**The use of waste bone of skipjack fish (katsuwonuspelamis) to be gelatin in north Sulawesi province**

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**Abstract**. Industrial processing of Skipjack fish in North Sulawesi has recently developed besides the processing of smoked fish, wood fish, etc. However, the fish waste is abundantly produced from the processing and thus, it still needs to be utilized more effectively. Skipjack fish is the most popular fish source with a production of 60.168 tons. As a result, the produced waste from industrial processing could reach ± 7460 tons/year. The purpose of this research was to determine the nutritive value of gelatin extracted from the waste bone of Skipjack fish. Meanwhile, this study used Complete Randomized Design with Statistic JMP shortcut software for ANOVA. Results showed that the highest rendemen value was 16.25% in gelatin with 5% HCL and the water content was 9.75%. The highest protein of the gelatin was 48.2% with the fat content of gelatin was 3% extracted from 1% HCL. The study concluded that the waste bone of Skipjack fish processed to be gelatin could be utilized as an additional food in food industry under the Indonesian Standard of food (SNI). Therefore, the processing of this industry could produce a lawful food which has a nutritious content and it might reduce environmental pollution.

**Keyword** : Gelatin, waste bone, additional food

1. **INTRODUCTION**

North Sulawesi Province has many fish processing industries, from traditional scale to modern scale, such as wood fish (katsubushi), smoked fish, and etc. Skipjack tuna production in north Sulawesi is 60.168 tons [1], and fish bone proportion to the fish body reaches 12.4% [2]. Hence, the solid waste production from the fish processing, such as fish bone, is estimated approximately 7,460 tons. If these wastes are not well used, it could give negative impact to surrounding human health. According to [3], bone waste is composed of collagen that will yield gelatin through hydrolysis. Gelatin can be utilized as stabilizer, gelling agent, binder, thickener, emulsifier, adhesive, and edible food coating.

 The use of the fish waste as gelatin is expected to be able to raise its economic value, reduce environmental pollution, can become alternatively safe raw material of gelatin, and can reduce the dependence of Indonesian’s industries upon imported gelatin. This study was aimed at knowing the nutritive value and the safety of gelatin product from the bone of skipjack tuna.

1. **MATERIALS AND METHOD**
2. **Materials**

This study used the fish bone of skipjack tuna (*Katsuwonuspelamis*) collected from UD. KaryaMandiriBersama, Bitung city, North Sulawesi Province, and smoked fish processing center in Manado city, North Sulawesi. Laboratory apparatus used were water bath, aluminium foil, erlenmeyer, beaker glass, pippet, filter paper, measuring glass, thermometer, cloth, oven, blender, pan, gas cooker, labeling paper, balance, and ruler. Besides, HCl and aquadest were utilized as well.

1. **Method**

This study was carried out in the laboratory of Fisheries Product Handling Technology and Processing, Faculty of Fisheries and Marine Science, and laboratory of Pharmacy, Faculty of Natural Science, Sam Ratulangi University, Manado.

1. ***Gelatin processing***. Fish bone gelatin processing step is demonstrated in Fig. 1. Fish bone was washed with water to take out flesh, dirt, and fat of the bone and degreased in the water pan at 80oC for 30 min. The clean fish bone was dried under the sunlight for 2 days and chopped into 2-3 cm size. Then, it was demineralized in 1%, 3%, and 5% HCl – containing Erlenmeyer, covered with aluminium foil, and labelled. The immersion was done for 36 hours. The bone was cleaned in running water and rewashed with neutral pH-distilled water, inserted in the glass beaker and moved into a water bath at 85± oC for 6 hours. Gelatin solution obtained was filtered through cotton-materialized cloth, and the extract was poured into a rectangular container. The extract was then inserted into an oven at 55oC for ±2 days. Gelatin sheet obtained was blended, put into a plastic sheet, and stored in a glass bottle.



Figure 1. Gelatin processing of skipjack fish (Modified from Haris, 2008)

*2.* ***Gelatin analysis***

Gelatin produced in this study was analyzed as follows:

***2.1.Rendemen****.* The analysis followed AOAC (1995). It was obtained from the ratio of dry weight of gelatin powder and net weight of clean dry fish bone using the following formula:

Rendemen = Dry weight/weight of clean dry fish bone x 100% (1)

***2.2. Water content.*** Gelatin water content was calculated using AOAC (1995), with the following formula:

Water content (%) = $\frac{B-A}{sample weight}x 100$ (2)

where A = cup weight + final sample (g) and B = cup weight + initial sample (g)

***2.3. Fat.*** Testing fat content of the gelatin used AOAC (1995) with the following formula:

Fat content (%) = $\frac{fat weight}{sample weight}x 100$ (3)

***2.4. Protein.*** As much as 0.2 g of sample was put in a 30 ml-kjeldahl flask, then added with 1.9 + 0.1 g K2SO4, and 2.0 + 0.1 ml of concentrated H2SO4. The sample was then destructed for 1-1.5 hours until the solution turned to clear. The solution was cooled, added with 8-10 ml of NaOH-Na2S2O3, and inserted into distillator.Under the distillator condenser was placed an erlenmeyer containing 5 ml of H3BO3 solution and several drops of red methyl indicator. The edge of condenser tube should be submerged in the solution to hold about 15 ml of distillate.The distillate was titrated with 0.02N HCl until the color turned to grey. Similar procedure was also done for the blank (without sample). The amount of sample titration (a) and blank titration (b) is expressed in ml of 0.02N HCl.

N content (%) = $\frac{\left(a-b\right)N HCl x 14.007}{sample weight}+100$ (4)

Protein content (%) = N content (%) x 6.25 (5)

**2.5 Amino acid.**  Qualitative testing of amino acid was done by observing color change referring to [6].

**2.6 Heavy metal.** Heavy metal was qualitatively detected through color change following [7].

**3. Data analysis**

The study used complete randomized design and ANOVA was applied for data analysis. If there was significant effect, Least Significant Difference test was used. For this, JMP shortcut statistic software was utilized.

**III RESULTS AND DISCUSSION**

**Rendemen**

Rendemen is one of the important parameters in gelatin production. It is the ratio of gelatin powder dry weight obtained and cleansed dry fish bone.

The present study indicated that the highest gelatin rendemen was obtained through treatment of 5% HCL concentration, 16.25 % and the lowest in 1% HCl treatment, 2.5%. High rendemen under 5% HCl treatment could result from proper hydrolysis of the bone collagen to gelatin so that rendemen obtained was higher. In contrast, immersion of the skipjack tuna bone in 1% HCl did not make the collagen be well hydrolyzed so that only some rendemen was gained (Fig. 1)**.** If compared with freshwater fish, such as nile tilapia (*Oreochromisniloticus*) [4], rendemen of marine fish is better. It could result from their different bone structure.

ANOVA showed that gelatin rendemen of skipjack tuna bone was significantly affected by 1%, 3%, 5% HCl treatments (P<0.05). The Least Significant Difference test reflected significant difference as well.



Figure 1. Gelatin rendemen of skipjack fish bone.

**Water content**

Water content is percent water content of a material based on fresh weight or dry. Water content of food material also determine the acceptability, freshness, durability, and can affect the appearance, texture, and the quality of food material. The highest water content of the gelatin was recorded in 3% and 5 % HCl treatment, 9.25% and 9.75%, while in 1% HCl the water content was 7.75%. Low water content could result from method application, drying time, low water content of the fish bone. Based on the Indonesia National Standard (SNI), the water content of gelatin is recommended 16% at maximum. Water content of skipjack fish bone extracted in HCl solution is presented in Fig. 2.

Compared with water content of freshwater fish bone, such as shark catfish, *Pangasius*, (9.26%) and nile tilapia (7.03%), the water content of gelatin is quite different from that of skipjack tuna that is still below commercial water content (12.53%). According to [8], high water content of the gelatin can reduce the gel strength, the viscosity, and the melting point of gelatin even though the decline is not significant.

ANOVA revealed that treatment of 1%, 3%, and 5% HCl immersion did not influence the water content of the skipjack fish bone gelatin obtained (P<0.05). It could be caused by gelatin feature of binding water, so that HCl treatment does not influence the water content of the gelatin.



Figure 2. Gelatin water content of skipjack fish bone.

**Protein**

Based on protein solubility, stromal protein can dissolve in acid/alkaline condition, and in fish, it occurs in the bone, skin, and scale called collagen. If the collagen is hydrolyzed it will produce gelatin.

The present study found that the highest protein content (48.2%) gained from collagen hydrolysis of skipjack fish bone used 1% HCl solution and the lowest protein content, 17.6%, was recorded in the treatment of 5% HCl (Fig. 3).

High protein content obtained in the treatment of 1% HCl solution is due to the collagen of skipjack fish bone being not well hydrolyzed so that less protein (collagen) contained is removed. It could be seen from hard structure of the fish bone after immersion process. In contrast, the use of 5% HCl treatment makes the collagen be properly hydrolyzed, and therefore, much protein is removed in washingphase after HCl immersion. It could occur since the skipjack tuna bone already turned very soft but not broken. Compared with protein in freshwater fish bone, that of marine fish bone is lower. It could result from different animal species (Ward and Court 1977).

ANOVA indicated that extraction and immersion of skipjack fish bone in 1%, 3%, and 5% HCl solution significantly affected the protein content of the gelatin from fish bone collagen hydrolysis (P<0.05). Least Significant Difference test also exhibited significant difference among treatments of 1%, 3%, and 5% HCl.



Figure 3. Gelatin protein content of skipjack fish bone.

**Amino acid and heavy metal of gelatin**

 The smallest unit of protein is amino acid, and protein quality is highly determined by amino acid. There are two amino acids, essential and non-essential one. The former cannot be produced by human body, and the latter can be formed by human body.

 Qualitatively, amino acid and heavy metals in the gelatin of skipjack fish bone are given in Table 1 and Table 2. There were 10 amino acids identified, but tryptophan was not identified (Table 1). The presence of amino acid, glycine and proline, can influence the quality of gelatin. According to [10], the higher glycine, proline, and hydroxyproline highly influence the gelatin quality and low content of these amino acids in the fish bone gelatin could result in low gelatin melting point. Heavy metal analysis also indicated no heavy metal content in the gelatin (Table 2).

Thus, the gelatin produced from bone waste of skipjack fish, particularly in UD. KaryaMandiriBersama, Bitung, and smoked skipjack tuna processing sites in Manado city, is safe to use as food material.

**Table 1. Qualitative test of amino acid (Fessenden, 1994)**

|  |  |  |  |
| --- | --- | --- | --- |
| No. | Amino Acid | Sample Concentration | Color |
| 1 % | 3 % | 5% |  |
| 1. | Aspartic acid | + | + | + | Clear |
| 2. | Glutamic acid | + | + | + | Clear purple + precipitate |
| 3. | Glycine | + | + | + | Dark purple |
| 4. | Tyrosine | + | + | + | Clearlight blue |
| 5. | Proline | + | + | + | Clear blue |
| 6. | Phenylalanine | + | + | + | Purple |
| 7. | Lysine | + | + | + | Clear |
| 8. | Histidine | + | + | + | Clear |
| 9. | Alanine | + | + | + | Light blue |
| **10** | **Tryptophan** | **-** | **-** | **-** | **No color change** |

**Table 2. Qualitative test of heavy metal (Fries, 1997)**

|  |  |  |  |
| --- | --- | --- | --- |
| No. | Sample concentration | Output | Remarks |
| 1. | 1% | **-** | No color changes  |
| 2. | 3% | **-** |
| 3. | 5% | **-** |

**Fat**

The present study revealed that amount of gelatin fat content was very few or even almost none (Fig. 4). High fat content, 3%, was gained from hydrolysis of skipjack fish bone collagen using 1% HCl solution and the lowest, 0.6%, was recorded in the use of 5% HCl solution. The lowest value of gelatin fat content of skipjack fish bone is lower than that in nile tilapia 1.36% reported by Harris (2008) and almost similar to that of commercial gelatin from snapper, 0.5%.

Moreover, sufficiently high fat content (3%) from hydrolysis 1% HCL could result from that the fat was not properly released yet at the degreasing process, and this condition would enable to affect the gelatin quality during the storage. Low fat content, 1.5 and 0.6%, respectively, at the treatment of 3% and 5% HCl could be caused by high fat content of the skipjack fish bone lifted to the surface and removed at the washing phase. HCl is a strong acid that can break fat in the skipjack fish bone. The lower the fat content of the gelatin is, the better the gelatin quality will be.

ANOVA also indicated that fat content of gelatin was not significantly influenced by immersion of the fish bone in 1%, 3%, and 5% HCl solution (P >0.05). It could result from other factor in the fish bone gelatin production process.



Figure 4. Gelatin fat content of skipjack fish bone.

**IV CONCLUSION**

The bone waste of skipjack tuna produced by fish industries in north Sulawesi could be taken to make gelatin due to its good nutritive value and safe to consume based upon Indonesia National Standard (SNI). Thus, the bone waste of the skipjack fish could be used as food additive for other products, such as fishball, sausage, and others in order to increase the fishermen’s income and prosperity. This utilization could also reduce environmental pollution from fish industries.

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