*Eco. Env. & Cons. 27 (2) : 2021; pp. (931-936)*
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*ISSN 0971–765X*

# Gelatin based on tilapia fish bones

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(Received 23 September, 2020; Accepted 5 November, 2020)

**ABSTRACT**

A research about gelatin synthesis and characterization from tilapia fish bone as basic material has been done. The aim of this research is to find out the influence of temperature variation on drying process on the gelatin creation process from tilapia fish bone in order to find the best temperature in the gelatin creation process. The process of the creation of the gelatin is started from cleansing the material (tilapia bone) from the dirt and flesh remains on the bone by soaking it in the boiling water for couple of minutes. After cleaning it, the tilapio bone is seeped and dried. After being dried, the fish bone is soaked in HCl 5% for about 48 hours long until *ossein* is formed. *Ossein* is cleansed until pH value is neutral and then be extracted using *waterbath* on 90 ºC temperature for 5 hours. The extracted product is filtered and dried in an oven on temperatures vary from 45 ºC, 50 ºC, 55 ºC, 60 ºC, and 65 ºC. The characteristic of the best gelatin sample and meet the standard criteria for the creation of the gelatin is shown by the sample on 45 ºC drying process where the rendemen value 4.11%, water content 6.31%, ash content 1.59%, melting point 24 ºC, gel point 10 ºC, and mollecular weight 385,336 g/mole. On the other hand, on the FT-IR test result, it has been found that sample shows functional groups of C-H stretching and bending, C=O stretching, O-H bending, O-H bending and N-H stretching as functional groups of gelatin creation.

***Key words****: Characterization, Gelatin, Tilapia fish bone, Drying temperature*

## Introduction

Indonesia imports gelatin from countries such as: France, Japan, India, Brazil, Germany, China, Argentina and Australia. Gelatin imports in Indonesia reached 6,200 tons in 2003 with a selling price in the domestic market reaching Rp. 60,000 to Rp. 70,000 per kilogram (Wahyuni *et al.,* 2009). Demand for gelatin is increasing with the demonstration of an increase in world gelatin production which is approaching 326,000 tonnes per year, where gelatin is obtained from pork skin (46%), from cow skin (29.4%), from cow bone (23.1%), and from other sources (1.5%) (Karim, 2008).

The use of gelatin from cows and pigs causes problems for its users, for cow gelatin is problematic for Hindus while pork gelatin is problematic for Muslims. Therefore, an alternative is needed to make gelatin using collagen sources from fish, namely on the skin or bones which is industrial waste. fish processing. Fish bones and skin are waste from the processing of fishery products that have not been utilized and will cause losses, especially environmental pollution if in large quantities. Gelatin can be taken from almost all types of skinned and prickly fish. Gelatin can be made from collagen from marine or freshwater fish so that it can support the development of land for fisheries culture that has not been improved.

Gelatin is a polymer of amino acids that can be obtained in collagen in animal skin and bone tissue (Matz, 1962). The amino acid composition consists of 2/3 glycine as the main amino acid of all the amino acids that make up and 1/3 is filled by proline and hydroxyproline (Charley, 1982). One of the fish that can be used for making gelatin is tilapia fish bones. In tilapia fish bones there is a water content of 10.27%, an ash content of 1.71%, and a protein content of 85.44% (Junianto *et al.,* 2006). This protein content can be converted into gelatin.

According to Parker (1982), gelatin has properties that can change reversibly from sol to gel form, can swell or expand in cold water, can form films, can affect the viscosity of a material, and can protect the colloid system. Gelatin behavior is influenced by pH, ash content, manufacturing method, thermal history, and concentration and gelatin contains 8-13% moisture and has a relative density of 1.3-1.4. In thermal history, research has been carried out on making gelatin with a variety of drying methods using vacuum drying and by using freeze dry where the best results are obtained from vacuum drying (Astawan, 2003). The preparation of gelatin has never been done using temperature variations in tilapia bone drying using an oven, so that the best drying temperature can be found.

## Materials and Methods

The process of making gelatin is done by cleaning fish bones from dirt and fish meat that are still attached by immersing them in boiling water for a few minutes. After that, the fish bones were drained and dried in the sun. Then the fish bones were immersed in 5% HCl for 48 hours until ossein was formed. Ossein was rinsed to neutral pH and then extracted using a water bath at 90 $^oC$ for 5 hours. The extraction results were filtered and then dried in an oven using temperature variations of 45 $^oC$, 50 $^oC$, 55 $^oC$, 60 $^oC$, and 65 $^oC$.

Furthermore, the gelatin samples that have been obtained were given various testing treatments to determine their characteristics. These tests include: Rendement to determine the efficiency level of a processing process and as a basis for calculating financial analysis and estimating the amount of raw material for production in a certain mass, testing the gelatin gel point to determine the temperature at which the gelatin solution forms a gel slowly when cooled, point testing melting of gelatin to determine the temperature when the gelatin that has formed gel melts when heated slowly, testing the water content of the sample needs to be known because it affects the quality and quality of the sample, ash content testing is carried out to determine the mineral content of the material and determine the purity of a food ingredient, determination Molecular weight which affects the macroscopic properties of a polymer (thermal properties, physical properties, mechanics, and optical properties) and can also determine the application of the polymer, FTIR testing aims to identify the content of the functional groups of the sample.

## Results and Discussion

### FTIR Testing

FTIR testing is used to identify functional groups contained in a compound based on its absorption of infrared rays, in this case the sample tested is tilapia fish bone gelatin. Gelatin in general has a structure consisting of carbon, hydrogen, a hydroxyl group (OH), a carbonyl group (C = O), and an amine group (NH).

From the infrared spectra in Figure 1 shows the presence of the OH functional group stretching vibrations at a wave- number around 3100-3500 $cm^{-1}$, while the OH bending is in the 1500-1300 $cm^{-1}$ area, the presence of OH groups is possible because there are still OH compounds from the water used for extracting gelatin. Stretching C = O is shown in the wave number area 1723-1700 $cm^{-1}$, while stretching NH is in the wave number area 3500-3300 $cm^{-1}$. Finally, for stretching and bending CH is shown in the wave number between 3000-2800 $cm^{-1}$. The transmittance peak of the infrared spectra obtained from the FTIR test of gelatin at a certain wave number is compared with the wave number that shows the functional groups of gelatin. Comparison of the functional groups of gelatin can be seen in previous research or by using the Handbook of Spectroscopic Data.

The identification results of gelatin FT-IR groups from tilapia fish bones are shown in Table 1, it can be seen that the characteristic groups of gelatin appear in several wave numbers. However, overall the FT-IR results of gelatin synthesis from tilapia fish bones have all the characteristic functional groups of gelatin, so it can be said that this extraction has succeeded in making gelatin.

### Molecular Weight Determination

Determination of the molecular weight was carried

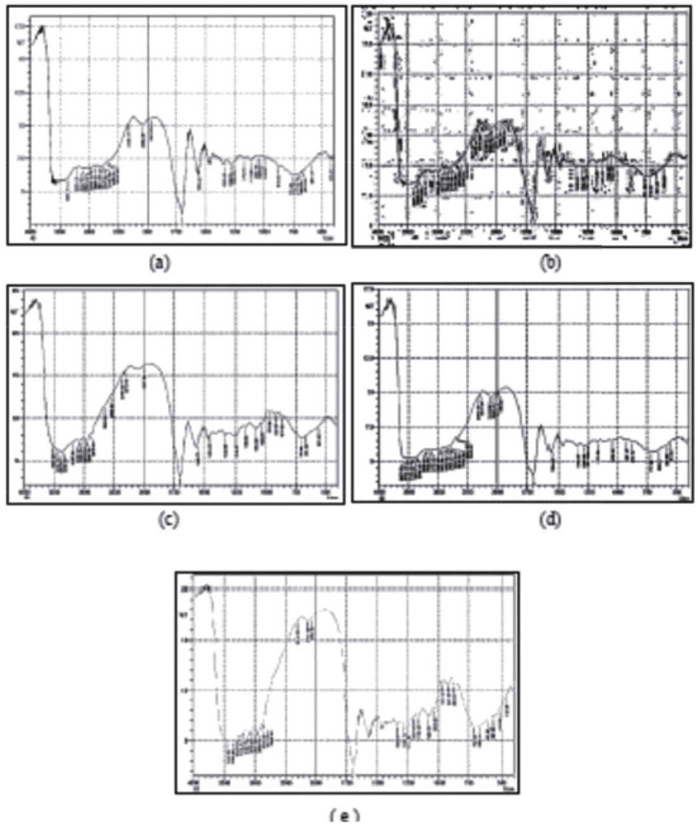

**Fig. 1.** IR spectrum of tilapia bone gelatin with drying temperature variations (a) 45°C, (b) 50°C, (c) 55°C, (d) 60°C, (e) 65°C

**Table 1.** Transmittance peaks obtained from gelatin drying variations

| Functional groups | Peak transmittance of each gelatin drying variation | | | | |
|---|---|---|---|---|---|
| | 45 °C | 50 °C | 55 °C | 60 °C | 65 °C |
| C-H stretching & bending | 2968.45 | 2983.88 | 2964.59 | 2953.02 | 2964.59 |
| C=Ostretching | 1701.22 | 1701.22 | 1697.36 | 1693.50 | 1693.50 |
| O-Hstretching | 3101.54 | 3219.19 | 3197.98 | 3122.75 | 3182.55 |
| O-Hbending | 1340.53 | 1340.53 | 1340.53 | 1340.53 | 1336.67 |
| N-Hstretching | 3367.71 | 3367.71 | 3365.78 | 3367.71 | 3363.86 |

out to determine the physical characteristics of gelatin, namely the average relative molecular weight of gelatin using an Ostwald viscosimeter which can flow a certain concentration of gelatin solution. The flow time of the gelatin solution was repeated four times then averaged for data processing on the relationship between concentration and viscosity reduction. So it can be obtained linear regression which has a value of $y = mx + n$ where n is a linear intercept which will be used to find the molecular weight as the intrinsic viscosity value [η].

Table 2 and Figure 2 show the relationship between the concentration of the gelatin solution from various variations of the gelatin drying process with the results of the reduction viscosity calculation. If the gelatin solution concentration is higher, the reduction viscosity will also be higher because the solution flow time is getting longer.

Table 3 shows the results of the calculation of molecular weight using the Mark-Houwink equation $[\eta] = K.M^a$, with a is 0.74 while the K value for cowhide gelatin is $1.66 \times 10^{-5}$ with $\alpha$ is 0.885. The lowest molecular weight obtained from gelatin from the drying process at 55 °C is M = 274,498.5154 g / mole, while the average molecular weight obtained from gelatin from other temperature drying pro-

**Table 2.** Relationship between gelatin concentration and reduction viscosity for each gelatin drying variation

| Gelatin concentration | Reduction viscosity (mL/g) for eachgelatin drying variation | | | | |
|---|---|---|---|---|---|
| | 45 °C | 50 °C | 55 °C | 60 °C | 65°C |
| 0.3% | 5.3679 | 6.9994 | 4.6536 | 6.3176 | 6.4799 |
| 0.4% | 10.4180 | 12.1530 | 10.4910 | 10.9659 | 11.3189 |
| 0.5% | 11.3733 | 12.9366 | 11.2127 | 11.9724 | 12.5129 |
| 0.6% | 13.6945 | 14.2465 | 12.3877 | 12.7286 | 13.0817 |

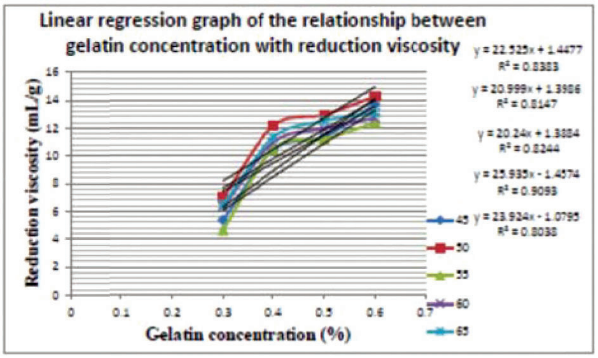


**Fig. 2.** Graph of linear regression relationship between gelatin concentration and reduction viscosity from various drying temperature variations

cesses is> 3.5x10$^5$. In the research of making gelatin from stingray bones, a maximum molecular weight of 551,128 g/mole was obtained and a minimum molecular weight of 123,187 g/mole was obtained. The average molecular weight of gelatin was > 5x10$^5$ g / mol. mole and the average molecular weight of gelatin from ossein obtained in acid immersion was 2.2x10$^5$ g/mol.

**Table 3.** Results of calculation of tilapia bone gelatin molecular weight

| Drying temperature variations | Gelatin molecular weight (g/mole) |
|---|---|
| 45°C | 385,336.3683 |
| 50°C | 382,437.0856 |
| 55°C | 274,498.5154 |
| 60°C | 364,753.9469 |
| 65°C | 367,815.4773 |

## Rendemen Testing

The optimal amount of rendemen will determine the efficiency of the treatment in making gelatin. The rendemen value of gelatin from various variations in the drying process is shown in Table 4 using the formula:

$$\text{Rendemen} = \frac{\text{Weight of gelatin}}{\text{Weight of raw bone shine}} \times 100\%$$

The highest rendemen value was produced by drying gelatin with a temperature of 60 °C and the lowest rendemen value was produced by drying gelatin at 45 °C. The difference in rendemenvalue of the two processes is not too high and there is no significant difference from each rendemen value because the factors that affect the rendemen of gelatin are variations in the fish species used, the temperature used in the extraction process, and the concentration of the HCl solution used in the immersion process.

**Table 4.** Tilapia fish bone gelatin rendemen test results

| Drying temperature variations | Gelatin rendemen (%) |
|---|---|
| Gelatin drying 45°C | 4.11 |
| Gelatin drying 50°C | 5.07 |
| Gelatin drying 55°C | 4.98 |
| Gelatin drying 60°C | 6.71 |
| Gelatin drying 65°C | 4.25 |

## Water Content Testing

The water contained in a material can affect the appearance, texture, taste and quality of the material as well as its acceptability, freshness, and durability. It can be seen the various water content of the tilapia fish bone gelatin manufacturing process using the drying temperature variations in Table 5 using the formula:

$$\text{Water Content} = \frac{(B - A)}{\text{Initial sample weight}} \times 100\%$$

Note: A = Weight of the cup + final sample (grams)
B = Weight of the plate + initial sample (g)

The low water content of tilapia fish bone gelatin with drying temperature variations is due to the drying process of gelatin using an oven with variations in temperature and long drying time. The long drying time causes the water in the gelatin to evaporate. The value of the water content obtained from the variation of drying temperature still meets the water content standard of SNI (1995), which is a maximum of 16% and also meets the standards of JECFA (2003) which is not more than 18%.

**Table 5.** Tilapia fish bone gelatin water content testing results

| Drying temperature variations | Water content (%) |
|---|---|
| Gelatin drying 45°C | 6.31 |
| Gelatin drying 50°C | 4.41 |
| Gelatin drying 55°C | 5.94 |
| Gelatin drying 60°C | 5.54 |
| Gelatin drying 65°C | 5.45 |

**Table 6.** The Results of testing the gelatin ash content of tilapia fish bone

| Drying temperature variations | Ash content (%) |
|---|---|
| Gelatin drying 45°C | 1.59 |
| Gelatin drying 50°C | 3.85 |
| Gelatin drying 55°C | 5.57 |
| Gelatin drying 60°C | 4.21 |

## Ash Content Testing

The value of the ash content of a material shows the amount of minerals contained in the foodstuff (Apriyanto *et al.*, 1989). Following the ash content obtained from the study can be seen in Table 6 by using the formula:

$$\text{Ash Content} = \frac{\text{Ash weight}}{\text{Initial sample weight}} \times 100\%$$

Only when making gelatin using 45 °C drying, the maximum value of ash content that meets the SNI (1995) requirements is 3.25% and falls within the standard range determined by the Food Chemical Codex (1996), which is not more than 3%.

The high and low levels of gelatin ash were influenced by the demineralization process, namely removing minerals which was done by soaking fish bones in 5% HCl for 48 hours. During immersion, there is a reaction between hydrochloric acid and calcium phosphate so that the calcium salt dissolves and the bones become soft (ossein). After soaking and washing, it is possible that there are still mineral components bound to collagen, which have not been released and are also extracted and carried away during the ashing process (Astawan and Aviana, 2003). also filtered so that it affects the results of the gelatin which has a high ash content.

## Melting Point Testing

The melting point of gelatin is the temperature at which the gelatin in the form of gel melts right after being heated slowly (Stainsby, 1977). The following results of gel point measurements are written in Table 7.

The high and low melting point of gelatin is influenced by the amino acid values of glycine and hydroxyproline, resulting in the formation of hydrogen bonds from gelatin to water in solution. The low content of the amino acids glycine and hydroxyproline causes the hydrogen bonding of gelatin to the water in the solution to be low, causing the melting point of gelatin to be low and vice versa.

**Table 7.** Tilapia fish bone gelatin melting point test results

| Drying temperature variations | Melting point (°C) |
|---|---|
| Gelatin drying 45°C | 24 |
| Gelatin drying 50°C | 24 |
| Gelatin drying 55°C | 23 |
| Gelatin drying 60°C | 23 |
| Gelatin drying 65°C | 23 |

## Gel Point Testing

Gelatin gel point is the temperature at which the gelatin solution changes form into a gel slowly when cooled at chilling temperature (Stainsby, 1977). The following results of gel point measurements are presented in Table 8.

Gelatin gel point is influenced by gelatin concentration, pH, and the size of the gelatin molecule (Stainsby, 1977). The higher the gelatin concentration, the faster the molecular bonds of gelatin will bind free water to the solution so that the formation of gel will be faster. Because the bonds of gelatin molecules and the length of the amino acid chain of gelatin are related, the longer the amino acid chain,

**Table 8.** Tilapia fish bone gelatin gel point test results

| Drying temperature variations | Gel point (°C) |
|---|---|
| Gelatin drying 45°C | 10 |
| Gelatin drying 50°C | 9 |
| Gelatin drying 55°C | 10 |
| Gelatin drying 60°C | 10 |
| Gelatin drying 65°C | 11 |

the faster the gel formation process.

## Conclusion

The best characteristics of the gelatin sample were shown by the gelatin sample with a drying temperature of 45 °C, where the gelatin sample had a yield of 4.11%, a water content of 6.31%, an ash content of 1.59%, a melting point of 24 °C, a gel point of 10 °C, and a molecular weight. 385,336 g/mol.

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