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Optimization of Extraction Time on The Characteristic of Gelatin from Scales of Red Snapper (*Lutjanus* sp.)

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Abstract. Fish fillet products that are increasingly popular have an impact on the accumulation of waste that can pollute the environment. Red snapper scales are one of the fishery byproducts that can be used as raw material for making gelatin because they contain collagen. The quality of the resulting gelatin can be affected by the extraction time, so research is needed to find out the optimal extraction time to get the quality of gelatin from red snapper scales that meet quality standards. This study uses a completely randomized design (CRD) with five treatments and four replications in each treatment. The treatment in this study was the use of different extraction times, namely one, two, three, four, and five hours. Data analysis was performed descriptively by comparing the results of testing the characteristics of red snapper scales gelatin with SNI and GMIA gelatin quality standards. The results showed that the extraction time of one hour was the optimal time in the process of extracting gelatin red snapper scales with yield of 7.38%, gel strength of 304.74 grams of Bloom, viscosity of 7.5 cp, water content of 14.02%, ash content of 1.23%, and pH 6.6. Red snapper scales can be used as raw material for gelatin to support the concept of zero waste in the fishery product processing industry.

1. Introduction

Gelatin is one of the products produced through the hydrolysis of collagen contained in the skin, bones and connective tissue of animals, including fish and poultry [1]. Gelatin has a very high protein content and low fat. Dry gelatin with 8-12% water content has a protein content of around 84-86%, 2-4% minerals, and contains almost no fat [2]. The content causes gelatin has a very important role in diversification of food ingredients, especially amino acid content. Gelatin contains nine of the ten types of essential amino acids needed by the body. The essential amino acid that is almost not contained in gelatin is tryptophan [3].

Fish fillet products that are increasingly popular have an impact on the accumulation of waste that can pollute the environment. According to Pan *et al*. [4] whole fish contains 20-25% edible meat and 75-80% waste that can be treated from the total weight of fish. The waste is in the form of head, skin, bones, stomach contents, and scales which are considered as low-value materials. According to Ninan [5], the use of fish byproducts for gelatin production as an alternative to mammalian gelatin raises several practical problems. First, fish collagen is very vulnerable to damage when compared to more stable mammalian collagen. Second, the raw material for the production of gelatin from fish that is the skin can experience rapid enzymatic and microbial damage when stored together with the rest of the byproducts including intestinal contents causing the quality of the resulting gelatin to vary.

Red snapper scales are one of the fishery byproducts that can be used as raw material for making gelatin because they contain collagen. Chemical compounds contained in fish scales are mostly albuminoids such as collagen (24%) and ichthylepidin (76%) of 41-13% organic protein [6]. Fish scales and bones are preferred in gelatin extraction because they produce large amounts of gelatin due to their high amino acid (proline) content compared to fish skins. The strength properties of the gel are almost the same as commercial pig skin and bone [7]. The hydroxyproline collagen retaining rate in fish scales is 96.10% [8].

Research on the pre-treatment conditions of the red snapper scales gelatin extraction procedure, starting from the acid hydrolysis method to the optimal therm distribution when red snapper scales gelatin extraction was carried out by Wahyuningtyas *et al*. [9]. The extraction time affects the viscosity and gel strength of the gelatin extracted from the skin of red snapper [10]. Optimal extraction time will cause collagen hydrolyzed and converted to gelatin optimally [11]. The results and quality of gelatin are apparently not only influenced by the species or origin of the extracted tissue but also by the extraction process, which may depend on pH, temperature, and time during pretreatment and extraction [12], therefore is necessary to compare gelatin extraction time to get quality that meets gelatin quality standards. This study aims to determine the optimal extraction time in the process of producing gelatin from red snapper scales (*Lutjanus* sp.).

2. Materials and methods

2.1. Materials

The raw material used for research is red snapper scales waste obtained from PT. Alam Jaya Surabaya, distilled water, and acetic acid (CH_3COOH) 3%.

2.2. Methods

This study used a Completely Randomized Design (CRD) consisting of five treatments and four replications. The treatment in this study was gelatin extraction time, which was one, two, three, four, and five hours.

Red snapper scales are washed first using running water until they were clean then drained, and stored in a tightly closed zip-lock plastic bag. The process of making gelatin consists of the processes of demineralization, neutralization, extraction, filtration, drying, and grinding. The process of making gelatin from red snapper scales waste uses the method of Wangtueai and Noomhornm which was modified in the demineralization and drying stages [13].

Soaking red snapper scales in the demineralization process using 3% CH_3COOH solution with a ratio of fish scales and CH_3COOH 1: 3. The demineralization process aims were to break down the structure of the triple helix collagen [3] and eliminate the mineral content contained in fish scales [11]. The demineralization process was carried out for 18 hours. Based on the research of Trilaksani *et al*. [14], the best gelatin, using a 3% CH_3COOH concentration and an immersion time of 18 hours.

The process of neutralizing fish scales using flowing water for one hour until the pH was neutral. The next step was the extraction process using distilled water. The extraction process aims were to continue the breakdown of the triple helix collagen structure into a water-soluble α-helix structure, namely gelatin [3]. The extraction process was carried out using a waterbath at a temperature of 75°C for one, two, three, four, and five hours with a ratio of fish scales and distilled water 1: 2.

The fourth step was t filtration process of gelatin solution using filter cloth and accommodated in glass moulds measuring 15 cm x 15 cm x 3 cm, then the filtrate is dried in an oven at 60°C [13] for 72 hours to obtain dry gelatin in sheet form . Gelatin sheets are ground using a grinder [11]. Next, gelatin of red snapper scales was stored in a sample bottle and tightly closed.

Testing of red snapper scales gelatin powder consists of FTIR and gelatin characteristics which include yield, gel strength, viscosity, water content, ash content, and pH. FTIR testing uses an infrared spectrophotometer to determine the functional groups of a product, so it can be seen that the resulting compound is gelatin [15]. The yield was obtained through a comparison between the weight of the red snapper scaled gelatin powder that was produced with the weight of the raw material for red snapper

scales [11]. Gel strength was measured using a texture analyzer [1]. Viscosity measurements were carried out at 60ºC using a viscometer [16]. Gelatin water content by calculating the ratio between the initial weight difference of gelatin with gelatin dry weight and gelatin initial weight [17]. Gelatin ash content by calculating the ratio between the weight of gelatin ash and initial gelatin weight [18]. Measurement of the degree of acidity (pH) is carried out at room temperature using a pH meter [1].

2.3. Analysis of data

Data analysis of gelatin characteristics consisting of yield, viscosity, gel strength, water content, ash content, and acidity (pH) using descriptive method. The gelatin characteristic data is compared with the Gelatin Handbook [19] and the Indonesian National Standard [20].

3. Results and discussion

3.1 Results

Based on the FTIR results of red snapper scales, there is an amine (NH) group at wave number 3257.24 cm^{-1} and CH group at wave number 2934.32 cm^{-1}. The carbonyl group (C = O) is in the wave number 1628.83 cm^{-1} and the hydroxyl group (OH) is in the wave number 1437.93 cm^{-1}. The data of FTIR gelatin red snapper scales test results are in Figure 1.

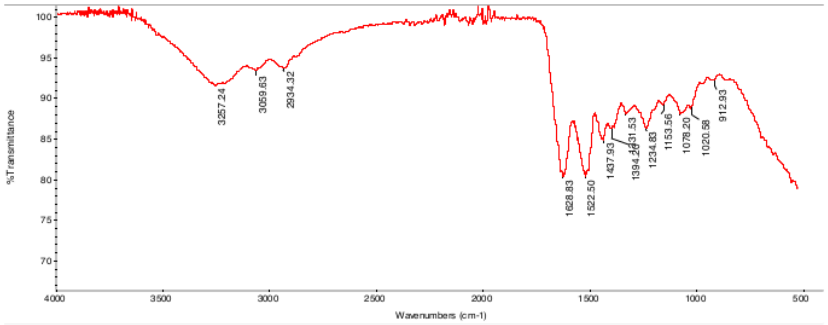

Figure 1. FTIR test results of red snapper gelatin scales

Table 1. Data on the results of testing the characteristics of red snapper scales gelatin

Characteristics	Extraction Time				
	1 h	2 h	3 h	4 h	5 h
Yield (%)	7.38	8.06	8.22	8.67	9.31
Gel strength (Bloom)	304.74	463.28	368.36	358.98	349.59
Viscosity (cp)	7.5	15.25	11.75	11.25	7.25
Moisture (%)	14.02	14.02	14.04	14.04	14.04
Ash (%)	1.23	1.39	1.59	1.62	1.84
pH	6.6	6.3	6.3	6.3	6.3

Based on the research results, the yield of red snapper scales gelatin was 7.38-9.31%. This value increases with increasing extraction time. The lowest strength value of red snapper scales gelatin was produced by one hour extraction time, which was 304.74 grams of Bloom, while the highest value of red snapper scales gelatin strength was found in the extraction time of two hours, namely 463.28 grams of Bloom. The lowest viscosity value of red snapper scales gelatin was produced by five hours of extraction time, namely 7.25 cp, while the highest viscosity value of red snapper scales gelatin was produced by two hours of extraction time, which was 15.25 cp. The water content of red snapper scales gelatin was 14.02-14.04%, and the ash content of red snapper scales was 1.23-1.84%. The gelatin ash

content of red snapper fish scales increased along with the increase in extraction time. The pH value of the extraction time of two hours, three hours, four hours, and five hours was relatively the same, namely 6.3, while the one-hour extraction time treatment was 6.6.

3.2 Discussion

According to Suptijah *et al*. [15], gelatin has a structure consisting of carbon (C), hydrogen (H), hydroxyl group (OH), carbonyl group (C = O), and amine group (NH). The results of FITR showed that the product produced by the study was gelatin. According to Hermanto *et al*. [21], the NH group is in the wave number area 3400-3200 cm^{-1}, the CH group is in the wave number area 3100-2800 cm^{-1}, and the C = O group is in the wave number area 1660-1600 cm^{-1}, while the OH group is in the wave number region 1500-1300 cm^{-1} [15].

The highest yield of red snapper scales gelatin produced by the treatment of extraction time of five hours, which is 9.31%. In contrast, the extraction of grouper fish scale gelatin [9] obtained a yield of 13.36% with pre-treatment using CH_3COOH (w/v: 1/10) for 24 hours. While the pre-treatment of lizardfish scales using 0.1-0.9% NaOH solution for 1-5 hours at 30ºC resulted in a gelatin yield of 9.1-10.9% [17]. The type of solvent used to convert collagen to gelatin can affect the yield value [11]. Acid solvents can convert triple-helical collagen fibres into single chains, whereas basic soaking solutions are only able to produce double chains [22]. Pre-treatment using a low concentration of the acid solution is sufficient to produce swelling and break the intra and inter molecular non-covalent bonds [23].

The results showed the strength value of the red snapper scales gelatin gel produced did not meet the quality standard of type A gelatin (gelatin made acidically) according to GMIA [19] which is 50-300 grams of Bloom according to the needs of users in various industrial sectors [24]. Gel strength is the main physical property of gelatin, because gel strength shows the ability of gelatin in gel formation [11]. The lowest strength value of red snapper scales gelatin gel and close to GMIA standard [19] is the treatment of one hour extraction time, which is 304.74 grams of Bloom (Table 1). Lizardfish scale gelatin extraction with 1: 2 w/v distilled water for 1-5 hours at 70-90ºC resulted in the strength of gelatin gel of 268 ± 5.39 grams Bloom [13]. The strength of the gelatin gel depends on the length of the amino acid chain. Perfectly hydrolyzed collagen can produce long polypeptide chains [22].

The viscosity value of red snapper scales gelatin according to the specified quality standard of gelatin, which is 1.5-7.5 cP [19]. The viscosity value of red snapper scales gelatin that meets the quality standard is a one hour extraction time treatment of 7.5 cP and a five hour extraction time treatment of 7.25 cP. The viscosity of red snapper scales gelatin [9] with extraction at 80ºC, a value of 7.14 ± 0.20 cP was obtained, while extraction of lizardfish scales gelatin at 70-90ºC was obtained viscosity values of 3.43-5.63 cP [13]. According to Ward and Courts [22], the conversion of collagen to gelatin is influenced by temperature, heating time and pH.

The moisture content value of the red snapper scales gelatin does not meet the quality standard type A gelatin according to GMIA [19], which is 8-13%, but it still meets the SNI gelatin quality standard (1995), which is ≤ 16% [20]. Table 1 shows the moisture content of red snapper scales from all treatments ranging from 14.02-14.04%. This shows the extraction time did not affect the value of the gelatinous water content produced. According to Winarno [25], the value of water content of a food can affect the appearance, texture, and taste and can determine acceptability, freshness, and durability of food ingredients.

The value of gelatin ash content of red snapper scales produced did not meet the quality standard of type A gelatin according to GMIA (2012) [19] which was 0.3-2.0% [19] but fulfilled the SNI quality standard (1995) which was ≤ 3.25% [20]. The lowest value of gelatin ash content of red snapper scales was produced by the extraction time treatment of one hour which was 1.23%, while the highest value of gelatin ash content of red snapper scales was produced by the five hour extraction time treatment of 1.84%. The minerals contained in the scales are calcium [6], so that the remaining calcium which does not dissolve in the acetic acid solution will also be extracted in the gelatin extraction process which causes the ash content to increase with increasing extraction time (Table 1).

The pH value of red snapper scales gelatin approaching normal is one hour extraction time treatment which is 6.6 higher than the other four treatments which have a pH value of 6.3 (Table 1). The pH value of red snapper scales gelatin with extraction at 80°C shows 6.10 [9]. According to Europan Pharmacopoeia standards [26], gelatin pH values range from 3.8-7.6 and gelatin with neutral pH will be more stable and become a widespread application of its use. The neutralization process with repeated washing after the demineralization process with the acid solution can increase the pH value [11]. According to Wahyuningtyas *et al*. [9], gelatin pH does not correlate with gelatin source or extraction temperature, but with the immersion solution used in the pre-treatment stage.

Based on the results of the study (Table 1), the extraction time of one hour is the optimal time because it produces gel strength and gelatin viscosity values close to GMIA [19] gelatin quality standards, which are 50-300 Bloom and 1.5-7.5 cp. According to Mariod and Adam [27], gel strength and viscosity are the main physical properties in assessing gelatin quality according to standard conditions, because it affects the application of gelatin in various products. Gel strength and viscosity depend on the distribution of molecular weight and amino acid composition.

4. Conclusion

One hour extraction time was the optimal time in the process of extracting gelatin from red snapper scales with a yield of 7.38%, gel strength of 304.74 grams of Bloom, the viscosity of 7.5 cp, moisture content of 14.02%, the ash content of 1.23%, and pH 6.6.

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