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Waste Skin of Hawaiian Ladyfish (*Elops hawaiensis*) Utilization as Gelatin Raw Material with Immersion Solution Combination

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ABSTRACT

Bontot from Domas Village, Pontang District, Serang Regency is a fish jelly product. The manufacture of Bontot produces fish skin waste with a percentage of 10% of the whole weight of the fish. This waste can be reduced by applying the concept of zero waste in the processing of bontot which is reused as a raw material in the manufacture of gelatin. This research aims to determine the best NaOH concentration in the manufacture of gelatin from the skin of Hawaiian ladyfish (*Elops hawaiensis*) and to determine the effect of NaOH immersion on the organoleptic, chemical, and physical qualities of the gelatin. This study used an alkaline solution (NaOH) with a concentration of 0.1%; 0.3%; and 0.5%, which is then followed by a 6% acid solution immersion process. The production of gelatin from the skin of Hawaiian ladyfish was carried out using a one-factor completely randomized design (CRD) with 2 replications (duplo) accompanied by non-parametric analysis which was carried out for organoleptic testing with a hedonic scale using the Kruskal Wallis test. The results showed that the best combination was 0.1% NaOH and 6% HCl with a hedonic value of 3.7 with a whitish-yellow color; odor hedonic value 3.13; 11% yield; gel strength 280.43 g bloom; viscosity 36.95 cP; water content 8.75%; ash content 0.58%; and a pH value of 6.88.

Keywords: Hawaiian ladyfish, Fish skin, gelatin, HCl, NaOH

INTRODUCTION

Bontot is one of the mainstay home industry fish gel products from Domas Village, Pontang District, Serang Regency. The processing of bontot in the village of Domas can produce fish skin waste of 10% of the total weight of Hawaiian ladyfish (*Elops hawaiensis*) which is estimated to be 84.5 kg/week. The amount and intensity of waste that appears can be reduced by applying the concept of zero waste (Haryati and Munandar 2010). The best solution that can be given to support zero waste activities in utilizing Hawaiian ladyfish skin waste is to process into gelatin raw material.

Gelatin is a protein derived from collagen which has broad functions in the food and pharmaceutical industries, including as a filler, emulsifier, binder, precipitant, and nutrient enrichment. Gelatin is flexible with a thin layer that is elastic, transparent, strong, and has high digestibility (Hastuti and Sumpe 2007). Most of the gelatin needs in Indonesia is supplied from foreign producers, including China, Thailand, Australia, Brazil, Bangladesh, and New Zealand (Nurilmala *et al.* 2017). Around 98.5% of gelatin traded globally comes from pork and beef (Karim *et al.* 2008). The gelatin raw material is known to contain non-halal ingredients, thus causing concern for Muslims to consume it. Raw materials from cattle will cause problems for people who follow the Hindu religion (Agustin 2013). In addition, the consumption of gelatin from cows in recent studies has resulted in reports of being able to transmit *Bovine Spongiform Encephalopathy* (BSE) or mad cow disease (Huang *et al.* 2019). Furthermore, Azziza *et al.* (2019) stated that the use of fish skin waste as a safe and halal raw material for gelatin is an alternative to increasing value-added in fishery industry waste while reducing pollution and reducing the dependence of industry in Indonesia on imported gelatin.

The manufacture of gelatin generally comes from the skin or bones that are rich in collagen soaked in acid or base. This research emphasizes the manufacture of gelatin using a combination solvent, starting with the immersion in alkaline solution (NaOH) and followed by immersion in acid solution (HCl). Wijaya *et al.* (2015) stated in their research that the immersion of NaOH solution before acid soaking was able to maximize the degreasing process, namely the process of eroding fat in the manufacture of tilapia gelatin. Puspawati *et al.* (2014) added that immersion with alkaline will trigger a deproteinization process that can dissolve non-collagenous proteins and remove color. For this reason, this research needs to be done by utilizing the waste skin of Hawaiian ladyfish as raw material for gelatin with a combination of soaking solutions.

The purpose of this study was to determine the best concentration of NaOH in the manufacture of gelatin from waste skin of Hawaiian ladyfish (and the effect of soaking NaOH on the organoleptic, chemical, and physical quality of gelatin from a waste of Hawaiian ladyfish skin.

MATERIALS AND METHODS

Methods

This study used the treatment of immersion in NaOH solution with a concentration of 0.1%; 0.3%; and 0.5% with a ratio of soaking the fish skin: solution = 1:3 for 18 hours, before being immersed again in an acid solution (6% HCl). Followed by the extraction process with a comparison (skin: water = 1:2) temperature 80˚C ± 3 for 2 hours, then filtered with calico cloth and dried using an oven ± 50˚C for 3 days. The best gelatin in this study was determined based on color, odor, yield, gel strength, viscosity, moisture content, ash content, and pH value.

Organoleptic test (Setyaningsih *et al.* 2010)

The hedonic scale is used for organoleptic tests with a scale of 1 (very dislike) to 5 (very much like). This test was conducted using a panel of 30 people. The parameters tested were odor and color. The test was carried out by giving a random code to the sample and assessed by the panelists using a score sheet.

Yield (Marzuki *et al.* 2011)

The yield is obtained from the dry weight of the gelatin flour produced by the weight of the fish skin used which is then entered into the formula

$$yield\ (\%) = \frac{dry\ weight\ of\ gelatin}{fish\ skin\ weight} \times 100\%$$

Gel strength (Tazwir *et al.* 2007)

The sample was weighed as much as 7.50 grams dissolved in 105 ml of water at a temperature of 60°C and stirred using a magnetic stirrer for 15 minutes until homogeneous. Then the sample was allowed to stand at room temperature for 15 minutes and cooled at 10±0.1°C for 17 hours. The sample was then measured the strength of the gel using a texture analyzer and expressed in g bloom.

Viscosity (Tazwir *et al.* 2007)

The sample was weighed 6.67 g, then dissolved in distilled water to a volume of 100 ml. The sample was heated at a temperature of 60◦C and measured using a viscometer.

Water Content Analysis (AOAC 2005)

The water content is obtained through calculations using the following formula:

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

Information:

A = weight of empty cups (g); B = weight of the cup filled with the sample (g); C = weight of the cup with dried sample (g).

Ash Content Analysis (AOAC 2005)

Determination of ash content was obtained by the dry ashing method. The principle of this analysis is to oxidize all organic substances at a high temperature (about 550°C), then proceed with the process of weighing the remaining substances (ash) after the combustion process.

$$\text{Ash Content (\%)} = \frac{B - A}{\text{The initial weight of sample}} \times 100\%$$

Information:

A = weight of the cup (g); B = weight of cup with ash (g).

pH (Tazwir *et al*. 2007)

Determination of the pH value of the sample can be done using a pH meter. The sample was weighed 1 g then dissolved in 100 ml of distilled water at 80°C, homogenized, and analyzed using room temperature.

Data Analysis

The production of gelatin from the skin of Hawaiian ladyfish was carried out using a one-factor completely randomized design (CRD) with 2 replications (duplo). Data were analyzed by analysis of variance/univariate analysis (ANOVA). Non-parametric analysis was conducted for organoleptic testing with a hedonic scale using the Kruskal Wallis test. If the results of the analysis show a significant difference effect, the smallest significant difference test (LSD test) is carried out.

RESULTS AND DISCUSSION

Organoleptic Test

Color Value

The color hedonic value obtained in this study ranged from 2.77 to 3.7 with the appearance of the color obtained from cream to brown. These results indicate an increase in the concentration of NaOH used will significantly reduce the value of the hedonic color of gelatin. The color of the gelatin produced from the 0.1% NaOH concentration meets the BSN (1995) standard which states that the color of the gelatin is colored as expected. NaOH concentrations of 0.3% and 0.5% resulted in a brownish gelatinous cream color that did not meet these standards.

The brownish cream color is produced from the combination of yellow and black brown. Black brown color will appear with the higher concentration of NaOH. This is because the high concentration of NaOH solution will continue to hydrolyze until the time of extraction so that the skin is denatured and mixed in the gelatin solution. Ayudiarti *et al.* (2007) stated that the color of fish gelatin will be darker than commercial gelatin because it is influenced by pigments from fish skin that cannot be completely removed.

Based on the analysis of Kruskal Wallis, the immersion of different concentrations of NaOH solution given as treatment was significantly different ($P<0.05$) (Figure 1). This shows that the concentration of NaOH treatment has a significant effect on the hedonic value of the gelatin color produced. The color of the gelatin is usually determined by the raw materials and the process used in the manufacture of gelatin. The whiter the gelatin color, the better and preferable it is because it will be easier to combine gelatin with other food ingredients without adding strong coloring agents to food products (Moranda *et al.* 2018; Shyni *et al*. 2014).

Odor Value

The results of organoleptic quality as indicated by the hedonic value, the smell of gelatin obtained in the study ranged from 3.03 to 3.13. Based on Kruskal Wallis analysis, each treatment was not significantly different ($P>0.05$). On average, the panelists assessed that the fish smell was still in the Hawaiian ladyfish gelatin in each treatment with the concentration of the given NaOH soaking solution. These results do not meet the standards of BSN (1995) which states that the smell of gelatin is normal. Choi and Regenstein (2000) stated that fish gelatin is rarely used and is not mass produced because of its dark size and fishy odor. Rahman and Jamalulail (2012) reported that fish gelatin does have a very strong fish odor compared to gelatin made from other raw materials such as gelatin made from chicken feet.

Yield

Yield is the percentage by weight of gelatin produced to the weight of the waste Hawaiian ladyfish skin used. The yield of gelatin from Hawaiian ladyfish skin waste is shown in Figure 3. Based on the analysis of variance, each treatment of immersion in NaOH solution with different concentrations was significantly different ($P<0.05$). The yield parameter value for each treatment is 11; 5.34; and 3.67%. The decrease in yield value occurred along with the increase in the concentration of NaOH given. Tazwir *et al* (2009) explained that the decrease in yield value was thought to be due to the gelatin washing process after immersion with NaOH was not optimal, so there was still NaOH solution left and the sample was hydrolyzed at the beginning. In addition, the reason that may occur again is the acid-base reaction between NaOH and HCl in the acid soaking process. This reaction can block the process of hydrolysis of collagen and the process of formation of ossein becomes less than perfect. Pangke *et al.* (2016) the extraction of tuna skin gelatin with a concentration of 0.6% NaOH solution got a yield of 4.14% while at a concentration of 0.3% the highest yield was 5.96%.

Gel Strength

The ability to form a gel (gel strength) is one of the physical properties that determine the quality of gelatin. Based on the analysis of variance, each treatment was significantly different ($P<0.05$). The gel strength obtained in this study ranged from 280.43 – 295.06 g bloom, with the highest gel strength at 0.5% NaOH concentration (Figure 4). Hawaiian ladyfish skin gelatin gel strength corresponds to type A gelatin and type B gelatin in the range of 50-300 bloom (GMIA 2012).

The gel strength in this study was included in the good category, where Rahmawati and Hasdar (2017) reported that a gel strength value below 50 bloom will make gelatin difficult to form a gel, while a gel strength value of more than 300 bloom will make gelatin difficult to form, so that it becomes stiffer and harder. Gelatinization (gelatinization) occurs due to immersion with alkaline solutions in the gelatin manufacturing process which can combine amino acid monomer chains and form a three-dimensional structure that will bind air to form a compact gel structure (Said *et al*.

2011). Gudmundsson and Hafsteinsson (1997) stated that gel strength can depend on the isoelectric point and can be controlled to some extent by adjusting the pH. Wijaya *et al.* (2015) reported in their research, that the highest average gel strength was obtained in the 0.6% NaOH immersion treatment, which was 86.47 blooms.

Viscosity

The viscosity obtained in this study ranged from 27.15 to 37.85 cP. Based on the analysis of variance, each treatment was significantly different ($P<0.05$). This shows that the concentration of the NaOH soaking solution affects the viscosity of the gelatin produced. The concentration of 0.5% NaOH solution resulted in a significantly different viscosity from the concentrations of 0.1% and 0.3%. However, this Hawaiian ladyfish skin gelatin viscosity value is not in accordance with the value of type A gelatin with a value of 1.50-7.50 cP and type B gelatin with a viscosity value of 2.00-7.50 cP in GMIA (2012).

Viscosity values that fall into the high category can be caused by the large molecular weight of the Hawaiian ladyfish skin gelatin. Tazwir *et al. (*2009) explained that the immersion treatment with NaOH solution was able to close the space around the polymer protein left by fats and other minerals, so as not to break the existing amino acid chain, this caused the gelatin obtained to have a larger molecular weight, this is shown by high viscosity value. Siburian *et al.* (2020) explained that the viscosity of gelatin is related to the average molecular weight and molecular distribution. The molecular weight of gelatin is directly related to the length of the amino acid chain. That is, the longer the amino acid chain, the higher the viscosity. Astawan and Avian (2003) reported that the alkaline immersion treatment resulted in greater quality than the acid treatment. This is because the soaking process with alkaline produces gelatin with long peptide chains so that the molecular weight becomes larger and the excessive viscosity becomes larger.

Water Content

Parameters of water content in gelatin will relate to shelf life, rancidity, and color of the gelatin produced. The water content obtained in this study ranged from 8.75 to 9.23%. The water content is related to the process of soaking with an acid solution after the alkaline solution is carried out. Ulfah (2011) reported that the acid solution given during immersion will diffuse into the raw material network so that the collagen structure contained will be more open and weaker, as well as produce a gelatin structure with weak bonds and make the water-binding capacity of gelatin less strong. The weak water holding capacity of gelatin will make water evaporate easily during the drying process.

Based on the analysis of variance, each treatment was not significantly different ($P>0.05$). The water content of gelatin will affect shelf life because it is closely related to metabolic activities that occur during the storage of gelatin such as enzyme, microbial, and chemical activity, namely the occurrence of rancidity and non-enzymatic reactions, causing changes in organoleptic properties and quality values (*Rachmania et al.* 2013).

Ash Content

The ash content obtained in this study ranged from 0.53 to 0.75% with the highest value at a concentration of 0.5% NaOH immersion solution. Based on the analysis of variance, each treatment was not significantly different ($P>0.05$). However, the content of ash content in the gelatin of Hawaiian ladyfish skin is still in accordance with the criteria of the GMIA (2012), type A gelatin ranging from 0.3-2.0% and type B gelatin ranging from 0.5-2.0%. The range of ash content of the Hawaiian ladyfish skin gelatin also still meets the quality standard of BSN (1995) with a maximum value of 3.25%.

The content of ash content in the gelatin of Hawaiian ladyfish skin is due to the treatment of immersion in NaOH solution will release Na^+ ions and soaking with HCl acid solution releases Cl^- ions. Na^+ and Cl^- ions trapped in the skin will bind to form NaCl salts which will affect the ash content of gelatin. Oktaviani *et al.* (2012) stated that the increase in ash content was caused by the presence of salt formed from Na^+ and Cl^- ions in a food product. The size of the ash content value is influenced by the washing and filtering process. Mineral components that have not been released during the washing and filtering process will be carried over to the ashing process (Febryana *et al.* 2018)

pH Values

The pH values obtained in this study ranged from 6.88 to 7.10. Based on the analysis of variance, each treatment was significantly different ($P<0.05$). Treatment of 0.1% NaOH solution concentration resulted in a significantly different pH value with 0.3% and 0.5% NaOH concentration solutions. The pH value of the Hawaiian ladyfish skin gelatin proves that there is a combined effect of NaOH and HCl when soaking which causes the pH of the Hawaiian ladyfish skin gelatin to be neutral. The pH range of fish skin gelatin is still in accordance with the GMIA (2012) standard for type B gelatin with a pH range of 5.00-7.50. Sompi *et al*. (2015) reported that the pH value of gelatin is caused by the small amount of acid that binds to skin proteins. The range of pH values indicates the process of penetration and washing of the skin before the extraction process runs perfectly so that contamination can be minimized. Gunawan *et al.* (2017) explained that a good washing process will cause less acid to be trapped in the skin so that the pH value will approach neutral.

CONCLUSION

The treatment of soaking fish skin with NaOH base soaking solution before soaking it with HCl acid soaking solution resulted in values that had a significant effect on color, yield, gel strength, viscosity, and pH. The best treatment combination produced was 0.1% NaOH and 6% HCl with a hedonic value of 3.7 with a whitish-yellow color; 11% yield; gel strength 280.43 g bloom; viscosity 36.95 cP; and a pH value of 6.88.

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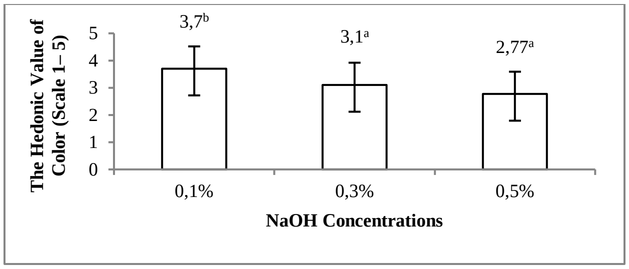

Figure 1. Histogram of the hedonic value of gelatin color with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$)

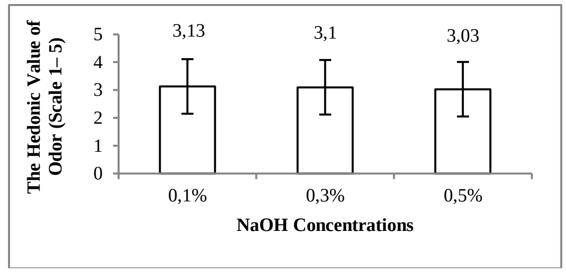

Figure 2. Histogram of hedonic odor of gelatin with different concentrations of NaOH solutions

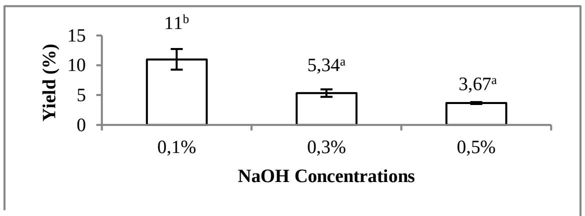

Figure 3. Histogram of gelatin yield with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$)

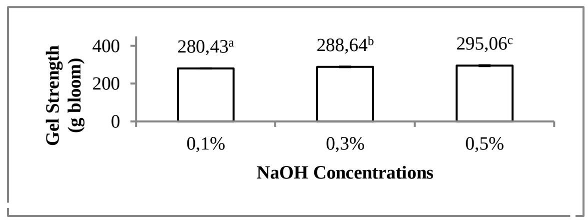

Figure 4. Histogram of gelatin gel strength with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$)

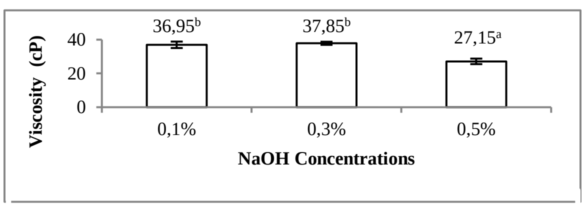

Figure 5. Histogram of gelatin viscosity with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$)

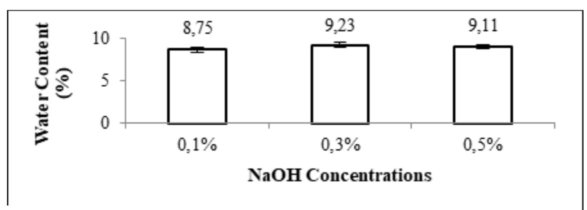

Figure 6. Histogram of water content of gelatin with different concentrations of NaOH solutions

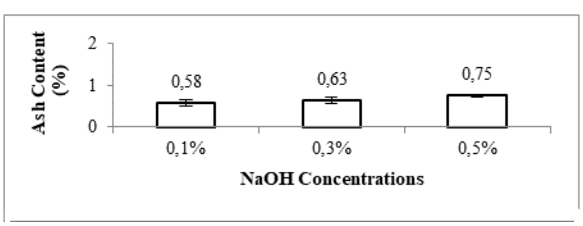

Figure 7. Histogram of gelatin ash content with different concentrations of NaOH solutions

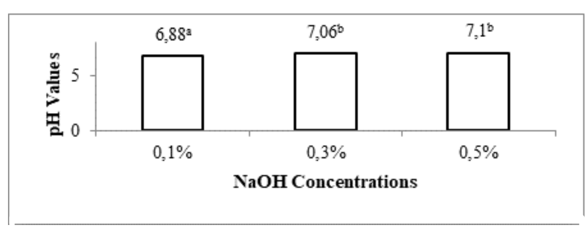

Figure 7. Histogram of gelatin pH values with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$).