

# Physical and Chemical Properties of Gelatin from Red Snapper Scales : Temperature Effects

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**Abstract**—The extent of applications in various fields makes the need for gelatin continue to increase in the global market. Fish gelatin is an alternative to mammalian gelatin and its use is more universal because it can be consumed by all religious followers. The high variability of fish gelatin properties is caused by the availability of many extraction methods to obtain it. This preliminary study was carried out to find the optimum range of gelatin extraction procedures using Red Snapper scales because it had not been widely studied, although it was reported that gelatin yield was not significantly different from the bone and skin part. The optimum condition of the extraction procedure was obtained by pre-treatment using 5 % CH<sub>3</sub>COOH with extraction temperature of 60 °C which produces 58.19% swelling of fish scales and yield of gelatin is 8.76% with the moisture quality of 6.68%, pH of 6.225, viscosity of 15.54 cP and the melting point of 60 °C. The functional groups of gelatin was also successfully confirmed by FT-IR spectra.

**Keywords**—Gelatin, Acid Pre-Treatment, Fish Scales, Extraction Temperature.

## I. INTRODUCTION

The use of gelatin biopolymers extracted from animal skin and bones in the food, pharmaceutical and biomedical industries has been widely reported[1]. 98% of the total use gelatin worldwide come from mammals, that is cows or pigs[2]. In recent years, gelatin from mammals has received serious attention because of the risk of infection with animal diseases such as bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE) and foot-and-mouth disease (FMD) which can be transmitted to humans[3]. In addition, cows are something that is glorified for Hindus, while pigs are not lawful and not kosher for Islam and Jews[4]. There is a high demand to replace the mammalian gelatin and that an alternative is of the aquatic animals, especially fish.

Control of collagen hydrolysis can be carried out chemically and enzymatically[5]. However, chemical hydrolysis is more in demand, mainly for producing industrial-scale commercial gelatin [34]. In addition, Zhang et al reported that due to the lability of acidity on crosslinks in the skin of the fish, pre-treatment using acid at low concentrations have generally been sufficient to produce a swelling and break the intra and inter-molecular non-covalent bonds[6].

Fish gelatin have different properties with those of mammalian gelatin. This is confirmed by Tkaczewska et al [7] that in addition to methods of pre-treatment, the quality of the fish gelatin is also dependent on the species of fish. It shows that the variability of fish gelatin product is very high.

Red Snapper Fish is one of the main fishery commodities in Indonesia. In 2017, the production of Red Snapper fish

increased dramatically, reaching 25,051 tons[8]. Red Snapper fish measuring 400-1000 grams can produce as much as 41.5% fillet meat and 58.5% waste[9]. Many studies has been conducted to utilize the waste as raw material of gelatin, especially of various body parts Red Snapper fish, such as skin[9]–[11], bones [10], [12], [13] and scales [14], [15]. The study resulted in gelatin with varied quality as a result different of pre-treatment and extraction conditions. This research was conducted as a preliminary study to obtain optimum conditions of the gelatin extraction procedure from fish scales red snapper (*Lutjanus sp.*).

## II. METHOD

### A. Preparation of Red Snapper Scales

Fish scales were stored in a chest freezer of -5°C and used for research within ± 2 months. Frozen Red Snapper scales were melted with running water for ±30 minutes, and were cleaned from sticking dirt. Next, fish scales were placed in a preheated oven at 70 °C until dry.

### B. Gelatin Extraction

The extraction was carried out according to the method of Sultana et al [10] with slight modifications. At first, dried Red Snapper scales were pre-treated with an acid solution using a ratio of scales and solution of 1:10 (w/v) for 24 hours. Three acids used were HCl (K), H<sub>3</sub>PO<sub>4</sub> (P) and CH<sub>3</sub>COOH (A). The three types of solutions varied in concentrations of 1, 3 and 5% (w/v); until optimum concentration was obtained (condition 1).

Furthermore, the fish scales were soaked using the optimum concentration with variations in the immersion time for 12, 24 and 36 hours, respectively; until optimum immersion time is obtained (condition 2). These conditions 1 and 2 were used as pre-treatment of fish scales before the extraction process. After that, the fish scales were neutralized using demineralized water. The extraction was conducted using conventional water bath with scales and water ratio of 1: 2, and it used the extraction temperature variation of 60, 70 and 80 °C. The extraction was filtered to obtain a filtrate and it cooled in a refrigerator temperature of 4-5 °C to form a jelly. The jelly then was dried at 60 °C to form gelatin film, which then be grinded to obtain powder.

### C. Swelling

The swelling was determined by calculating the percentage of dried fish scales and fish scales swell after immersion[10].

### D. Yield

The yield was determined by calculating the percentage of dried fish scales and gelatin powder produced[10].

### E. Moisture

1 gram of gelatin powder was placed in a petri dish and heated at 105 °C for 2 hours. The gelatin powder was weighed and the percentage of the weight loss value was calculated[16].

### F. pH

Gelatin pH measurements were carried out by making a gelatin solution with a concentration of 6.67 % (w/w). The solution was homogenized with a magnetic stirrer and heated at 70 ° C. The pH of the solution was measured at room temperature using a pH meter[17].

### G. Viscosity

Viscosity was carried out by measuring the gelatin solution with a concentration of 6.67% (b / b). The value of viscosity was expressed in units of centipoise (cP)[16].

### H. Melting Point

The melting point was measured by entering 0.01 gram of gelatin powder into the capillary tube and tested using the Thermo Scientific melting point test instrument[16].

### I. Functional Group Analysis

Gelatin crushed with KBr, and formed into pellets. Then, the sample analyzed by functional group uses the FT-IR instrument brand SHIMADZU with a wavelength of 4000-400  $\text{cm}^{-1}$ . The FT-IR spectra were used to confirm the constituent functional groups of gelatin.

### J. Data Analysis

The data obtained was analyzed statistically and descriptively. The effect of treatment on parameters can be known by the analysis of the One Way Analysis of Variance (ANOVA).

## III. RESULTS AND DISCUSSION

### A. Preparation of Red Snapper Scales

Preparation of fish scales as the main raw material was the initial stage in this study. Fish scales stored in a chest freezer of -5 °C were good for research in less than two months[18]. Clean scales of fish were dried at 70 ° C. Drying at these temperatures was carried out to prevent early denaturation of collagen, as mentioned in Alhana et al study that the temperature of denaturation of collagen depends on the type of fish species[19].

### B. Acid pre-treatment

The acid solution used for soaking fish scales aims to remove calcium salts and other minerals[20], impurities (proteoglycan, blood, mucus, sugar, fat etc.) and convert collagen into the optimum form for gelatin extraction[21]. The raw material-treatment with acid process allows for the swelling, the swelling scales due to the interaction of the acid solution ( $\text{H}^+$  ions) which cause changes in the tertiary structure of proteins by breaking some hydrogen bonds between the protein chain[6]. The presence of swelling is indicated by the addition of the percentage increase in the mass of fish scales after immersion[22]. Proposed reaction mechanism that occurs at this stage is shown in Figure 4.

### Variation of Concentration of Immersion Solution

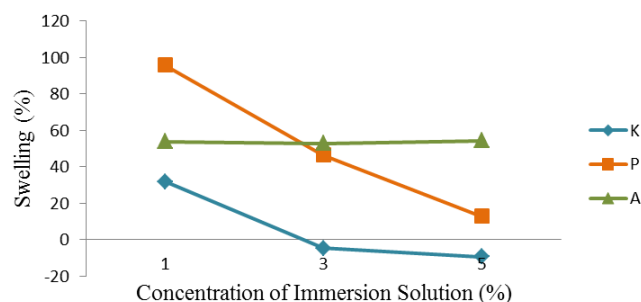


Figure 1. The plot of the concentration of immersion solution versus swelling

The results of the One Way Analysis of Variance (ANOVA) at this stage shows that in the same immersion time, variations in the concentration of the soaking solution had a significant effect ( $P < 0.05$ ) on the swelling.

The effectiveness of the immersion process is marked with the large of swelling[20]. Figure 1 shows the sequence of the level of effectiveness of the immersion process with an acid solution based on largest swelling of each type of solution. It can be seen that the values follow this trend :  $\text{H}_3\text{PO}_4$  1 % (v/v) (P 1) >  $\text{CH}_3\text{COOH}$  5 % (v/v) (A 1) >  $\text{HCl}$  1 % (v/v) (K 1).

The immersion of fish scales using  $\text{HCl}$  3 and 5% (v/v) shows negative value of swelling. This means that there has been excessive collagen solubility in the solvent as has been stated by Suwardi et al[22]. Concentration and strength of the acid are too high lead to the termination of more hydrogen bonds the among triple-helix and cause greater volumes of collagen which dissolves into the solution of pre-treatment, causing decrease in protein and yield of the resulting gelatin[6]. The selection of concentration at this stage of pre-treatment to obtain best extraction conditions for gelatin is very important.

The results of the One Way Analysis of Variance (ANOVA) at this stage shows that variations in immersion time had a significant effect ( $P < 0.05$ ) on the swelling.

Figure 2 shows the sequence of the level of effectiveness of the immersion process with an acid solution based on largest swelling of each type of solution that is successively as follows : P 1 for 24h (P 2) > A 1 for 36h (A 2) > K 1 for 24h (K 2). According to Sumbono et al[10], in the acid process and at the same immersion time (Figure 1), the soaking solution is obtained by the highest swelling with the highest acidity (K 1), because  $\text{Cl}^-$  anions are more electronegative than  $\text{PO}_4^{3-}$  and  $\text{CH}_3\text{COO}^-$ . This resulted in a larger dipole moment solvent lead to the gravity of the positively charged ions also become larger. Larger attractive force which resulted in the ability to disrupt and break some hydrogen bonds within and between molecules present on collagen are also getting bigger. The more broken hydrogen bonds will make collagen more strained coil, in order to obtain a larger number of swelling.

After going through the process of immersion, fish scales washed with demineralised water. Suwardi et al reported that washing amino acid salts with water to neutral pH can eliminate the mineral content and  $\text{Cl}^-$ ,  $\text{H}_2\text{PO}_4^+$ ,  $\text{CH}_3\text{COO}^-$  ions on amino acid salts released with water[22].

### Variation of Immersion Time

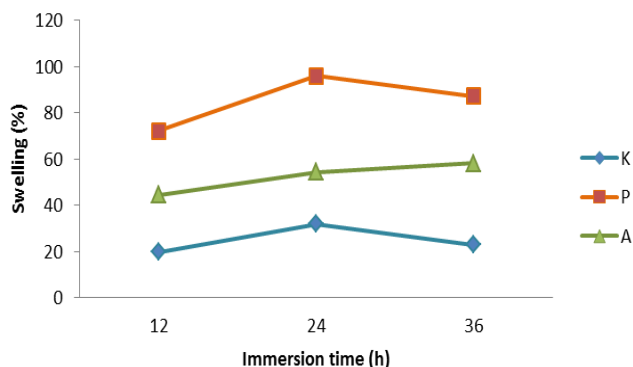


Figure 2. The plot of the immersion time versus swelling

### C. Gelatin Extraction

Extraction was carried out by hydrolysis method using conventional waterbath with a ratio of scales and water of 1: 2. In addition, it was also used variations in temperature, i.e. 60, 70 and 80 ° C. According to Fatimah et al[23], in addition to the treatment with acids, the tropocollagen will also be denatured by heating. This extraction process causes the triple-helix molecule loses its stability and breaks down into 3- $\alpha$  chain. The  $\alpha$ -chain is a helic peptide chain that is right-hand or clockwise[24]. Extraction with warm water damage crosslinking which subsequently can damage hydrogen bonds, a stabilizing factor for collagen structure. During extraction, helical triple structures are denatured into single water-soluble chains, small polymers or fragments.

The extract was filtered to obtain a filtrate and stored in a refrigerator temperature of 4-5 ° C to form a jelly. During the cooling process, there was a partial recovery of the helix in collagen fibers that lose conformation during the heating process. Gelatin are always in the formed of jelly due to its water trapped in the matrix chain. The structure of gelatin changes during gelation as a chain undergoes different spatial settings and interactions. These characteristics depend on gelatin concentration, temperature, and activation energy for the formation of secondary structures. Then, the jelly was dried at 60 ° C to remove the water contained in dissolved gelatin so that gelatin films are formed with the strength of the  $\alpha$ -chain bond structure which is stronger as the state of type B in the Ahmed et al[25] report. Subsequently, the film gelatin reduced in size to form gelatin powder.

### Variation of Extraction Temperature

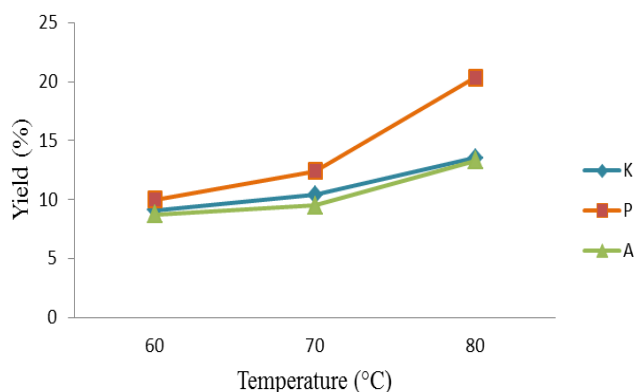


Figure 3. Plot of extraction temperature versus yield

In the extraction process after pre-treatment with an acid solution, OH<sup>-</sup> ions from electron-rich H<sub>2</sub>O attack the carbonyl group in amino acid tissue, while the H<sup>+</sup> ion reacts with

excess H<sub>2</sub>O to form H<sub>3</sub>O<sup>+</sup> (Figure 5). The presence of an OH<sup>-</sup> group bound to the carbonyl group causes resonance and the intra-molecular bond becomes weak. Crosslinking triple-helix breaks down into a crosslinking chain- $\alpha$ ; which is exemplified in the form of amino acids alanine and glycine [22].

Table 1. The Yield of Gelatin from Several Parts of Red Snapper Fish

No	Source	Yield (%)		
		K	P	A
1.	Skin	2.63	3.97	2.10
2.	Bones	2.92	2.71	0.49
3.	Scales	15.12	13.50	13.36

The results of the One Way Analysis of Variance (ANOVA) at this stage (Figure 3) show that the extraction temperature variation had a significant effect (P <0.05) on the yield of the gelatin produced. In the same soaking solution used in the pre-treatment process and with the same extraction time, it is known that there is an increase in yield to temperature, which is true for all types of soaking solutions. This is similar to the report submitted by Kaewruang et al [26] that with the same extraction time there is a trend of increasing the yield of gelatin to increase the extraction temperature used. This was also confirmed by Muyonga et al [27] and Kittiphattanabawon et al [28] which reported an increase in the yield of gelatin from fish Nile perch skin and blacktip shark fish skin due to higher extraction temperature. A higher temperature used in the extraction process will provide a higher energy also to damage the hydrogen bonding as a stabilizer matrix of collagen in fish scales. As a result, more intensive denatured collagen formed and produce a higher yield of gelatin as well.

Gelatin extraction effectiveness shown by the yield of the resulting gelatin[29], can be is also characterized by the amount the swelling as a result of the immersion process[20]. Figure 3 shows the sequence of the level of effectiveness of the extraction process with pre-treatment acid based on the largest yield of each type of solution is : P 2 at 80 ° C (P 80) > K 2 at 80 ° C (K 80) > A 2 at 80 ° C (A 80). This was related to the events occurring in the pre-treatment process because the H<sup>+</sup> ion from the acid solvent acts to break the hydrogen bonds so that the collagen coil stretches which results in a more optimum thermal distribution at the time of extraction. Consequently, more peptide bonds are broken.

Table 1 shows that the yield of bone gelatin and Red Snapper skin (from data from research conducted by Sumbono et al [10] is lower than the yield value of gelatin made from Red Snapper scales. It can be concluded that gelatin made from fish scales Red Snapper has a great potential as one alternative to mammalian gelatin prospective.

### D. Moisture

Determination of moisture is performed to determine the amount of water that is bound by the solid component of a material. The water content in a material able to determine the appearance, texture and survivability of such materials to attack by microorganisms, so that the water content is one of the important variables related to the quality and duration of storage gelatin. This is because gelatin is a hydrocolloid compound soluble in water and can absorb water in large enough quantities.

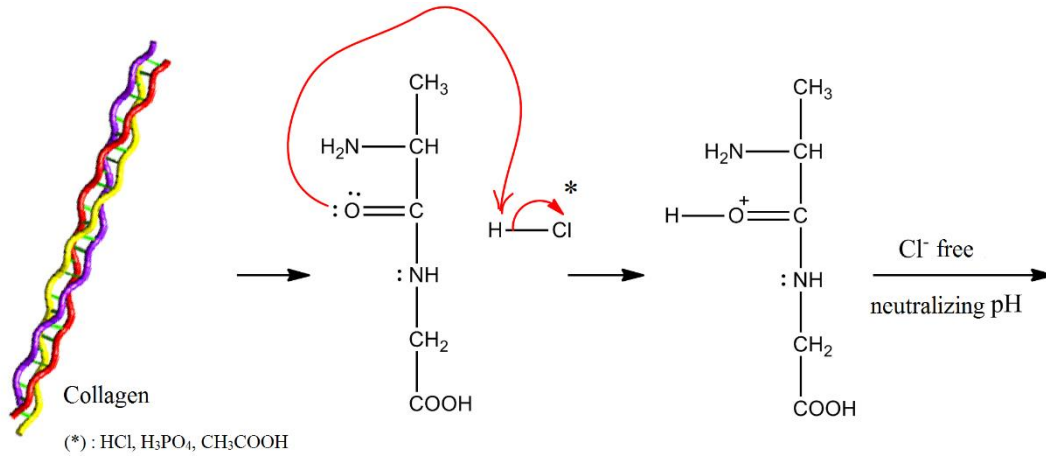


Figure 4. Proposed reaction mechanism in the acid pre-treatment process

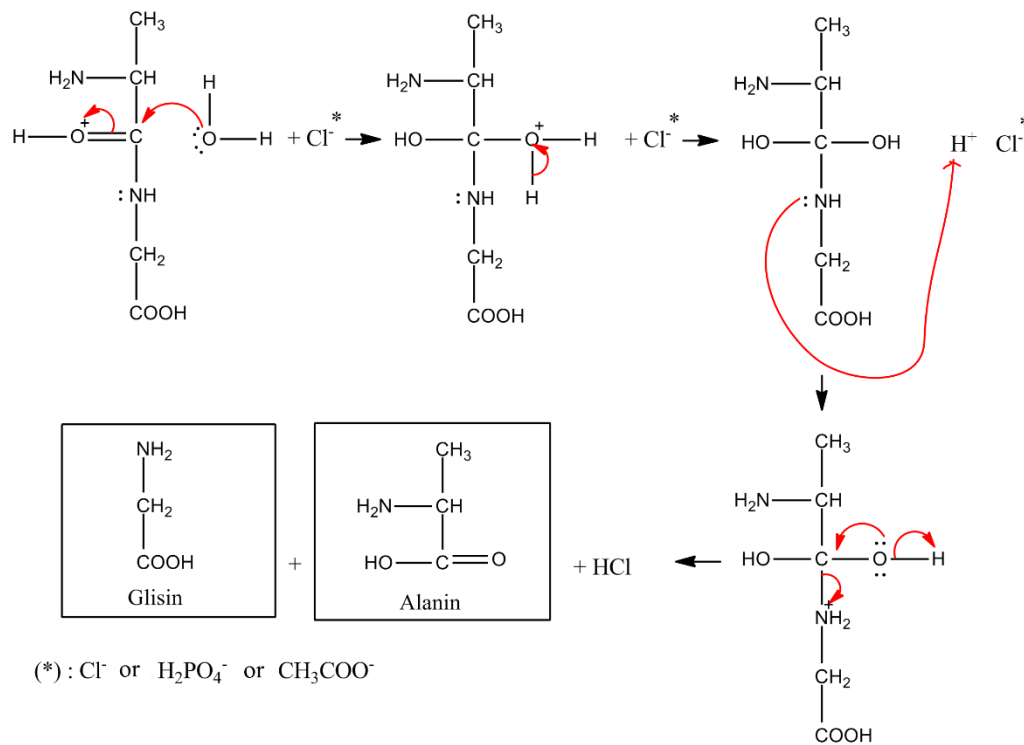


Figure 5. Proposed reaction mechanism in the extraction process

Table 2 shows that the average moisture of gelatin produced in this study is 3.37-10.38%, and the majority is lower than the control, that is commercial bone gelatin made from beef (9.33%). The water content also has met the Indonesian National Standard (SNI) gelatin, i.e. a maximum of 16%.

The moisture of the gelatin is not affected by temperature extraction, but is influenced by several factors, including the type of solution, the source of the gelatin and the process of drying and storage prior to analysis[10].

**E. pH**

Measuring the pH value of gelatin solution is very important because it affects the properties of other gelatin, namely viscosity and gel strength[17], and affects the application of gelatin products[30].

The pH of gelatin solution did not correlate with the source of gelatin or extraction temperature, but with the soaking solution used in the pre-treatment stage.

Table 2. Physical Properties of Gelatin

Moisture (%)	60 °C	70 °C	80 °C
K	4.44 ± 0.00	3.37 ± 0.00	3.87 ± 0.00
P	10.38 ± 0.00	8.61 ± 0.00	7.27 ± 0.00
A	6.68 ± 0.00	6.86 ± 0.00	9.40 ± 0.00
Control		9.33 ± 0.00	
pH	60 °C	70 °C	80 °C
K	5.51 ± 0.20	5.40 ± 0.00	5.75 ± 0.20
P	5.08 ± 0.18	4.52 ± 0.04	4.25 ± 0.06
A	6.23 ± 0.04	5.92 ± 0.04	6.10 ± 0.00
Control		5.83 ± 0.12	
Viscosity (cP)	60 °C	70 °C	80 °C
K	13.14 ± 0.43	10.01 ± 0.23	6.02 ± 0.15
P	7.55 ± 0.12	7.29 ± 0.22	4.58 ± 0.16
A	15.55 ± 0.36	12.33 ± 0.25	7.14 ± 0.20
Control		6.88 ± 0.04	
Melting point (°C)	60 °C	70 °C	80 °C
K	33.00 ± 0.00	33.00 ± 0.00	33.50 ± 0.50
P	54.00 ± 0.00	42.00 ± 0.00	65.00 ± 0.00
A	64.50 ± 0.50	65.00 ± 0.00	65.00 ± 0.00
Control		83.00 ± 90.00	

Table 2 shows that the gelatin produced by pre-treatment solution is actually higher than P and K, as the pH of the solution A were higher than the other two solutions. This is similar to Sumbono et al [10] reported that the differences were due only to the use of the type and concentration of the soaking solution. The lower of pH (the more acidic) the soaking solution, the lower the pH of the gelatin solution.

Gelatin pH obtained in this study is 4.245–6.225. The pH was not significantly different when compared to controls, namely commercial gelatin made from bovine bones (5.835). The pH also meets the European Pharmacopoeia standard on GME [31], of which the pH value of gelatin ranges from 3.8–7.6. Gelatin with a neutral pH will be more stable and become very wide application.

#### F. Viscosity

Viscosity is also one of the important physical properties of commercial gelatin. Gelatin with low viscosity result in short and brittle gel; while gelatin with high viscosity provides a stronger and longer chain[32]. For many applications, the gelatin with high viscosity are preferred and are sold at a higher price. Some research suggests that fish gelatin solution has a higher viscosity than the pork and beef gelatin[32]. The best viscosity in this study was obtained from pre-treatment gelatin using solution A, which was 15.54 cP which was better than the control - commercial gelatin made from beef bone (6.88 cP).

Table 2 shows that there is a downward trend in the gelatin solution viscosity due to higher extraction temperature. In addition, there is a correlation between pH viscosity, where the higher the pH of the gelatin, the higher the viscosity. The viscosity of gelatin with pre-treatment P is lower than K and A which may be caused by the presence of low molecular weight peptide chains as a result of excessive hydrolysis of collagen during the acid pre-treatment stage. This is similar, as indicated by the swelling P compared to C and A in the pre-treatment stage. The effect of extraction temperature and pH on viscosity was confirmed by Lin et al [32] who reported that the viscosity of gelatin depends on concentration, temperature, pH and additional salt, and partly controlled by the average molecular weight and molecular size distribution of proteins; which means that it is dependent on pre-treatment methods gelatin.

#### G. Melting Point

The melting point is the other important properties of gelatin. As a thermo-reversible gel, gelatin gel will begin to melt when the temperature rises above a certain point[2]. Gelatin has a unique characteristic among hydrocolloids in thermo-reversible gel formers with a melting point below or close to body temperature, which is very important in pharmaceutical food applications[1].

The melting point value of gelatin in this study is presented in Table 2 which shows the difference in melting point of gelatin due to variations in pre-treatment conditions but does not show any difference due to the increase in extraction temperature. Gelatin treated with solution K shows a lower melting point than the other two solutions. The lower the pH of the soaking solution, the lower the melting point of the gelatin produced. This is similar to the events that occurred at the stage of pre-treatment, which obtained the greatest swelling with solution soaking K. Collagen coils due to the pre-treatment of K are more tenuous than P and A, so the thermal distribution is more optimum at extraction and results

in more hydrogen bonds being broken. At least the hydrogen bonds make the collagen structure is unstable and has a low ability to withstand thermal, so it becomes a low melting point. The best melting point in this study was obtained from pre-treatment gelatin using solution A, at 65 °C which was closest to the control - commercial gelatin made from cow bone (83–90 °C).

#### H. Functional Group Analysis

The FT-IR spectra are analyzed to confirm the functional groups of gelatin structure come from Red Snapper scales (*Lutjanus sp.*). There are four areas that commonly appear in the FT-IR spectra, i.e. amide A, amide I, amide II and amide III [27], that the difference is only in the location of the structure. The FT-IR spectra also used to study about secondary structure change of the functional groups of gelatin[26]. All gelatins are characterized using FT-IR to prove that gelatin contained the constituent functional group as contained in control - mammalian gelatin come from bovine.

Based on the overall FTIR spectra obtained in this study (Figure 6-9), it can be seen that there are five areas of a typical gelatin fish, i.e. amide A, amide B, amide I, amide II and amide III, and it shows there are slight difference in the spectra. The five functional groups are also reported in gelatin come from bone and skin of Red Snapper[10], unicorn leatherjacket skin[26], and bighead carp scales[33].

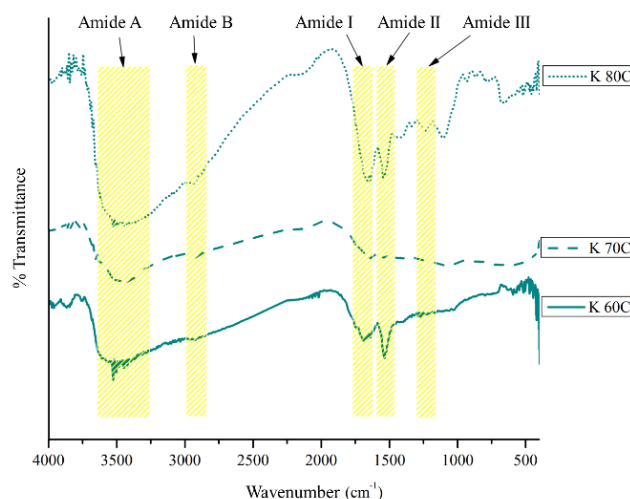


Figure 6. FT-IR spectra of gelatin using K pre-treatment

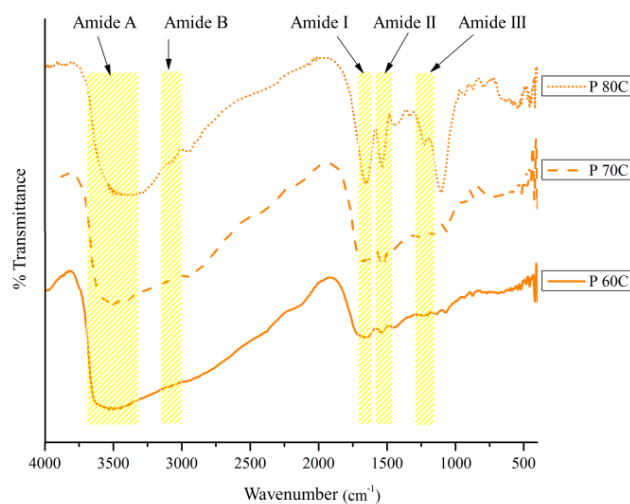


Figure 7. FT-IR spectra of gelatin using P pre-treatment.

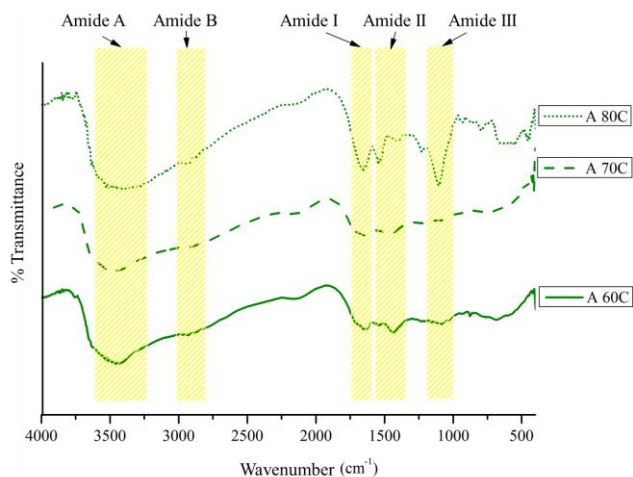


Figure 8. FT-IR spectra of gelatin using A pre-treatment.

Table 3.

The Wavenumber of Functional Group of Gelatin Using Acid Pre-Treatment

Functional group (cm <sup>-1</sup> )	Temperature variation (°C)		
	60	70	80
<b>HCl (K)</b>			
Amide A	3525	3427	3446
Amide B	2922	2924	2929
Amide I	1639	1653	1658
Amide II	1535	1541	1545
Amide III	1269	1199	1230
<b>H<sub>3</sub>PO<sub>4</sub> (P)</b>			
Amide A	3468	3500	3356
Amide B	-	2924	2937
Amide I	1651	1635	1658
Amide II	1535	1537	1535
Amide III	1230	1238	1226
<b>CH<sub>3</sub>COOH (A)</b>			
Amide A	3427	3441	3367
Amide B	2931	2922	2929
Amide I	1633	1639	1658
Amide II	1546	1533	1545
Amide III	1230	1209	1226
<b>Control</b>			
Amide A		3446	
Amide B		2929	
Amide I		1643	
Amide II		1539	
Amide III		1242	

In this study, the area of Amida A is absorbed at wavenumbers 3525-3356 cm<sup>-1</sup>. Amida A shows stretching-NH in pairs with hydrogen bonds, and is absorbed at wavenumbers 3440-3400 cm<sup>-1</sup>. Amide A from the triple-helix polymer is observed at very high frequencies, around 3400-3300 cm<sup>-1</sup>, compared to the frequency of Amide A in polypeptides and other proteins[26], [33].

The Amide B area is absorbed at wavenumber 2960-2874 cm<sup>-1</sup>. The Amide B area represents the asymmetric stretching-CH vibration as well as NH<sub>2</sub>. [33] reported that the Amida B area in the range of 3100-3000 cm<sup>-1</sup>, so gelatin come from Red Snapper scales is classified as low in Amide B. This is occur due to the interaction of NH<sub>3</sub> groups among peptide chains.

The Amide I area is absorbed at wavenumber 1683-1631 cm<sup>-1</sup>. It is confirmed by Muyonga et al [27] who reported that the Amida I area of gelatin is absorbed at wavenumber 1700-1600 cm<sup>-1</sup>, the most important area in analyzing secondary structure of proteins in FT-IR spectra. Amida I represents a combination of stretching-C=O vibrations which are

hydrogen bond couple with COO, stretching-CN, CCN deformation and bending-NH. Amida I is characteristic of the gelatin coil structure. The difference in Amida I spectra in all gelatin samples produced is due to the conformational differences of the polypeptide chain, similar with the Tu et al report [35] that Amida I area is useful in studying material naturalness and change in protein conformation.

The Amide II area is absorbed at wavenumber 1548-1442 cm<sup>-1</sup>. This is confirmed by Kewruang et al [15] who reported that the Amide II area of gelatin is absorbed at 1560-1500 cm<sup>-1</sup>. Amida II vibration mode is related to the combination of stretching-CN and bending-NH of the peptide group [35].

The Amida III area is absorbed at wavenumbers 1269-1080 cm<sup>-1</sup> and confirmed by Tu et al [35]. Amida III is a complex vibrational mode of peak combination of stretching-CN and bending-NH associated with amides and significant absorption arises as vibrations of wagging-CH<sub>2</sub> from the glycine backbone and proline side chain. The Amida III of the overall gelatin spectra indicates a disorder of the gelatin molecule structure associated with the loss of the triple-helix state [15].

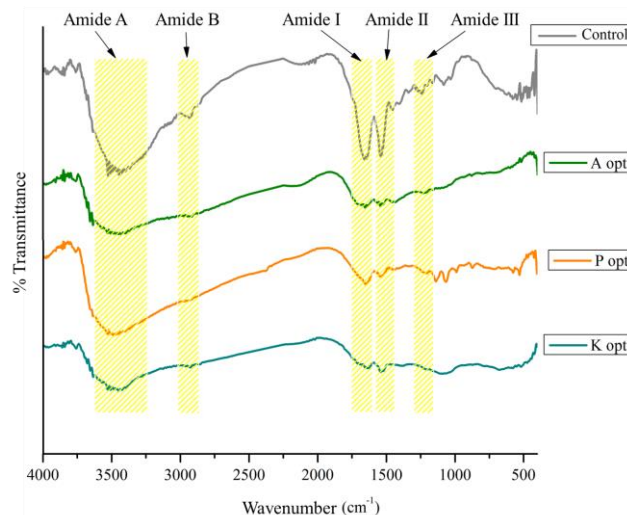


Figure 9. FT-IR spectra of gelatin using acid pre-treatment

#### IV. CONCLUSION

The preliminary study to obtain optimum conditions of the extraction procedure gelatin from fish scales red snapper (*Lutjanus sp.*) by acid hydrolysis method has been successfully carried out. Excessive denaturation of collagen structure into dissolved gelatin is very important to prevent because it can affect the quality of the gelatin produced. It is concerned with coil stretch of collagen which results in a more optimum thermal distribution at the time of extraction, thus determining the pre-treatment conditions are best suited to be important. Variations in temperature carried out to determine the properties of gelatin, such as moisture content, pH, viscosity and melting points, which are important properties to ensure widespread application. FT-IR spectra of gelatin also has confirmed its constituent functional groups, namely amide A, amide I, amide II, and amide III groups, which are also contained in commercial gelatin from mammals.

#### ACKNOWLEDGMENT

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