



## Skin and Bone Fish Waste Utilization for Gelatine Preparation by Acid Treatments: A Review

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### ABSTRACT

Gelatin is a high molecular weight polypeptide produced from collagen. Collagen found in connective tissue in animals such as skin, bones, cartilages, and veins. Fish skin is a fishery waste product that contains the highest collagen which is hydrolyzed into gelatin. Fish skin is a fishery waste product that contains the highest collagen which can be hydrolyzed into gelatin. Gelatin can be divided into two types, type A and type B gelatin. Type A is hydrolyzed using the acid process and type B is hydrolyzed using a base process. The special properties of gelatin are changing reversibly, forming films, expanding in cold water, and affecting the viscosity of the material and protecting the colloidal system. Gelatin is used in the addition of food ingredients because of its high nutritional value, thickening agent, clotting agent, emulsifier, stabilizer, elastic, foam-forming, thin coatings, and rich in nutrition.

Keywords: gelatin, hydrolysis, fish skin, collagen.

## INTRODUCTION

The Indonesian government is improving the industry in the field of marine and fisheries. One of the industries contained in the fisheries sector in Indonesia is the Tilapia fillet industry, one of the Tilapia fillet industries. Tilapia is a type of freshwater fish consumption that is quite popular among aquaculture fishers. A large number of fish culture production can cause a high rate of waste production because 1.24% of fish production is waste. It is estimated that almost 7.3 million tons of fish processing by-products waste are produced annually (Karim & Bhat, 2009). Waste in the form of skin and bones is the biggest by-product of around 20% of the total body weight of fish that have the potential to produce collagen (Fransiskha, 2016).

Utilization of fish skin waste as a source of gelatin is an effort in handling waste and can also increase the added value of fish skin waste because so far, the utilization of tilapia skin is still limited. Fish skin contains the protein that has high enough collagen, which holds the potential to be used as gelatin (Maryani et al., 2010).

Gelatin is a type of protein derivative from collagen fibers that can be extracted from the skin. Collagen is the major insoluble fibrous protein in the extra cellular matrix and connective tissues (Sekat et al. 2016). Gelatin has unique characteristics which include the ability to turn from sol to gel, be amphoteric, and maintain colloidal properties. According to Maryani et al., (2010) gelatin contains 19 amino acids linked by peptide bonds to form long polymer chains. Gelatin is a polymer composed of repeated units of amino acids such as glycine-proline or glycine-proline-hydroxyproline. The amino acids are interconnected through peptide bonds to form gelatin. The gelatin contained amino acids which are mostly contained among others glycine (26.4% -30.5%), proline (16.2% -18%), hydroxyproline (13.5%), glutamic acid (11.3% - 11.7%), and alanine (8.6% -10.7%).

The need for gelatin is increasing annually. Pig-based gelatin is selling in the market. Much research is constantly being done just to get the best gelatin sources. This is supported by several reasons, firstly gelatin from pigs causes problems for Muslim and Jewish populations because pigs are forbidden animals consumed, and the Hindu community does not consume products from cows. Secondly, safety reasons are related to the plague of Bovine Spongiform Encephalopathy (BSE). Third, there are allergic reactions in some people to products from pigs and cows (Badii & Howell 2006). Therefore fish waste such as bones, scales and skin which contain many collagen are now becoming safer alternative choice (Rochima et al. 2016).

## FISH SKIN WASTE

Fishery waste is gas, solid, or liquid waste produced from a production process both industrial and household, which is no longer useful and has no economic value and can even be detrimental. Fisheries industry waste in the form of innards is a part that has a high content of protein and unsaturated fats (Bhaskar et al., 2008). Waste from fishery products, in the form of skin, fish bones, fish head, and internal organs are usually left wasted so that it can pollute the environment even though from the waste part there is a skin containing collagen which if further processed can produce gelatin. Collagen is composed of skin waste which, if hydrolyzed, will produce gelatin (Agustin, 2015). According to Abun., (2006) fisheries waste has potential in the form of proteins and so on which can be processed chemically and biologically to produce products that are more useful and of economic value. Waste in the form of bones and skin of fish is the biggest waste which amounts to around 20% of the total body weight of fish which has the potential to be produced into collagen or gelatin (Fransiskha, 2016).

## COLLAGEN

According to Nurhayati et al., (2013), collagen is the main structural component of white connective tissue covering almost 30% of total protein in the body. There are about 25 types of collagen that have been identified, namely types I to XXV (Olsen et al., 2003). Collagen types have been identified in fish only type I and V. According to Nagai et al., (2002), type I collagen is found in skin, bones, and scales of fish, while type V collagen is found in connective tissue in the skin, tendons, and muscles of fish which also contains collagen type I. The content of collagen in tissue depends on the protein content, because collagen is the largest component in tissue protein, and the protein content in animal tissue depends on the type of animal, for example, large animal skins contain higher protein content compared to small animal skins. Collagen molecules are composed of twenty amino acids that have slightly different shapes depending on the source of the raw material. The main amino acids in collagen are amino acids glycine, proline, and hydroxyproline. Fish can be used as raw material for gelatin because, in certain parts of the fish, such as bones and skin, there is collagen which is by using the addition of acid or alkali treatment and the

heating process causes the collagen can be converted into gelatin. The collagen content of hard fish ranges from 15-17%, whereas in cartilage fish ranges between 22-24% (Nurilmala, 2004).

## GELATIN

Gelatin is a type of protein derivative from collagen fibers that can be extracted from the skin. Gelatin has unique characteristics which include the ability to turn from sol to gel, be amphoteric, and maintain colloidal properties. According to (Yuliani & Marwati, 2015) gelatin is a high molecular weight polypeptide produced from collagen, where collagen is abundant in the connective tissue of animals such as skin, bone, cartilage, and veins. Gelatin can be made from ingredients that contain collagen, such as skin, scales, and bones. Chen et al., (2016) stated that the content of collagen in the skin and scales is higher than bone. Another advantage of making gelatin with skin raw material is a faster process that is the process of acid immersion.

Gelatin contains 19 amino acids that are connected by peptide bonds to form long polymer chains. Gelatin compound is a linear polymer composed of repeat units of the amino acid glycine-proline-proline or glycine-proline-hydroxyproline. Gelatin does not contain tryptophan but contains a small amount of tyrosine and cystine.

Gelatin is divided into two types, gelatin type A and B. Gelatin type A is produced from the acid process, which is generally applied to the skin, where collagen molecules are young, whereas type B gelatin is produced from the acid and base process, which is generally applied to the bones and skin of cattle because the triple-helical collagen molecule is older so the cross bonds are denser and more complex (GMAP, 2002). Therefore, it can be said that the acid process is used for relatively soft raw materials, while the alkaline process is applied to relatively hard raw materials.

### Physical and Chemical Properties of Gelatin

The quality of gelatin is very much determined by the physical, chemical, and functional properties that make gelatin has a unique character (Fahrul, 2005). The physical and chemical properties of gelatin are influenced by raw material, animal age, collagen type, manufacturing method, tissue type, species, and collagen characteristics (Sompie et al., 2012). Age of animals that get older can increase the rendamen, ash content and gelatin fat obtained (Muyonga, 2004). While the use of high temperatures and long extraction causes a decrease in the value of viscosity, gel formation ability, and physical properties of gelatin (Godmundson, 2002).

According to Budavari (1996), the physical and chemical properties of gelatin include: colorless or slightly yellow, transparent, brittle, odorless, tasteless; in the form of sheets, flakes, or flour; soluble in hot water, glycerol and acetic acid; and insoluble in organic solvents. The physical properties of gelatin are gel strength, viscosity, gel point, melting point, emulsion activity, and stability, and degree of white, while the chemical properties of gelatin include water content, ash content, fat content, protein content, and pH.

General physical properties and specific mineral content in gelatin can determine the value of gelatin quality. Gelatin quality standard (GQS, 1995) can be seen in Table 1. Gelatin requirements for food based on FAO standards are presented in Table 2.

**Table 1.** Gelatin Quality Standards

Characteristics	Terms
Color	Not pale yellowish
Smell, taste	Normal
Water content	16% maximum
Ash Levels	3.25% maximum
Heavy metal	Maximum 50 mg / kg
Arsenic	Maximum 2 mg / kg
Copper	Maximum 30 mg / kg
Zinc	Maximum 100 mg / kg
Sulfite	Maximum of 1000 mg / kg

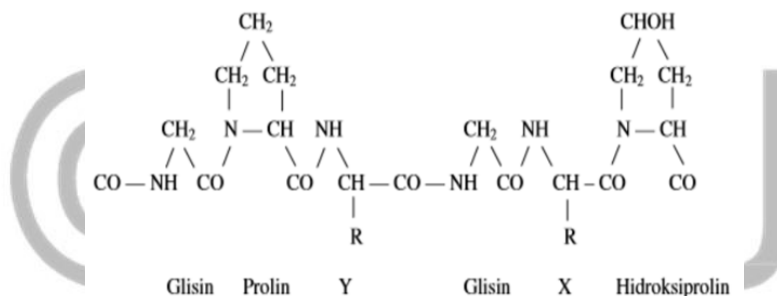
Source: GQS 06-3735-1995

**Table 2.** Gelatin requirements based on FAO

Characteristics	Terms
Ash Levels	No more than 2%
Water content	No more than 8%
Sulfur Dioxide	Not more than 40 mg / kg
Arsenic	Not more than 1 mg / kg
Heavy metal	Not more than 50 mg / kg
Lead	Not more than 5 mg / kg
Limits of Microbial Contamination	
<i>Standard Plate Count</i>	Less than 104 / g
<i>E. coli</i>	Less than 10 / g
<i>Streptococci</i>	Less than 102 / g

Source: JECFA (2003)

Generally, the gelatin has a structure, namely -Ala-Gly-Pro-Arg-Gly-Glu4Hyd-Gly-Pro-. The chemical structure of gelatin can be seen in Figure 1 (Grobben et al., 2004). The letter Y indicates the presence of the amino acid Proline, while the letter X indicates the presence of the hydroxyproline amino acid. Glycine is the most type of amino acid in gelatin, which is 26-27%, proline is 14-17%, hydroxyproline is 12-14%, and the rest is other amino acids. While the hydroxyproline content is an amino acid that affects the strength of the gel, the higher the amino acid hydroxyproline, the higher the strength of the gel produced and vice versa (Jaswir, 2007).



**Fig 1.** Gelatin Chemical Structure (Grobben et al, 2004)

Amino acids are the structure that forms proteins. Amino acids can be obtained from food after being absorbed through the blood and partially synthesized in the body. Among the twenty amino acids commonly found in proteins, there are essential and non-essential amino acids (Suhardjo 1987).

Non-essential amino acids are amino acids that can be synthesized by the body, by converting one amino acid into another amino acid in the body's cells, which consists of alanine, asparagin, aspartic acid, cysteine, glutamic acid, glycine, ornithine, proline or can be in the form of hydroxyproline, serine, and tyrosine. While essential amino acids are amino acids that cannot be synthesized by the body but are highly needed by the body and must be supplied in the finished form, consisting of isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, arginine, and histidine (essential for children). Between cow skin gelatin and fish skin gelatin, there is a difference between the amino acids proline and hydroxyproline. In fish skin gelatin, the amount of amino acids is lower than in cow skin gelatin. This results in a low hydrogen bonding of gelatin to water, thereby affecting the gel point and melting point of gelatin (Azwar 2008).

**Gelatin Rendemen**

The yield is one of the important parameters in assessing the effectiveness of the gelatin production. The greater the yield produced, the more efficient the treatment given (Yuliani and Marwati, 2015). The results of Yuliani and Marwati's research (2015) stated that an increase in HCl concentrations of 4-8% caused a decrease in the yield value. This is comparable to the research conducted by the author using 1-4% acetic acid on the skin of tilapia that the greater the concentration of acid given, the smaller the yield obtained. The results of the study of Jamilah

et al., (2011) Rendemen produced in *Pangasius sutchi* skin gelatin was 11.17%, while in the study of See et al., (2010) with the same fish which was 10.78%. The yield of gelatin from the skin of African catfish (*Clarias gariepinus*) was also known from a previous study of only 11.4% (Alfaro et al., 2014). Low yields occur due to imperfect collagen hydrolysis or loss of collagen Characterization of Washing Gelatin Extraction (Shyni et al., 2014). The yield value varies depending on the proximate composition of fish skin, collagen content and the number of components dissolved in the skin, species and age of the fish, and the extraction method used (Songchotikunpan et al., 2008).

## Water Content

Water content is an important parameter of a food product, because water content is closely related to the shelf life of gelatin, low water content will affect the quality of gelatin, especially in rancid gelatin and colors that are less bright. The results of the study of Darwin et al. (2018) the range of gelatin water content extracted from tilapia fish bones was 6.35 - 9.45%. Based on the results of research Yuliani and Marwati (2015) stated that the concentration of hydrochloric acid solution as a marinade solution and the duration of soaking affect the water content of the gelatin of mackerel fish produced, with a range of values between 6.88-8.56%. The results of Trilaksani et al. (2012) showed that the water content of red snapper skin gelatin was 10.19%. The water content is lower than that of commercial gelatin water (12, 21%) and laboratory standard gelatin (11.45%) based on Nurilmala's test results (2004). The difference in the value of water content can occur due to the difference in the acid used and due to the more acid ( $H^+$  ion) in the soaking solution and the longer soaking, causing the collagen structure to be more open, thus the less water trapped physically in the collagen matrix structure that causes the water content is getting lower (Yuliani and Marwati 2015). Warinangin et al. (2005) stated that acid treatment on the conversion of collagen to gelatin is much faster compared to base treatment. The difference in the value of water content can occur due to the difference in the acid used and due to the more acid ( $H^+$  ion) in the soaking solution and the longer soaking, causing the collagen structure to be more open, thus the less water trapped physically in the collagen matrix structure that causes the water content is getting lower (Yuliani and Marwati 2015). Warinangin et al. (2005) stated that acid treatment on the conversion of collagen to gelatin is much faster compared to base treatment. The difference in the value of water content can occur due to the difference in the acid used and due to the more acid ( $H^+$  ion) in the soaking solution and the longer soaking, causing the collagen structure to be more open, thus the less water trapped physically in the collagen matrix structure that causes the water content is getting lower (Yuliani and Marwati 2015). Warinangin et al. (2005) stated that acid treatment on the conversion of collagen to gelatin is much faster compared to base treatment. The water content values still meet the standards Gelatin GQS 1995 with a maximum moisture content of 16%.

## Ash Levels

Ash content aims to determine the mineral content of the material and to determine the purity of a food ingredient. About 96% of food consists of organic matter and water, while 4% consists of mineral elements (Winarno, 1997). The results of Suptijah et al. (2018) stated that the ash content of the catfish skin produced was 0.19%, almost the same as *Pangasianodon gigas* ash content of 0.25% and *P. hypophthalmus* by 0.23%, but slightly lower compared to *Pangasius sutchi* is 0.73% (Thitipramote and Rawdkuen, 2011; See, Hong, Wan & Babji, 2010). Low ash content less than 0.5% is a good quality raw material for the manufacture of collagen and gelatin, because the demineralization process can reduce the amount of ash produced (See et al., 2010). This value also fulfills GQS requirements (1995), which is a maximum of 3.25% and included in the standard range of gelatin ash content determined by Food Chemical Codex (1996), which is no more than 3%.

## Gel Strength

During the extraction process, the gelatin molecule develops, heat will open bonds to the gelatin molecule and the liquid which was originally free to flow into a thick solution. The strength of the gel depends on the length of the amino acid. If the condition of collagen has been completely hydrolyzed, then the strength of the gel can be increased (Rares et al, 2017). The statement is by the results of the author's research using tilapia skin with acid treatment, that the higher the content of amino acid gelatin, the greater the strength value of the resulting gel. This happens because the hydrolyzed collagen produces a long polypeptide chain. Gelatin gel can be stable in the presence of pressure from outside the hydrogen bonds, due to covalent bonds.

Pertiwi et al. (2018) stated that the measurement of gel strength in gelatin from catfish bone obtained was 364.19 bloom. The strength of the gel in the study was greater than that of Mahmoodani et al. (2014) namely 254.7 bloom and the research of Iqbal et al. (2015) which is 136,439 bloom. That is because the gel strength in this study

is quite high. After all, the sample testing is done after the gelatin process is extracted and filtered with filter paper and put into the refrigerator at 10 ° C for 17 ± 2 hours (until the liquid gelatin has formed a gel), so the concentration gelatin is more concentrated (Pertiwi et al., 2018). Gel strength is a physical property of gelatin that is related to the application to food products. The strength value of gelatin gel to be applied to confectionery products is > 175 bloom (Damayanti, 2007).

### Viscosity

Gelatin viscosity shows the flow of molecules in a solution in water, simple organic liquid, and aqueous suspension. Colloid systems in solutions can be increased by thickening the liquid, resulting in the absorption, and developing colloids (Haris, 2008). Viscosity is related to the average molecular weight gelatin and molecular distribution (Trilaksani et al., 2012). The weight of the gelatin molecule is directly related to the length of the amino acid chain. The longer the amino acid chain is contained, the higher the viscosity value will be.

## MANUFACTURING GELATIN

Hydrolysis of collagen to gelatin can occur chemically by immersion in a certain time with an acid or base. In addition to chemical means, hydrolysis can also occur enzymatically with the help of specific collagenase enzymes (Oktaviani et al., 2017). In principle, the process of making gelatin can be divided into two kinds, such as the acidic process and the basic process. The difference between these two processes lies in the immersion process (Fahrul, 2005).

In the manufacture of gelatin, the treatment of raw materials in the form of animal collagen with dilute acid or with a base cause cross-cutting of protein bonds, the structure becomes broken and the pieces dissolve in water. These pieces of water-soluble protein chains are called gelatin. The quality of gelatin produced depends on the concentration of acid or base used, temperature, and length of soaking time (GMIA, 2012). In principle, the process of making gelatin can be divided into two types, namely the acidic process and the basic process. Based on this, gelatin is classified into two types, namely gelatin type A and type B:

- Gelatin type A (Acid)

Gelatin type A is obtained through the immersion of raw materials using dilute acid. This method is suitable for collagen raw materials obtained from young animals or from the skin. The cross-linking in collagen is still weak so to break the bond is enough with a dilute acid solution. Soaking can be done using 2-6% hydrochloric acid for 24-72 hours at room temperature (GMIA, 2012).

- Gelatin type B (Base)

Type B gelatin is obtained by a conditioning process using a base solution. The raw material is from bone or collagen which is a bit old. Depending on the alkaline concentration and temperature used, the soaking process can take several days to months. If using a 1% NaOH solution at a temperature of 20°C, the conditioning process can take several days. But if you use a lime solution can be more than 1 month. Although this process is very long, it produces high purity gelatin.

The fish skin gelatin extraction process is categorized as type A gelatin, so in this study acidic solution was used as a marinade solution. According to Ward and Courts (1977), acids can convert triple-helix collagen fibers into single chains. In the acidic way, the skin of the fish is soaked with acetic acid until the skin is swelling, this occurs because of the breaking of the cross bonds between the collagen polypeptide chains and the breaking of several bonds in the polypeptide chain (Nasution et al, 2018). This acid causes repulsion between collagen molecules. With the loss of bonding power, warm water is able to effectively penetrate the matrix (Ahmad & Benjakul, 2011).

The process of producing fish skin gelatin begins with the collection of raw fish skin waste taken from the byproducts of the fish industry. Then the raw material for fish skin obtained is processed degreasing, namely the process of removing meat, dirt, and fat that is still attached to the skin of fish. This process is carried out at a temperature of 80°C for 2 minutes, until the remaining meat can be separated from the skin and the use of these temperatures following the solubility of the fat and the coagulation temperature of albumin, which ranges from 32-80°C (Pertiwi et al, 2018). The use of temperatures over 8 ° C can reduce the amount of collagen produced. 2 minutes in the degreasing process is the optimum time to reduce the amount of fat found in the skin. Then do the cutting and soaking using acid with a skin ratio of 1: 2 acid for 24 hours. After that, washing is done with running water to neutral pH and extraction with waterbath for 2 hours at 80 ° C using distilled water with a skin ratio of 1: 3 distilled water. After the extraction process, the sample was filtered with whatman filter paper No. 41 and in the oven for 48 hours at 50°C. After the dry sample has been smoothed gelatin using a blender. After that the necessary analysis is carried out.

## THE USE OF GELATIN

Gelatin is used almost throughout the food and pharmaceutical industries because of its distinctive feature, melting in the mouth. Gelatin has a melting point of 27-34°C because gelatin is called miracle food. Gelatin is also biodesive which is well used in mucoadhesive delivery systems that aim to increase the concentration of drugs in the digestive tract (Racmania et al, 2013).

Gelatin is used for food processing, cosmetics, and microbiological media (Rahayu et al., 2015). Gelatin is used in the addition of food ingredients because of its high nutritional value, as a thickening agent, clotting agent, emulsifier, stabilizer, elastic, foam maker, thin coating, and rich in nutrition. Gelatin is a product that is much needed in various industries, namely food and non-food industries. According to Siti Miskah, et al (2010) gelatin has different functions in each addition in a product, including:

1. Food products in general, gelatin functions as a thickening agent, clotting, emulsifying, stabilizing, elastic, forming foams, thin coatings, and rich in nutrients.
2. Processed meat products, gelatin serves to increase the water-binding capacity, consistency, and stability of sausage, corned beef, and ham products.
3. Processed dairy products, gelatin works to improve the texture, consistency, and stability of products in yogurt, ice cream, sour milk, and cottage cheese.
4. Bakery products, gelatin serves to maintain moisture and adhesives.
5. A beverage product, gelatin functions as a purifier for juice, juice, and wine.
6. A fruit product, gelatin functions as a coating to cover the pores of the fruit to avoid drought and disturbances by microbes to maintain freshness and long storage.
7. Candy products and the like, gelatin functions to regulate the consistency of bite power, hardness, texture, moisture, and stickiness.

## CONCLUSION

Gelatin is a type of protein derivative from collagen fibers that can be extracted from the fish skin. Gelatin has unique characteristics as the ability to turn from sol to gel, be amphoteric, and maintain colloidal properties. The collagen found in the fish skin and bones is bigger than the rest of the fish. Making gelatin type A or using acid is easier than using a base treatment.

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