



## **Fish Bone Gelatin**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

The purpose of this review article is to examine the method of making gelatin, the characteristics of gelatin from the results of research that has been carried out in Indonesia and the benefits of fish gelatin. Based on a review of various articles and other literature, it can be concluded that fish bone gelatin can be extracted by the acid method. The production of fishbone gelatin consists of 4 stages, the preparation of raw materials includes removal of non-collagen components from raw materials, conversion of collagen to gelatin, purification of gelatin by filtering and finally drying and powdering. Fishbone gelatin can be applied to both the food and non-food industries.

*Keywords: Gelatin; circular economy; application; gelatin extraction; conversion.*

## **1. INTRODUCTION**

The development of the fish processing industry in Indonesia is currently experiencing an increase, such as the frozen fish fillet industry, which produces processing waste in the form of fish bones [1]. So far, fish bones have only been used as animal feed, so it only increases the

economic value slightly, even fish bones are discharged without use [1]. Based on this, it is necessary to make an effort to utilize fish bone waste into something more useful, one of which is by processing fish bone waste into gelatin. Utilization of fish bone waste into gelatin aims to reduce the impact of environmental pollution. In addition, the use of fish bones as raw material for

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gelatin is a cleaner production of fish processing. Cleaner production is a processing concept to reduce the impact on environmental pollution [2].

The use of pork skin and bones as raw material for gelatin is not appropriate when applied in a country where the majority of the population is Muslim, such as Indonesia [3]. This is because pigs are prohibited animals for consumption, while the use of gelatin from cow raw materials is also still a concern because of the disease outbreaks carried by livestock, including anthrax disease and mad cow disease [4]. Therefore, it is necessary to develop gelatin products with other animal raw materials, including fish.

Bone is one of the binding weaves. Bone consists of cells, fibers and fillers. The bone fillers are protein and mineral salts, such as 58.3% calcium phosphate, 1.0% calcium carbonate, 2.1% magnesium phosphate, 1.9% calcium fluoride and 30.6% protein. Bone contains approximately 50% water and 15% red and yellow marrow. Marrow consists of 96% fat [5]. The bone which has been taken for fat consists of organic matter and inorganic salts in a ratio of 1: 2 [5]. The removal of organic matter by heat does not cause a change in the overall bone structure, but will reduce bone weight, because bones lose some of its organic matter. The high protein content in fresh bones is a good medium for processing into raw materials for gelatin production [6].

The proportion of fish bones to fish bodies reaches 12.4 %. In 2003, 900 tons of fish bones were produced from the tilapia fillet industry [7]. Generally, the yield of gelatin from fish bones is around 12 %, so it is estimated that the gelatin that can be obtained from 6,703 tons of fish bones is 804.6 tons [7]. Indonesia imported 2,715,782 kg of gelatin with a value of US \$ 9,535,128 from various countries (France, Japan, India, Brazil, Germany, China, Argentina and Australia) to fulfill domestic supply [8]. Increasing the productivity of gelatin in the country is needed. The things that support the level of gelatin productivity include the availability of raw materials and the selection of the right hydrolysis method, so that it can produce gelatin that meets Standar Nasional Indonesia.

Many researches related the utilization of fish as an alternative source for producing gelatin and the optimization of the process have been carried out. Improvement of the value added of tuna (*Thunnus* sp.) bone waste to gelatin and analysis

of physico-chemical properties [9]. Production of gelatin from fish bones and its use as a base material for making capsule shells [2]. Effect of defatting and extraction temperature on the characteristics of snakehead fish bone gelatin [10]. The effect of several types of acid solutions (HCl, H<sub>3</sub> PO<sub>4</sub>, and CH<sub>3</sub> COOH) on the manufacture of gelatin from the skin of swamp sepat fish [11]. The use of acetic acid solvent in the demineralization process of milkfish bones (*Chanos chanos*) on gelatin production at concentrations of 2%, 4%, 6%, and 8% [12]. Therefore, this review article aims to examine the meaning of gelatin, the method of making gelatin, the characteristics of gelatin from the results of research that has been carried out in Indonesia and the benefits of fish gelatin.

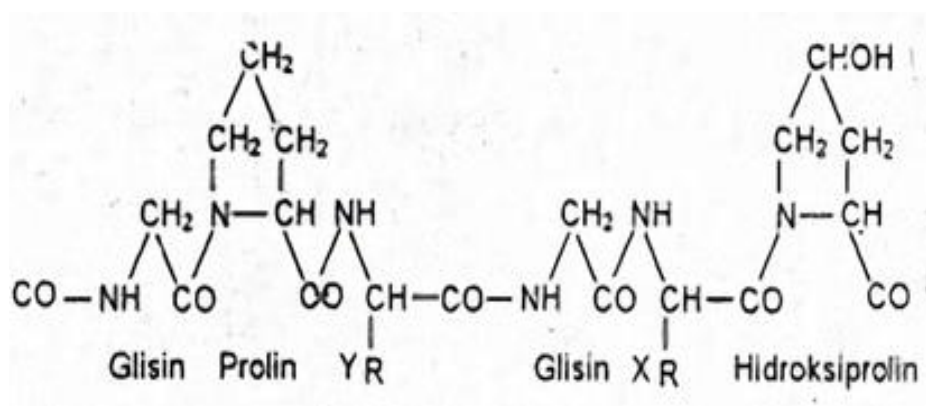
## 2. GELATIN

Gelatin comes from the word "gelatus" which means sturdy, strong and physically frozen. The term gelatin was popularized in 1700. Gelatin has a dry, dense, tasteless and transparent form [13]. Gelatin is a protein resulting from hydrolysis of bone and skin collagen. Gelatin is one of the ingredients that is increasingly being used, both for food and non-food products. This is related to its benefits, among others, as a stabilizer, gelling agent, binder, thickeners, emulsifiers, adhesives, food wrappers [14].

Amino acids are bonded to each other via peptide bonds to form gelatin. In Fig. 1, you can see the gelatin chemical structure in the form of Gly-XY where X is generally the amino acid proline and Y is generally the amino acid hydroxyproline. The absence of tryptophan in gelatin causes gelatin to not be classified as a complete protein [15].

The average gelatin molecular weight ranges from 15,000 - 250,000 Da. The molecular weight of gelatin is around 90,000 Da while the average molecular weight of commercial gelatin ranges from 20,000 - 70,000 Da [16].

Gelatin is divided into two types based on different processing processes, namely type A and type B. In making gelatin type A, the raw material is treated with immersion in an acidic solution so that this process is known as the acid process. Meanwhile, in the manufacture of type B gelatin, the treatment applied was alkaline treatment. This process is called the alkaline process [17].



**Fig. 1. Gelatin chemical structure**

Source: Grobden et al. [15]

Fish gelatin is categorized as type A gelatin [18]. Economically, the acid process is preferable to the alkaline process. This is because the immersion in the acid process is relatively shorter than the alkaline process.

The process of converting collagen into gelatin involves the following three changes: First, the breaking of a number of peptide bonds to shorten the chain, second is breaking or disrupting a number of camping bonds between chains. The three changes in chain configuration [19].

Gelatin is soluble in water, acetic acid and alcohol solvents such as glycerol, propylene glycol, sorbitol and mannitol, but insoluble in alcohol, acetone, carbon tetrachloride, benzene, petroleum ether and other organic solvents [2]. Gelatin dissolves easily at a temperature of 71.1°C and tends to form a gel at a temperature of 48.9°C [20]. The heating is carried out to dissolve the gelatin at least 49°C or usually at a temperature of 60-70°C [21].

Gelatin has properties that can change reversibly from sol to gel form, swell or expand in cold water, can form films, affect the viscosity of a material, and can protect the colloid system [22]. Such properties make gelatin preferable to materials such as xanthan gum, Carrageenan and pectin [17].

### 3. THE METHOD OF MAKING GELATIN

In principle, the process of making gelatin is divided into two, namely the acid process and the alkaline process. The difference between these two processes lies in the immersion process [23]. Based on the strength of the protein cross-

covalent bonds and the type of material extracted, the application of organic acids and bases and other extraction methods such as hydrolysis time, pH and temperature will vary [24].

Making gelatin base is less effective for gelatin production, because at the same time the amount of collagen hydrolyzed by alkaline solutions is much less than acidic solutions [25]. Therefore, soaking with an alkaline solution takes much longer than the acid process to hydrolyze collagen.

Acid preparation of gelatin is generally more suitable for fish bones. However, the type of acid solution used can vary greatly, both organic and inorganic acid solutions. Acid is able to convert triple helical collagen fibers into single chains, while alkaline immersion solutions are only able to produce double chains [26].

The process of making gelatin is divided into four stages: The first stage of raw material preparation includes removing non-collagen components from the raw material. The second stage is the conversion of collagen to gelatin. The third stage is purification of the gelatin by filtering. The last stage is drying and powdering [27].

The first stage is the process of washing or cleaning the bones. This process aims to remove or remove impurities, especially the remains of meat that stick to the bones. Washing or cleaning can be done by heating with boiling water for 1-2 minutes. After that, degreasing is carried out, which is the process of removing fat from the bone tissue. This process is carried out by

immersing the bones in water with the optimum temperature (between the melting point of fat and the coagulation temperature of bone albumin). The optimum degreasing process is a temperature of 32-80°C so as to produce optimum fat solubility [14].

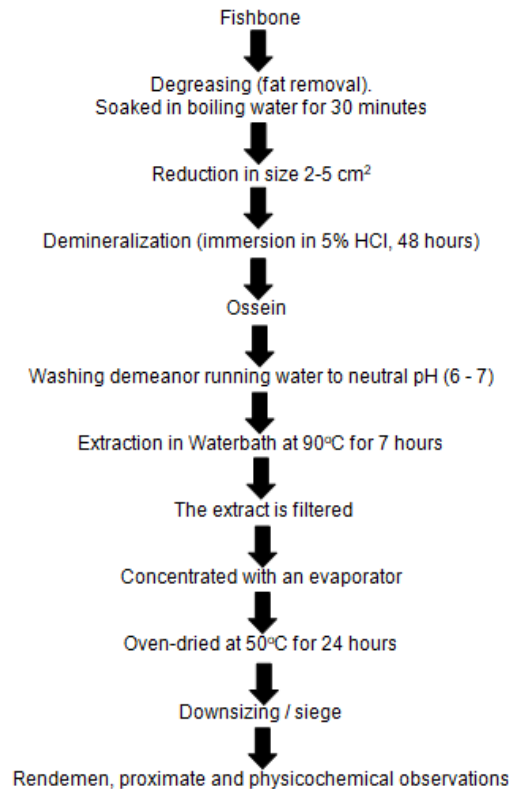
Furthermore, the demineralization process is carried out, namely the process of removing calcium and salt in the bones, resulting in a soft bone called ossein where there is collagen in it. Ossein is soft bone that contains collagen and other proteins [27]. The acid used in the demineralization process is hydrochloric acid with a concentration of 4-7%. The inorganic acids used are hydrochloric acid, phosphoric chloride, and sulfuric acid [14]. Meanwhile, according to Hinterwaldner [27], this demineralization process should be carried out in an acid-resistant container for several days to two weeks.

The next step, the ossein was extracted with heated water. Extraction aims to convert collagen into gelatin. The minimum temperature in the

extraction process is 40 - 50°C [6] to 100°C. Extraction of bone collagen is carried out in an acidic environment at pH 4-5 because generally this pH is the isoelectric point of non-collagen protein components, so that it is easily coagulated and removed [27]. If the pH is lower it needs fast handling to prevent further denaturation [17].

Then the extracted gelatin solution is concentrated before drying. Concentration is done to increase the total solid of the gelatin solution so that it speeds up the drying process. This can be done using a vacuum evaporator, then dried in an oven at a temperature of 40-50°C [6] or 60-70°C [28]. Reducing the size is carried out to further expand the surface of the material so that the process can take place more quickly and perfectly. Therefore, the resulting gelatin is more reactive and easier to use [17].

Schematically, the stages in making fish-based gelatin can be seen in Fig. 2.



**Fig 2. The process of making gelatin from fish bones**

Source: Junianto et al. [2]

## 4. RESEARCH RESULTS ON "CHARACTERISTICS OF GELATIN FROM FISH BONES"

According to research by Farida et al. [29] on "Characteristics of Gelatin Made from Raw Skipjack Fish Bone (*Katsuwonus pelamis*) Using Different Types of Acid". The types of acids used were 4% HCl, 4% CH<sub>3</sub> COOH and 4% H<sub>2</sub> SO<sub>4</sub>. The research results are as follows:

### 4.1 Sensory Test

Furthermore, the organoleptic parameters are as follows:

#### 4.1.1 Color

The color of the gelatin produced from this study is colorless to yellowish, where the color can be influenced by the freshness of the raw material at the time of making the gelatin. This is in line with the statement of Gunawan et al. [30], which states that the brightness of this gelatin can be determined by the raw material and at the time of the manufacturing process. Gelatin can be colored like this because of the drying process that can be affected by the raw material, but it can also be affected by the immersion process during the process with the compounds used [31].

#### 4.1.2 Aroma

The aroma produced in this study is the aroma of volatile compounds, where these compounds come from the raw material for the manufacture, namely Skipjack Fish which does not have a distinctive aroma so that the acidic compounds given can have a significant effect on the resulting gelatin aroma. This study is in line with Pratama et al. [10] where fish contains volatile compounds including aldehydes, alcohols, hydrocarbons and ketones.

While the results obtained for the characteristics for the chemical test are as follows:

### 4.2 Chemical Analysis

#### 4.2.1 Water content

The results of this research regarding the water content do not meet Standar Nasional Indonesia standards, because the yield obtained is only 16%, which can happen due to the bonds formed between the gelatin molecules and the water, which is still weak, so that water can be trapped

in the gelatin through its structure. This statement is supported by Jones [32] where the lower the concentration of gelatin in a solution, the weaker the bonds between the molecules will be. The difference in each treatment, the value of the water content can be influenced by the amount of collagen that can form in the gelatin, so this can cause the water content to decrease.

#### 4.2.2 Ash content

The results of the ash content in this study get a result of 3.2% where these results can still meet the standards of Standar Nasional Indonesia [33]. In the results regarding the ash content, the success and quality level of the extraction process are said to be successful.

#### 4.2.3 Protein content

The results of this study regarding the ash content were gelatin when immersed in acetic acid, the highest yield was 46.88% and the lowest was sulfuric acid with a yield of 27.08%, this was because the protein contained in gelatin using acetic acid was able to hydrolyzing collagen and acetic acid can break down the fibers in collagen more and more quickly without damaging the gelatin [34].

The results obtained for the pH degree of acidity were 4.81 for acetic acid, for 3.34 for hydrochloric acid and 2.93 for sulfuric acid. According to Standar Nasional Indonesia [33], said that a good standard for pH is 3.8-6.0. The difference in pH value that occurs is due to the difference in the acid. In this study, the highest pH was obtained by acetic acid, and the lowest by sulfuric acid, this could occur because acetic acid is a strong acid when compared to hydrochloric acid or sulfuric acid. In addition, during the immersion process, collagen swelling occurs due to the remaining solution that cannot react to the previous one and cannot be absorbed by collagen.

While the results obtained for the yield in this study can be seen in the following table:

### 4.3 General Yield

The results obtained for the yield in this study were that the highest yield was sulfuric acid at 1.83% and the lowest was acetic acid at 1.04%. This can happen because there is the amount of H<sup>+</sup> ions in which it can hydrolyze the collagen in the skipjack tuna bones and can be different when the acid used is different.

**Table 1. Average Sensory Gelatin Test [29]**

Parameter				
Handling	Color ±	Category ±	Flavor ±	Category
CH <sub>3</sub> COOH ±	6.12 ± 0.04	White	5.27 ± 0.12	Less Fragrant
HCl ±	6.08 ± 0.04	Yellowish white	5.33 ± 0.06	Less Fragrant
H <sub>2</sub> SO <sub>4</sub> ±	6.16 ± 0.11	Bright white	5.26 ± 0.12	Less Fragrant

Information: \* soaking for 48 hours at a concentration of 4%

**Table 2. Chemical analysis of gelatin [29]**

Handling				
Parameter	CH <sub>3</sub> COOH ±	HCl ±	H <sub>2</sub> SO <sub>4</sub> ±	Standar Nasional Indonesia [33]
Water Content%	3.99	6.82	13.32	Maximum 16%
Ash Content%	2.84	3.19	2.27	Maximum 3,2%
Protein%	46.88	35.42	27.08	87.25%
PH	4.81	3.34	2.93	3.8-6.0

Information: \* soaking for 48 hours at a concentration of 4%

**Table 3. The result of the analysis of gelatin general yield [29]**

Handling	Basic Weight (g)	Material Sample Weight (g)	General Yield%
CH <sub>3</sub> COOH ±	600	6,2516	1.04
HCl ±	600	7,3150	1.21
H <sub>2</sub> SO <sub>4</sub> ±	600	11,0142	83.1

Information: \* immersion at the concentration of 4%

So, it can be concluded that the Cakalang fish bone gelatin is the best in the color sensory test with immersion using sulfuric acid with a value of 6.2, while for the resulting aroma test using hydrochloric acid attenuation which has a yield of 5.4%, for coars water is 13.32%, ash content is 3.19%, protein content is 35.42%, PH is 4.81, and the total yield obtained by the yield is 1.83%. The type of acid used had no significant effect on the type of fish bones used.

According to research by Atma et al. [35] gelatin was successfully extracted from catfish bones using pineapple waste in pre-treatment conditions (immersion of fish bones in pineapple waste) for 32, 48, and 56 hours with the main extraction (immersion in warm water) for 5 hours at a temperature 75°C. The Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used to analyze the gelatin existence. Fish bone gelatin has molecular weight ranging from 100-150 kDa and > 225 kDa. The hydroxyproline content of gelatin was 10.9–16.3 mg/g. Gelatin of Pangasius catfish bone extracted with pineapple waste has gel strength of 64.83 g.bloom g, hardness of 4.96 N, cohesiveness of 0.88, springiness of 1.03 mm, gumminess of 4.36 N, and chewiness of 2.78 N. Viscosity and pH of gelatin solution obtained were 3.17 cP and 4.52 respectively. The

proximate characteristics obtained were moisture 8.59%, ash 0.95%, crude protein 47.60% and fat 7.71%.

According to Panjaitan [36] research used hydrochloric acid with various concentrations of 1, 3, 5, 7, 9 and 11% (v / v) in the demineralation process for 4 days. Proximate analysis carried out in the form of measurement of water content, ash content, protein content, and fat content and adjusted to the predetermined method. The research results are presented in Table 4.

**Table 4. Yield of tuna fish bone gelatin with variations in the concentration of Hydrochloric acid [36]**

Acid Concentration (%)	Yield (%)
1	1.65
3	5.03
5	4.19
7	1.98
9	0.56
11	0.22

It was found that the highest yield of 5.03% was produced from hydrochloric acid with a concentration of 3%. The characteristics of the resulting gelatin had a water content of 8.59%, an ash content of 8.02%, a fat content of 0.27%,

a protein content of 80.2% with a molecular weight of gelatin in the range of 45 kDa and above 100 kDa and the strength of gelatin. is 167.8450 gram bloom.

It was concluded from this study that the highest gelatin yield was obtained from 3% hydrochloric acid concentration with 8.59% moisture content, 8.02% ash content, 0.27% fat content, 80.2% protein content. To find better results, further research is needed on the application of the resulting gelatin.

## 5. BENEFITS OF FISH BONE GELATIN

The food industry uses the gelatin industry a lot, including in products that require the formation of foam (whipping agent), usually in the manufacture of ice cream, while products that need to stabilize the results, this gelatin functions as a stabilizer. There are products that require gelatin to increase viscosity and also function as a binder, emulsifier and thickener [37]. In the field of photography, gelatin is used to extend the shelf life of storing photos, namely as a photoresist that can avoid (coating) from the presence of sensitive light [38].

Gelatin applications in food ingredients include gelling agents, thickeners, emulsifiers, foaming agents, edible films and stabilizers. In the pharmaceutical field, gelatin is widely used for the manufacture of soft and hard capsules [39]. Gelatin has the characteristic of being bright yellow or transparent to white, in the form of sheets, powder or like flour, stems, like leaves, soluble in hot water, glycerol and citric acid and other organic solvents. Gelatin can expand and absorb water 5-10 times its original weight [40].

## 6. CONCLUSION

Based on a review of various articles and other literature, it can be concluded that making gelatin acidically is generally more effective for fish bones, because at the same time the amount of collagen hydrolyzed by acid solutions is much more than alkaline solutions. However, the type of acid solution used can vary widely. The production of fishbone gelatin consists of 4 stages, the preparation of raw materials includes removal of non-collagen components from raw materials, conversion of collagen to gelatin, purification of gelatin by filtering and finally drying and powdering. Fishbone gelatin can be applied to both the food and non-food industries.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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