 (<3%) and GMIA 2019 (0.3 - 2%) standards. The result shows that ash was relatively higher than the research results of [23, 31] but lower than the results of [24-25, 29-30]. A lower ash content indicates that the demineralisation process is more optimal. The ash obtained by the researchers was also compared to the red snapper skin gelatin from the research of [38] which is 3.0%, and [39] which is 2.14%. The relatively high ash content indicates that the demineralisation process is not optimal owing to the use of a low acid concentration [36].

3.1.5 Protein

The average protein content of gelatin from red snapper skin was 53.40 ± 3.15%, lower than the research results [33], with an average gelatin protein between 84 and 86%, as well as [23-25, 29-31]. The protein content, results of this research are also lower than that of red snapper skin studied [27], which amounted to 87.063 ± 0.62%. The use of too high a temperature during the extraction process causes the protein contained in gelatin to denature. An increase in extraction temperature can trigger denaturation of proteins so that they can turn into non-protein compounds [17].

3.1.6 Viscocity

The viscosity value of red snapper skin gelatin is 9.24 cP, lower/not meeting SNI 8622:2018 standards (>15 cP) and GMIA 2019 standards (15 – 75 cP); and [11] namely 17.4 cP; [17] and [39] are 26.00 cP respectively, but higher than [26] which is 7.30±0.36 cP. Gelatin viscosity is strongly influenced by protein content, gelatin molecular weight, and extraction temperature. Low viscosity indicates low protein content. The quality of nanofibre membranes is greatly influenced by gel strength and gelatin viscosity. Low viscosity is directly proportional to gel strength. Low viscosity and gel strength indicate that the amino acid content in gelatin is low, especially proline and hydroxyproline which are responsible for making the gel. Low viscosity causes the membrane structure to become more fragile, so that the tensile strength and elongation of the membrane become low.

3.1.7 Gel Strength

The gel strength of the gelatin produced by the researchers was 99.37 bloom. The results obtained by the researchers met the SNI 8622:2018 standard (>75 bloom) and the 2019 GMIA standard, namely between 50 and 300 bloom. The results obtained by the researchers were the lowest compared to research conducted by [11], namely 211.90 ± 5.26 bloom, [26] namely 312.5 bloom. Gel strength is greatly influenced by gelatin protein/amino acid content, especially the presence of the amino acids proline and hydroxyproline. A low gel strength indicates that the levels of proline and hydroxyproline in gelatin are low; therefore, the gel formation process is hampered. The protein in gelatin can be assumed to be the basis for determining gel strength because it is greatly influenced by the presence of amino acids in gelatin. A low gel strength results in membranes with low porosity and water resistance. Low gel strength also reduces the physical stability and macromolecular structure of the membrane.

3.2 Membrane profile testing

Observations of the profile (size of fibrin threads) forming the membrane were analyzed in stages, starting with observations with a light microscope to detect the appearance of fibrin threads, followed by detailed observations using electron microscopy, functional group analysis and membrane solubility tests (see Figures 1, 2, 3).

Table 1. Characteristics of red snapper skin gelatin

Materials of Fish	Extraction Method	Yield (%)	pH	Moisture (%)	Ash (%)	Protein (%)	Viscosity (cP)	Gell Strength (bloom)
Red Tilapia Skin [17]	Acid extraction	5.51	-	3.87	0.61	90.32	26.00	-
Red Tilapia Skin [26]	Acid extraction	31.11	5.69	8.96	-	87.54	7.30	211.90
Red snapper fish bones [39]	Acid extraction	15.03	-	4.64	2.14	90.67	-	200.33
Red Tilapia Skin [11]	Acid extraction	5.32	5.45	10.19	0.40	88.88	17.40	312.50
Red snapper skin*	Acid extraction	12.27	5.05	3.45	1.46	53.40	9.24	99.37

Note * = Researcher's results

3.2.1 Microscopic observation

The results of observation of membrane-forming fibril threads with a light microscope from each treatment are shown in Figure 1. Many fibrin threads were observed in the control sample (p0) and p1. The number of fibrin threads observed indicates that the membrane formation process with electrospinning is very good. In sample p2, fibrin yarns were almost invisible. In sample p3, no fibrin yarn formation was observed. Factors that affect electrospinning with organic polymers are the polymer content, solvents used, and

environmental conditions during the process. The success factor of electrospinning is determined by the bond between the materials used to make the electrospinning solution. Samples p2 and p3 showed that the materials used were not perfectly homogenised, so that the fibrin threads formed were very few and almost not observed. The results of the observation of fibril threads obtained are that sample p1 is the sample with the best results, where the nanofibre threads formed are close to the condition of the p0 treatment, so only sample p1 will be tested further.

3.2.2 Scanning electron microscope (SEM) test

The SEM test used an electric current of 10 kV with a distance between the sample and the sensor of 10 mm. The test was conducted with magnification of the electron microscope scale (500, 1,000, 5,000 and 10,000). SEM test results can be seen in Figure 2. Control/p0 treatment samples with 500 magnification (A1), 1,000 magnification (A2), 5,000 magnification (A3), and 10,000 magnification (A4). Treatment sample p1 (ratio of red snapper skin collagen and pullulan, 25%:75%) with 500 magnification (B1), 1,000 magnification (B2), 5,000 magnification (B3), and 10,000 magnifications (B4).

SEM observation results showed that the nanofiber formed in the control treatment (p0) looked uniform. The membrane formed has a size smaller than 1 μm. Observation with a magnification of 500 shows that the membrane surface is quite tight and there are few craters formed due to splashing during the electrospinning process. Observation with a magnification of 1,000 there are white spots on the membrane. According to [40], white spots on the membrane are the remaining membrane/gelatin that is not dissolved completely. Observations at a magnification of 5,000 and 10,000 showed a uniform membrane with the distribution of several white dots.

Observation of sample 25/75 (p1) at 500 magnification, there are many black spots, which are cavities in the membrane caused by the presence of sparks during the electrospinning process [40]. Observation at a magnification of 1,000, white spots were observed because of an inhomogeneous mixture of gelatin and pullulan. Observations at a magnification of 5,000 showed that the nanofiber on the membrane had a high density, white spots were also visible on the membrane surface. Observations at a magnification of 10,000 showed that the nanofiber threads formed had a uniform size (<1 μm). According to [41], the small size of nanofiber is suitable for application in the medical field. The resulting membranes have the potential to be used in drug securement, diagnostic imaging, and tissue engineering.

3.2.3 Fourier transform infrared (FTIR) test

FTIR tests were carried out on a spectrum of 4,000 - 400 cm^{-1} for 64 scans with a resolution of 4 cm^{-1} (see Figure 3). Figure 3 shows the comparison of the FTIR results of treatment p0 with p1. Figure 3b shows the FTIR results of the p0 treatment and Figure 3c shows the FTIR results of the p1 treatment.

Based on the results of FTIR analysis, 5 (five) functional groups were recorded in the control treatment sample (p0), namely aliphatic phosphate, phenolic, aliphatic secondary amine, alkyl monosubstitution, and aliphatic hydrocarbon. The aliphatic phosphate group was detected at a spectrum of 3,423.66 cm^{-1} with absorption at a spectrum of 1,242 cm^{-1}. According to [42], the absorption in the spectrum of 1.242 cm^{-1} shows that the sample has a double covalent functional group 2 (two) bonded with hydrogen (P = O). The spectrum of 1,242 cm^{-1} is in the spectrum range of 1,250 - 1,115 cm^{-1}, where in that vulnerability the

chemical bond formed is relatively strong. The absorption in the spectrum of 3,423.66 cm^{-1} shows that there is a hydrogen bond derived from the hydroxyl functional group on phosphate. Aliphatic phosphate groups function as therapeutic ingredients for wound healing, especially wounds to bones [43]. Phenolic groups were detected in the spectrum range of 3,600 - 3,350 cm^{-1}. According to [42], phenolic compounds were detected at a spectrum of 3,670 - 3,550 cm^{-1}. The absorption in the spectrum of 1,153.94 cm^{-1} proves that there is a phenol functional group on the membrane.

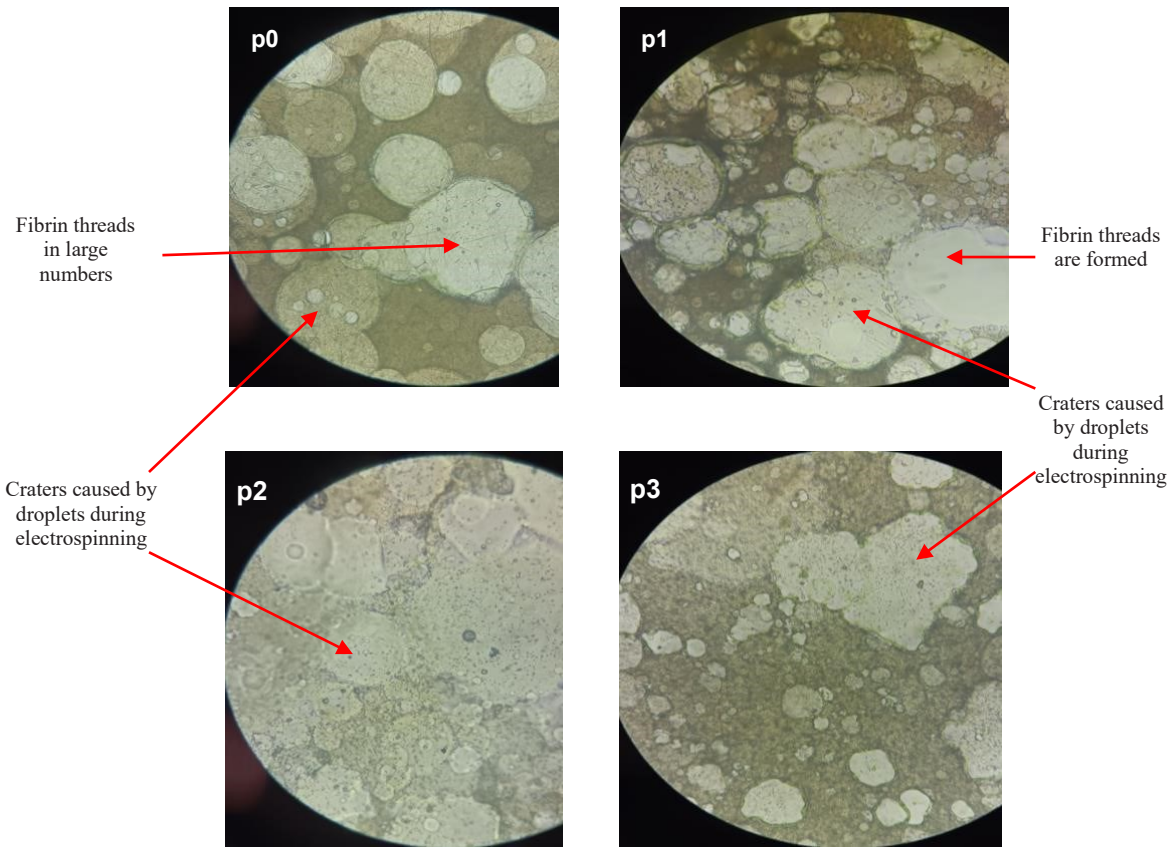

Fig. 1. Results of membrane observations with a light microscope

Description: p0: control treatment, membrane with a ratio of bovine gelatin & pullulan (50% : 50%) p1: membrane with a ratio of gelatin & pullulan (25% : 75%) p2: membrane with a ratio of gelatin & pullulan (50% : 50%) p3: membrane with a ratio of gelatin & pullulan (75% : 25%).

The presence of phenolic groups indicates that the membrane has antimicrobial capabilities [44]. Secondary aliphatic amine groups were detected in the spectrum of 3,320 $cm^{-1.}$ The absorption in the 3,320 cm spectrum^{-1} indicates that the secondary amine group has an N-H bond [42]. The presence of secondary aliphatic amine groups is reinforced by the presence of absorption in the spectrum of 1,654 cm^{-1} and 1,546 cm^{-1} which means there are CO and $_{NH2}$ bonds. The presence of secondary aliphatic amine groups indicates that the membrane produced has a slight antioxidant reaction. Monosubstituted alkyl groups were detected at 3,320 cm^{-1} and 2,130 cm^{-1} spectra. The absorption at these wavelengths indicates the membrane has strong carbon bonds (H-C∶C-H).

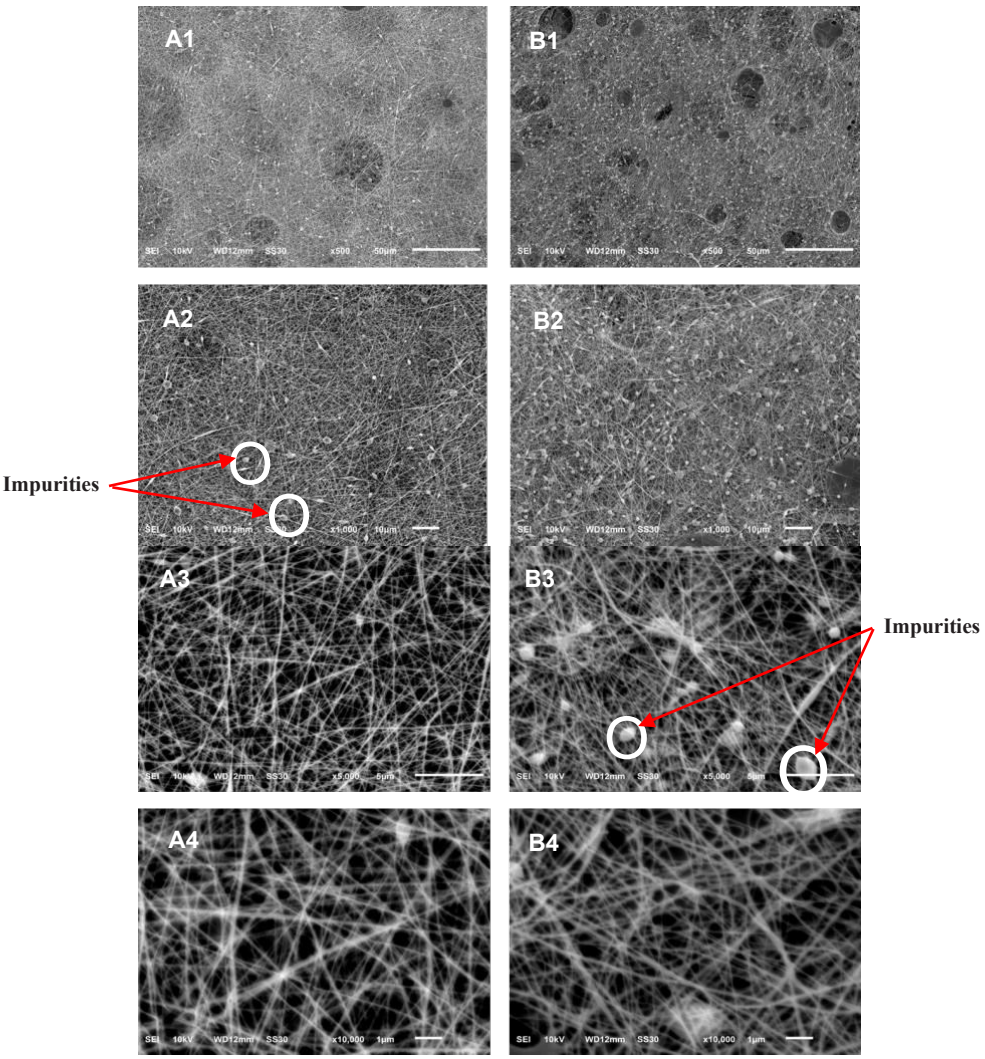

Fig. 2. Observation results with SEM

Description: A1: SEM test of control sample (p0) with a magnification of 500; A2: SEM test of control sample (p0) with a magnification of 1,000; A3: SEM test of control sample (p0) with a magnification of 5,000; A4: SEM test of control sample (p0) with a magnification of 10,000; B1: SEM test of test sample (p1) with a magnification of 500; B2: SEM test of test sample (p1) with a magnification of 1,000; B3: SEM test of test sample (p1) with a magnification of 5,000; B4: SEM test of test sample (p1) with a magnification of 10,000

The absorption of at 2,130 cm spectrum^{-1} is only used as an indicator of the presence of monosubstituted alkyl groups. The presence of alkyl groups monosubstitution is indicated by the presence of absorption in the 650 cm spectrum^{-1}. According to [42] the absorption in the spectrum of 680-610 cm^{-1}, indicates that there is a C-H alkyl bond (alkyl monosubstitution) which functions to help synthesize drug [45]. Aliphatic hydrocarbon groups were detected at 2,929.78 cm^{-1} spectrum, indicating the presence of hydrocarbon

CH_2 bonds. Absorption at 1,454 cm^{-1} and 1,335 cm^{-1} spectra, indicating the presence of CH_2 bonds that form a cross-link formation. The absorption in the spectrum of 720 cm^{-1}, indicates the presence of a bond structure that forms a rocking structure. Aliphatic hydrocarbon groups have benefits in drug securement, tissue scaffolding and drug transportation because they have biocompatibility and adjustable degradation time [46].

Based on the results of FTIR analysis, the p1 treatment sample has 4 (four) functional groups, namely aliphatic phosphate, aliphatic secondary amine, alkyl monosubstitution, and aliphatic hydrocarbon. Aliphatic phosphate groups were detected in the spectrum of 3,404.90 cm^{-1} and reinforced by absorption in the spectrum of 1,019.90 cm^{-1} and 1,280 cm^{-1}. According to [42], the absorption in the spectrum of 1,019.90 cm^{-1} indicates that the membrane has an organic phosphate ester bond (P-O-C) and in the absorption spectrum of 1,280 cm^{-1} indicates that there is a phosphate double bond with oxygen (P=O).

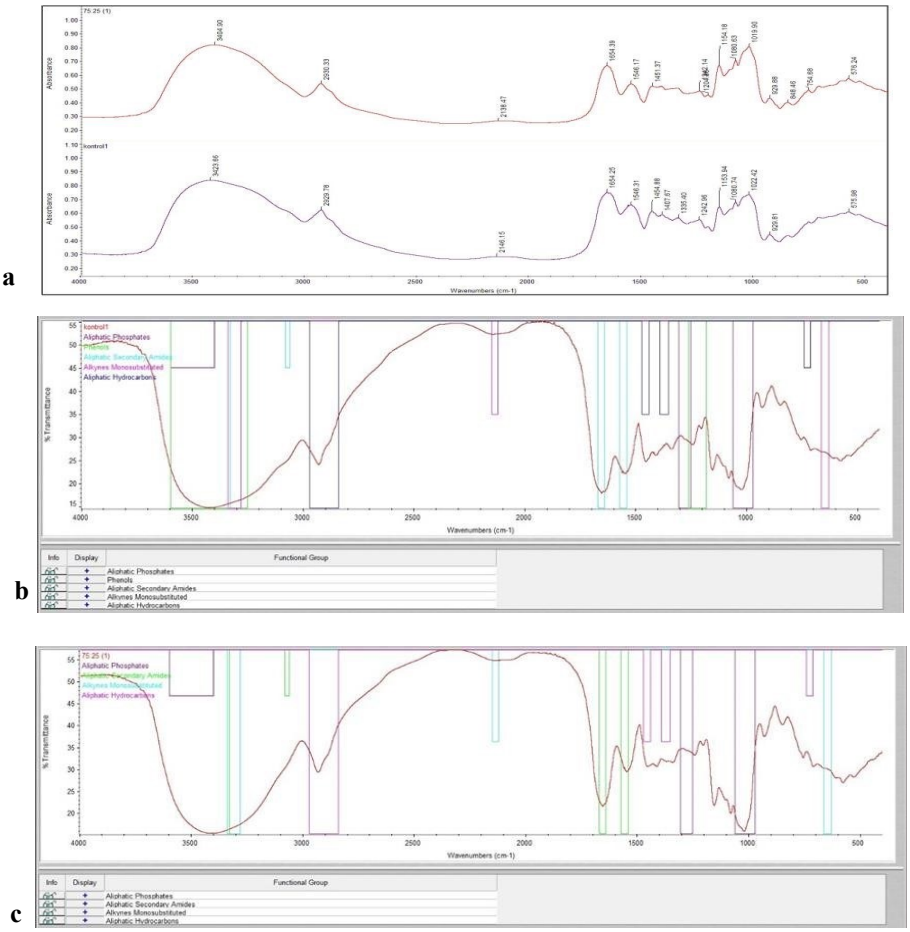

Fig. 3. FTIR analysis results

Description:

a : Comparison of FTIR results of control treatment (p0) and treatment p1

b : Results of graphic analysis of control treatment (p0) with IR

c : Results of graphic analysis of treatment p1 with IR

The absorption in the spectrum of 3,404 cm^{-1}, shows that there is a hydrogen bond derived from the hydroxyl group in the phosphate compound. Aliphatic phosphate groups function as therapeutic ingredients for wound healing, especially wounds found in bones [43]. Secondary aliphatic amine groups were detected in the spectra of 3,320 cm^{-1} and 3,070 cm^{-1}, which indicating the presence of secondary N-H bonds in the sample. The presence of secondary aliphatic amine groups is reinforced by the absorption in the spectra of 1,654 cm^{1} and 1,546 cm^{-1}, which indicates the presence of CO and NH_2 bonds in the sample. The presence of secondary aliphatic amine groups indicates that the membrane produced has a slight antioxidant reaction. Monosubstituted alkyl groups were detected at 3,300 cm^{-1}, 2,100 cm^{-1}, and 650 cm^{-1} spectra. In the spectrum of 3,300 cm^{-1}, there was a strong carbon bond (H-C∶C-H). The spectrum of 2,100 cm^{-1} shows the presence of carbon C∶C asymmetric bonds. Absorption at 2100 cm^{-1} spectrum was used as an indicator because H-C∶C-H bonds were detected, indicating the presence of hydroxyl groups. The absorption at 650 cm^{-1} spectrum indicates the presence of C-H alkyl bonds which signify the presence of H-C∶C-H bonds. Monosubstituted alkyl groups function to help drug synthesis [45]. Aliphatic hydrocarbon groups were detected at spectra of 2.930 cm^{-1}, 1.451 cm^{-1}, 1.242 cm^{-1}, and 7.20 cm^{-1}. The spectrum of 2930 cm^{-1} shows that there is a CH $bond_2$. The spectrum of 1,451 cm^{-1} indicates the presence of a CH bond $structure_2$ which forms a cross structure. The spectrum of 1,242 cm^{-1} shows the presence of t-butyl group. The absorption in the 720 cm $spectrum^{-1}$ shows the presence of CH_2 groups that form a rocking structure. Aliphatic hydrocarbon groups have benefits in drug delivery, tissue scaffolding and drug transportation because they have biocompatibility properties and adjustable degradation time [46].

3.2.4 Solubility test

The results of the membrane solubility test into the SBF (Simulated Body Fluid) solution as shown in Table 2. The results of the membrane solubility test (control treatment samples / p0 and p1) showed a rapid degradation process, namely only in an interval of 4.67 ± 0.58 seconds (p0) and 9.33 ± 3.21 seconds (p1). The results obtained show that the membrane samples treated with p0 and p1 have a faster solubility time than the results of [47]. The fast solubility rate indicates that the resulting membrane has a brittle texture. Research by [48] on immersion for 180 minutes, showed a degradation rate of 80%. Research [20] takes 45 minutes for complete membrane solution. The research results obtained were compared with [49] with a scaffold membrane after soaking in SBF solution for 8 weeks, only experienced a weight loss of 11%. Based on the standards of the [50], the membrane in the form of capsules must dissolve in less than 5 minutes, thus the researcher's membrane (9.33 ± 3.21 seconds) has met the standards of the Ministry of Health. The solubility of the membrane in a short time interval (seconds) is concerned that the drug delivered/released is not on target so that it requires more precise optimization.

Table 2. Results of solubility test in SBF solution

Sample	Dissolve time (s)	[47]	[50]
Control (p0)	4.67±0.58	4 minutes	< 5 minutes
Sample 25/75 (p1)	9.33±3.21		

4 Conclusions

The characteristics of red snapper skin gelatin are: yield (12.27 ± 3.37%); pH (5.05 ±0.06); moisture (3.45 ± 0.20%); ash (1.46 ± 0.44%); protein (53.40 ± 3.15%); viscosity 9.24 cP; and gel strength value was 99.37 bloom. Based on the results of observations with a light microscope, the percentage of membrane nanofiber yarns formed (largest to smallest) respectively resulted from treatment p0 > p1 > p2 > p3. SEM observation showed that the size and shape of the yarns in p1 and p0 treatments were relatively the same (<1 μm). FTIR observation showed that treatments p0 and p1 had the same 4 functional groups (aliphatic phosphate, aliphatic secondary amine, alkyl monosubstitution, and aliphatic hydrocarbon). The p0 treatment was superior because it had 1 additional functional group, namely phenol. In the solubility test, the sample from treatment p1 dissolved longer (9.33 ± 3.21 seconds) than p0 (4.67 ± 0.58 seconds). Treatment p0 & p1 were the best treatments compared to p2 and p3.

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