



## Extraction of collagen bone fish (*Thunnus albacares*) into gelatin with CH<sub>3</sub>COOH treatment

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Open Access Research Journal of Biology and Pharmacy, 2022, 04(02), 015–021

Publication history: Received on 06 January 2022; revised on 08 March 2022; accepted on 10 March 2022

Article DOI: <https://doi.org/10.53022/oarjbp.2022.4.2.0027>

### Abstract

Indonesia is one of the countries in Southeast Asia with the largest Muslim population in the world and the number is estimated at around 86%. This amount is actually a separate opportunity in marketing halal products and one of them is gelatin from fish skin. Gelatin is a protein extracted by partial hydrolysis of collagen, with the main component being protein derived from the skin of meat or fish, animal or fish bones and hides. The purpose of this study was to produce good quality fish skin gelatin with acetic acid treatment and soaking time of 12, 24 and 48 hours. The method used in this research is the experimental method on a laboratory scale. The results obtained in this study were that the rendemen of fish skin gelatin ranged from 7.50 to 7.60% with CH<sub>3</sub>COOH treatment. Meanwhile, the best pH value is acetic acid immersion with a time of 48 hours with a pH value of 2.8-6.2. Furthermore, the best organoleptic values for attributes (color, texture and scent) are as follows; 6.5; 6.2 and 6.5 with 48 hours of immersion.

**Keywords:** Collagen, CH<sub>3</sub>COOH; Gelatin extraction; Yellowfin tuna skin

### 1. Introduction

Gelatin is a polypeptide obtained by thermal hydrolysis of collagen. Gelatin has a very wide application in various industries such as the food, pharmaceutical, cosmetic and photographic industries. In the food industry, gelatin is widely used as an emulsifier, stabilizer in emulsion systems. The quality of gelatin for a particular application depends on the structural properties, as well as whose physicochemical properties are strongly influenced not only by the species or tissue from which it is extracted, but also by pretreatment and extraction methods.

Gelatin is a protein component produced from the hydrolysis of collagen from bones and skin which has been widely used for various industrial purposes, both food and non-food because it has unique properties, and can change reversibly from sol to gel form, and can expand in cold water, and can form a film, can affect the viscosity of a material, and can protect the colloid system. At a temperature of 71 °C, gelatin can be easily dissolved in water and can form a gel at a temperature of 49 °C The potential for Gelatin development in North Maluku is promising, this is due to the proliferation of fish companies operating in this area for the export scale of tuna and skipjack tuna. The number of Maddihang Loin Fish Filing Companies for export scale continues to increase every year, along with the demand for yellowfin stock in several countries continues to grow. Yellowfin loin is exported to several countries, for example, Japan, Korea and Europe, which continues to increase. This is because the quality of yellowfin tuna in North Maluku is of fairly good quality and the quantity is quite abundant. The results of the filtering, if left unchecked, will actually cause problems for the environment. These problems arise as a result of poor waste management. The habit of fishermen and

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industry is usually this waste, dumped into the sea, buried or burned so that it can pollute the environment if it is in large enough quantities.

Seeing this phenomenon, the idea arose to utilize yellowfin tuna skin waste, as a source of minerals, especially calcium and phosphorus which have been implemented several years previously in the form of bone powder and fortified into food products and used as raw materials for drugs as supplements or herbs. This is in accordance with research that this fish bone waste has a high mineral content as a suitable material as a natural calcium source (Malde *et al.* 2010). This is reinforced by research (Talib *et al.* 2009) that yellowfin tuna bone waste contains minerals, especially calcium and phosphorus which are quite high so that it is very suitable to be used as a substitute material in food products, feed or as a supplement. Another benefit that can be developed from yellowfin tuna bones is gelatin, the gelatin in the bones and fish skin is quite high when processed and can be used for additional food products. The protein composition of fish meat consists of 65–75% myofibrils, 20–30% sarcoplasm and 1–10% stroma. Stromal protein is found in the bones, scales, skin, fish fins and it is a solid waste that has not been utilized properly by the fishery product processing industry.

According to proportion of fish bones to the fish body reaches 12.4% (Marsaid and Atmaja, 2011). Thus, solid waste in the form of bone from the processing is estimated 7,460 tons. If these wastes are not used properly, they can pollute the environment which can interfere with the health of the surrounding community. It is known that these wastes have just been processed into fish meal as animal feed and by utilizing fish bone waste into gelatin as a food additive with high economic value, can be a solution to the problem of waste from the fisheries processing industry.

The potential of abundant fish skin and bone waste can be an opportunity to develop halal gelatin because Indonesia is an Islamic country with the largest Muslim population in the world, which is 86%. This number is an opportunity as well as a challenge in the future because the need for gelatin for the food and pharmaceutical industries continues to increase along with the times that continue to experience dynamics. The need for gelatin raw materials continues to increase, while obtaining halal gelatin is very difficult because it must be exported from various countries in the world. The issue of halal food source products is often a polemic in Indonesia, namely the circulation of information about non-halal food and medicine ingredients. According to the theory, gelatin is a protein obtained from the partial hydrolyzation of collagen, which is usually derived from skin, bones, hides, and connective tissue from animal bodies (Domb *et al.*, 2014). Until now, the demand for gelatin continues to increase, especially in the food, non-food and pharmaceutical industries. So far, the extraction of gelatin generally comes from the skin or bones of cows and pigs. The raw material source of gelatin which is sourced from pork or other types of materials that are not halal is actually a problem in Indonesia because the majority of the population is Muslim. Other ingredients commonly used to make gelatin are cow skin or bones, but they also cause problems because they are associated with mad cow disease or bovine spongiform encephalopathy (BSE).

The above description indicates that to meet the needs of gelatin in Indonesia is not easy, it must require extra labor to meet the needs of gelatin in the country. One alternative to meet the needs of halal gelatin in Indonesia is to utilize gelatin sources derived from fish. Utilization of gelatin derived from fish bones or skins is very appropriate to be developed in the future. One type of fish that can be used as raw material for gelatin is yellowfin tuna. Yellowfin tuna is a type of fish that has hard bones and contains collagen ranging from 15-17% (Rachmania *et al.*, 2013). Based on the analysis of the situation above, the utilization of yellowfin tuna bone waste as an alternative to halal gelatin sources is very appropriate to be developed considering the abundant potential of fish skin.

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## 2. Material and Methods

### 2.1. Place and time of research

This research took place at the Fishery Product Processing Laboratory, University of Muhammadiyah North Maluku, sedangkan waktu pelaksanaan penelitian dilakukan pada bulan September-Desember 2021.

### 2.2. Tools and Materials used in Research

The raw material used is yellowfin tuna (*Thunnus albacares*) skin. The material used in this research is  $\text{CH}_3\text{COOH}$ . The tools used in this study were: basin, bucket, kitchen knife, cutting board, ruler, heating oven, LPG gas, litmus paper and a sieve. For drying the material, a cabinet dryer is used. As well as other tools used consist of one unit of collagen extraction equipment: jar, 1000 ml beaker, cool box, filter and calico cloth. While the tool used for organoleptic test is a score sheet.

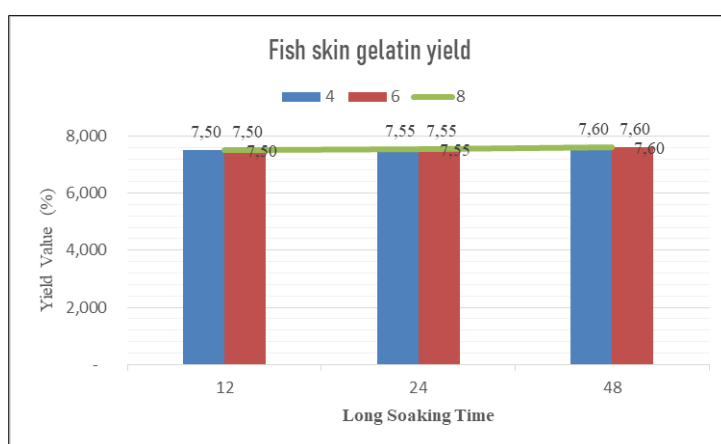
### 2.3. Research procedure

Yellowfin tuna skin is washed with clean and running water and remove the remaining fish skin that is still attached, the next process is to reduce the size by cutting it into small rectangular pieces. The next process is immersion of the sample using a  $\text{CH}_3\text{COOH}$  solution with a concentration of (0,48%), with a ratio of 1:4 with immersion time (12, 24 and 48 hours). The next process after soaking is the fish skin is washed with distilled water 3 times until the pH is neutral and ossein will be obtained. To produce quality collagen, ossein is extracted again with a ratio of 1:3 using a water bath with a duration of 6 hours at a temperature of  $85^\circ\text{C}$  to obtain collagen extract. After getting the collagen extract, the material is ready to be placed on a baking sheet, then heated or in the oven at  $50^\circ\text{C}$  for 24 hours. The last step is to test the gelatin product with the test parameters are rendemen and organoleptic which includes (color, texture and aroma) and pH value.

## 3. Results and discussion

### 3.1. Rendemen

Rendemen is one of the important parameters in calculating the initial weight and final weight of a food product. Rendemen can be used as an important parameter to determine the economic value of a particular product. The Rendemen of yellowfin tuna skin in the manufacture of gelatin resulted in a smaller rendemen along with the length of treatment time. The Rendemen of gelatin produced depends on the length of immersion, the longer the immersion, the stronger the ability to absorb so that it can affect the weight of fish skin gelatin. The complete rendemen of yellowfin tuna skin gelatin can be seen in Figure 1.



**Figure 1** Average rendemen of yellowfin tuna skin gelatin

The results of the calculation of the rendemen of gelatin ranged from 7.50 to 7.60%. The lowest gelatin rendemen value was found in the  $\text{CH}_3\text{COOH}$  treatment at 12 hours of immersion and 4% acetic acid concentration, while the highest was in the same treatment with 48 hours of soaking and 8% acetic acid concentration with a value of 7.60%. The treatment of immersion time and concentration of acetic acid can have a significant effect on the rendemen. This is presumably due to the increasing concentration of acetic acid ( $\text{CH}_3\text{COOH}$ ) so that the collagen structure is more exposed and causes the amount of collagen to be hydrolyzed so that the extracted gelatin will increase.

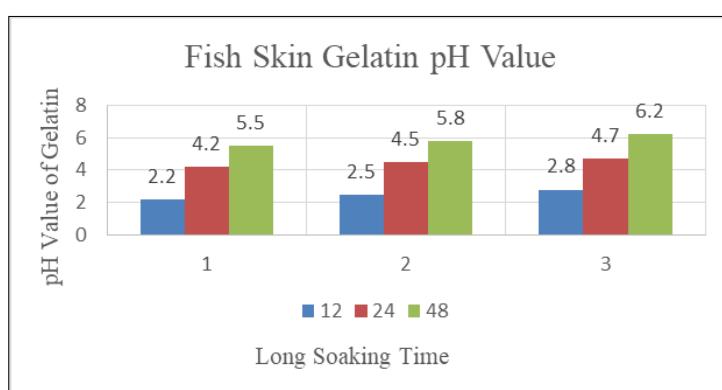
This is in accordance with research (Trilaksani, 2012) on red snapper skin gelatin with a rendemen value of 16.8%. While in the study (Yenti, 2015) on gelatin from the skin of the sepat rawa fish using the immersion method with different solutions of HCl,  $\text{H}_3\text{PO}_4$  and acetic acid with the same concentration of 2% for 24 hours. Another study was conducted by (Nirmala, 2017) with a rendemen value of 17.0% with heating for 6 hours. The solvent used was distilled water with a temperature of  $75^\circ\text{C}$  and the rendemen value was 17.0%. The rendemen produced with acetic acid solution is 3.51%. The difference in the rendemen of gelatin produced is due to differences in the extraction method, both concentration and type of solvent used to remove non-collagen proteins and also the type of raw material used (Potaros et al., 2009). The high concentration of acid and the long soaking time used can produce good gelatin.

The high rendemen value with  $\text{CH}_3\text{COOH}$  immersion treatment is thought to be influenced by the high gelatin component and the low level of other components, such as the presence of mineral salts related to the ash content of the material. In line with Karim and Bhat (2008) if the gelatin component is low and the non-gelatin component is high,

it will reduce the quality of the gelatin. The low rendement value of gelatin with  $\text{CH}_3\text{COOH}$  immersion treatment is thought to be caused by the nature of the acid which belongs to the weak acid group so that it is not able to completely decompose collagen fibers. In accordance with the research of Wulandari et al., (2013), the low rendement of gelatin is thought to be due to collagen denaturation at high temperatures during the extraction process and the collagen fibers have not been completely broken down into gelatin. Based on the results of the study, it was found that the gelatin obtained from the research with the  $\text{CH}_3\text{COOH}$  immersion treatment had a very low rendement when compared to previous studies.

### 3.2. Test pH Value

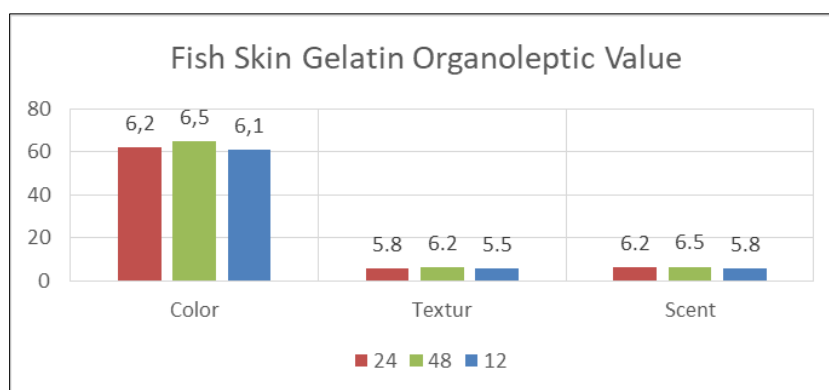
The pH value in this study with the duration of immersion in acetic acid on day one to day three showed a significant difference. The lowest pH value was found in the acetic acid immersion treatment for 12 hours with a pH value of 2.2 on day one, while the highest was in the acetic acid immersion treatment for 24 hours on the third day with the highest value at pH 6.2. The measurement of the pH value in this study was intended to determine the degree of acidity in three washings of fish skin material before being processed into fish skin gelatin. The level of acidity in fish skin material with a pH value is presented in Figure 2.



**Figure 2** The pH value of yellowfin tuna skin material

The use of solvents used can affect the high and low degree of acidity of the product. The pH value can also be used as an indicator to physically or chemically determine the agar in a given material. The pH value measured in fish skin gelatin products is very significant. This is because the use of  $\text{CH}_3\text{COOH}$  to clean and dissolve fish skin has a very positive impact. According to Nurilmala et al (2021) about the physical and chemical characteristics of the skin gelatin of catfish, tilapia and tuna, respectively ( $5.56 \pm 0.15$ ;  $5.67 \pm 0.06$  and  $5.65 \pm 0.02$ ). Furthermore, according to Purnawijayanti (2001) stated that the degree of acidity (pH) is very favorable for the growth of destructive bacteria and pathogens with a pH value of more than 4.6 to pH 7. The results of the research on fish skin gelatin showed that the pH value of fish skin gelatin would increase along with the washing process which was carried out repeatedly. This is when compared to SNI GMIA, 2019 with a pH value range of 3.8-7.5, the pH value in this study is still in accordance with what has been determined.

### 3.3. Gelatin's Organoleptic Characteristics



**Figure 3** Average yield of gelatin with  $\text{CH}_3\text{COOH}$  treatment

Organoleptic characteristics of yellowfin tuna skin gelatin treated with  $\text{CH}_3\text{COOH}$  solution were carried out by 15 semi-trained panelists. The results of the tabulation of organoleptic values for the attributes of color, texture, aroma are presented in Figure 3.

### 3.3.1. Gelatin Color

The results of this research on yellowfin tuna skin gelatin products indicate that the color of the resulting product is in accordance with the tests carried out by panelists with an average color value in the range of 6.1-6.5. Color is one of the factors to determine product quality visually so that it can be an attraction for consumers. Color will give an impression before the product is consumed and attractive colors can give the impression of being expensive and elegant according to the product.

### 3.3.2. Gelatin Texture

Texture is an indicator to determine the quality of food because it gives satisfaction to consumers. Therefore, it is important that the texture of gelatin greatly affects the quality of the product. Texture is closely related to the crunchy taste of the resulting product (Winarno, 1995). Assessment of texture comes from the touch by the skin surface, usually using the fingertips so that the texture of a material can be felt, texture includes smooth, dense, rough. The indicator for determining the texture organoleptic numbers is in the numbers (1) to (7) starting from the number of dislikes to numbers that really like. The organoleptic test value on gelatin texture samples with  $\text{CH}_3\text{COOH}$  extraction treatment was in the range of 5.5-6.2 (Figure 3), with the highest value on gelatin with an immersion time of 48 hours with a value of 6.2.

### 3.3.3. Aroma Gelatin

Aroma can be interpreted as one of the five senses that can be observed with the sense of smell or smell which produces a rotten or fragrant aroma. The sense of smell or smell reaches the olfactory tissue into the nasal cavity together with the air. Sensing in this way can give an idea that the smell or smell is absolute that can be judged by the human senses (Winarno, 1995). The indicators for determining the aroma are unscented (1) to very flavorful (7). The organoleptic test value on gelatin aroma samples with  $\text{CH}_3\text{COOH}$  extraction treatment was in the range of 5.8-6.5 (Fig. 3), with the highest value in the 48-hour immersion treatment with a value of 6.5.

### 3.3.4. Pair Comparison Test

The results of the physical appearance of gelatin with  $\text{CH}_3\text{COOH}$  immersion treatment with a length of time (12, 24 and 48 hours) the results were not much different, but the panelists preferred gelatin with an immersion time of 48 hours. The appearance of color with the 48 hour immersion treatment was brighter in color when compared to other treatments which included (color, texture and aroma) in the form of dry powder and had a distinctive fish odor. The research results are compared with commercial products or products sold in the market produced by (PT. Brataco) with bone and cowhide gelatin as raw materials, the color and odor attributes of the two gelatin products are slightly different.

Judging from the aroma aspect, the aroma produced from yellowfin tuna skin gelatin in this study is thought to come from volatile compounds derived from gelatin raw materials which are very distinctive. Primary Research et al. (2013) showed that the volatile compounds in fish skin generally consist of several constituent components including aldehydes, alcohols, ketones, and hydrocarbons. These volatile compounds will interact with the proteins contained in fish during the processing process, causing a specific or distinctive fish aroma found in fish skin gelatin products. The physical form of gelatin is presented in Figure 4.



**Figure 4** Appearance of Gelatin; (a)  $\text{CH}_3\text{COOH}$  (b) Commercial Gelatin

Commercial gelatin tends to be yellowish white in color and almost odorless. Meanwhile, gelatin with CH<sub>3</sub>COOH immersion research has the shape and size of the granules and the color resembles commercial gelatin. Gelatin from research with CH<sub>3</sub>COOH immersion treatment is gelatin that most closely resembles commercial gelatin, compared to gelatin from other studies. So that in terms of physical appearance examination in this study, it was found that the gelatin from the research with CH<sub>3</sub>COOH immersion treatment had a high degree of similarity with commercial gelatin compared to gelatin from other studies. Gelatin products should have a main structural component consisting of white connective tissue, which contains almost 30% of the total protein in vertebrate and invertebrate organ tissues (Setiawati, 2009).

Gelatin is a product that has water soluble properties so it can be used for industrial purposes (Wahyuni and Paranginangin, 2008). Fish skin gelatin produced from this study when compared with commercial gelatin, commercial gelatin has a fairly high degree of whiteness. This is because it affects the type of solvent used. Solvents contribute greatly to the quality of fish skin gelatin products. This is because the main ingredient of gelatin is protein, which contains 85 to 95%.

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#### 4. Conclusion

The best yellowfin tuna skin gelatin was made using CH<sub>3</sub>COOH treatment with a soaking time of 48 with the highest yield value ranging from 7.50 to 7.60%. Meanwhile, the best pH value was soaking in acetic acid for the same 48 hours with a pH value of 2.8-6.2. Furthermore, the best treatment organoleptic values for attributes (color, texture and aroma) are as follows; 6.5; 6.2 and 6.5 with the same duration of immersion time.

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#### Compliance with ethical standards

##### *Acknowledgments*

We would like to thank the entire research team who participated in this research. We also thank you for the research grant program batch V of the PP Muhammadiyah Diktilitbang assembly for funding this program.

##### *Disclosure of conflict of interest*

No conflict of interest.

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