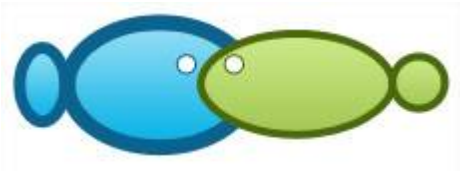

Use of tuna bone waste as raw material for gelatin

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Abstract. The aim of this study was to use tuna bone waste as a raw material for gelatin extraction by utilizing the acetic acid derived from palm vinegar, and to determine the physicochemical characteristics of the extracted gelatin. The quality of gelatin was tested by determining the yield, viscosity, pH, and proximate composition using the completely randomized design (CRD) method and varying palm vinegar concentrations of 3%, 4% and 5%. The result showed that the concentration of acetic acid had a significant impact on proximate composition levels, yield, and viscosity but had no effect on the pH value. The gelatin exhibited specific physical and chemical properties, including yield (ranging from 5.9 to 10%), viscosity (2.83 to 4.30 cPs), water content (12.53 to 14.32%), ash (5.69 to 6.62%), protein (70.82 to 73.85%), fat (1.78 to 3.24%), and pH (4.57 to 4.83). Based on the physical and chemical tests, 4% acetic acid was the best treatment, which produced the lowest fat and ash levels of 5.69% and 1.78%, respectively. However, the protein content was not significantly different from the 5% concentration treatment at 73%.
Key Words: palm vinegar, economic value, fish bone, tuna gelatin.

Introduction. Tuna is one of the fisheries resources with important economic value, due to its meat, which serves as a crucial raw material in the tuna loin industry. However, other parts of this fish, aside from the meat, often end up wasted. According to Al Khawli et al (2019), Xu et al (2019), Maschmeyer et al (2020), and Rajabimashhadi et al (2023), certain fish waste components, namely the skin, scales, bones, skull, swimming bladder, and remaining viscera, retain high economic value due to abundant collagen and minerals. Jakhar et al (2012) stated that skin and bones, comprising 20-30% of the total fish body, can be used as raw material for gelatin processing. This waste is an excellent raw material for the preparations of collagen and gelatin from marine by-products. The preparation satisfies the kosher and halal requirements as well as consumers concern for bovine spongiform encephalopathy (BSE), while increasing the economic returns for the fishing industry (Astawan et al 2002).

Gelatin, a protein derivative derived from collagen fibers in skin, bones, and cartilage, is an important additive in the food and non-food industries, which is obtained from collagen by heat denaturation (Siburian et al 2020; Chandra et al 2023). In principle, gelatin is extracted from the hydrolysis process of collagen as one of the constituent components of fish skin and bones (Tazwir et al 2007). This product is available in flour and sheet forms, and when immersed in water, it expands and softens, showing the ability to absorb water 5-10 times the overall weight. Gelatin dissolves in hot water, and when cooled it forms a gel (Park et al 2008).

The production of gelatin comprises the acid and the alkaline processes, with the difference between both found in the soaking process (Trilaksani et al 2012). The acid and alkaline soaking processes produce types A and B gelatin, respectively (De Wolf 2003). Economically, the acid process is more preferred than the base due to the

relatively shorter soaking duration as stated in preliminary studies (Grossman & Bergman 1991).

Commercial gelatin is generally processed from bone and skin of livestock, specifically cows and pigs (Akbar et al 2017). However, the use of gelatin obtained from the bones and skin of pigs and cows is subject to strict religious limitations, specifically in Islam, where the consumption of pork is forbidden, and in Hinduism, where products made from cows are not tolerated (Zulpahmi et al 2022). The use of bone and fish skin is an appropriate alternative, as these components contain collagen, a fiber-shaped protein found in connective tissue (stroma). When boiled in water combined with the treatment of acids and bases, collagen transforms into gelatin.

Preliminary studies on gelatin processing from fish bone used several types of acids as reagents in the extraction process. Hydrochloric acid (HCl) was used by Kusumawati et al (2008), while Fatimah & Jannah (2008) and Istiqlaal (2018) utilized citric acid and lontar vinegar, respectively. It is possible to explore other types of organic acids as reagents in the gelatin extraction process. One such organic acid is acetic acid derived from fermented water, specifically, the water of sap obtained from palm fruit (*Arenga pinnata*).

The use of natural raw material as a source of organic acid in gelatin extraction has not been previously reported. Therefore, this study examined the characteristics of gelatin extracted from tuna bones using acetic acid from palm vinegar.

Material and Method. This study was conducted in the laboratory of the Fishery and Marine Science Faculty, Universitas Negeri Gorontalo, Gorontalo and the laboratory of the Agency for Applying Quality and Diversification in Fishery Products, Gorontalo, from January to July 2022.

Tools and material. Tuna fish bones and palm vinegar were obtained from the fishing industry at TPI Gorontalo City and Dulamayo village, Bone Bolango district. The material used for gelatin quality testing were distilled water, H_2SO_4, NaOH, $CuSO_4$, boric acid, cresol Bromine indicator green-methyl red, NaCl, Na_2SO_4, methyl ester (Pudak Scientific), and HCl (Merck). The tools used for gelatin extraction were a knife, cutting board, extractor, evaporator, drying oven, and sieve. In addition, the equipment for chemical analysis was an analytical scale, oven, furnace, desiccator, pumpkin kernel, soxhlet, burette, thermometer, porcelain cup, and pH meter.

Study methods. This stage was carried out in two steps, namely extraction of gelatin from fish bones using 3 different concentrations of acetic acid and chemical characterization of gelatin produced.

Gelatine extraction. Gelatin was extracted through several stages in accordance with the method proposed by Tazwir & Ayudiarti (2011). The stages comprised preparation of raw material, immersion for demineralization, washing, extraction, filtration, and drying. The soaking process was carried out in palm vinegar with an acetic acid concentration of 3%, 4%, and 5% respectively.

The stages used to prepare gelatin were as follows: (1) the raw substance was cleaned from the remnants of meat, (2) the bone was cut into small sizes of approximately 1-1.5 cm, (3) the bone fragments were soaked in sugar palm vinegar solution for 30 days until ossein was formed, (4) the ossein was washed to neutral pH (6-7) then extracted at 80ºC for 6 hours, (5) the material was filtered and dried at 55ºC, and (6) the substances were grounded to gelatin powder.

Calculated gelatin yield. Gelatin yield was expressed as a percentage based on the ratio of the weight of gelatin powder and the weight of the cleaned fish bones (Jamili et al 2016).

Physical characterization of gelatin. The physical properties of gelatin included viscosity and pH value.

Viscosity. This measurement was in accordance with the modified procedure of GMIA (2019). The viscosity test used a Brookfield viscometerwith 6.67% (w/v) gelatin solution measured for viscosity at 40°C at a speed of 30 rpm using the 61st spindle. The measurement results were multiplied by a conversion factor.

pH value. Gelatin pH was measured using a pH meter (Hanna) by the study by Tinrat & Sila-asna (2017). Gelatin powder was dissolved in distilled water (1% (w/v)) for 5 minutes. The electrode of the pH meter was inserted into the gelatin solution, and the results read on the LCD screen of the pH meter.

Chemical characterization of gelatin. The AOAC method (2005) analyzed the extracted gelatin for its chemical characteristics. The process was conducted using an oven (Memmert), a furnace (Neycraft JFF 2000, Germany), a Kjeldahl (Gerhardt KB 8, Germany), and a soxhlet (Gopal, Ind) to determine its moisture, ash, protein, and fat contents.

Statistical analysis. The experiment was carried out by soaking fish bones in palm vinegar with acetic acid concentrations of 3%, 4%, and 5% for 30 days. The obtained results were analyzed using ANOVA with a confidence level of 95%.

Results

Acetic acid palm vinegar levels. The fermented nira water (*A. pinnata*) produced palm vinegar with an acetic acid content of 5.79%. The fermentation was carried out for 1 month with spontaneous fermentation methods and took place in facultative aerobics, which means allowing less oxygen into the process.

Physical characterization of gelatin

Yield value. The gelatin yield, representing the amount of gelatin obtained from the extraction process, serves as an important indicator of the effectiveness of the palm vinegar extraction. Based on the analysis results, the resulting gelatin yield value is 5-10%, as shown in Figure 1.

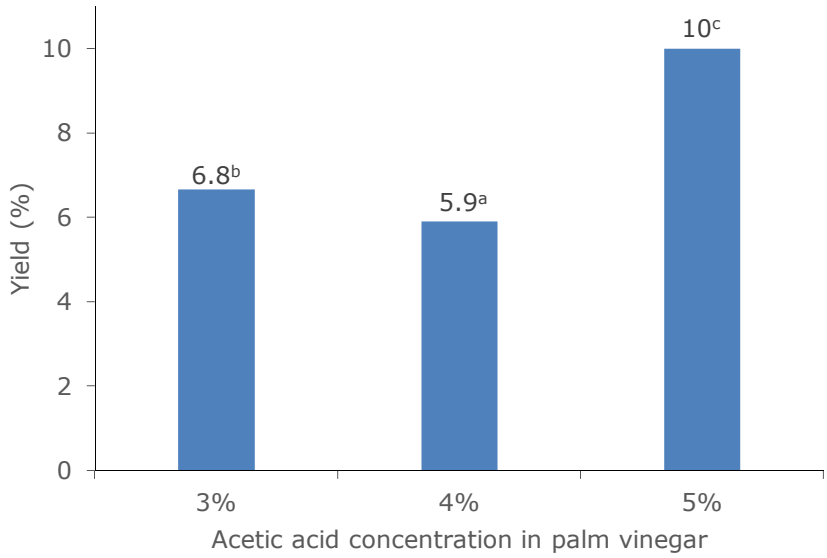

Figure 1. Yield of gelatin of tuna bone (note: different letters show significant differences between treatments).

Figure 1 shows that the yield of gelatin obtained from 3%, 4%, and 5% treatments is 6.8%, 5.9%, and 10%, respectively. The variance analysis results show that the concentration of acetic acid in palm vinegar significantly affects the yield. Furthermore, the Duncan analysis result illustrated that the yields produced at each treatment concentration had varying value due to the ability of acetate to hydrolyze the proteins in fish bones. The high yield value which is 10% at an acetic acid in palm vinegar concentration of 5%, was obtained because acetic acid in palm vinegar can hydrolyze the proteins found in fish bones. According to Ebrahimi et al (2022), other organic acid compounds in palm vinegar are benzoic and butanoic acids.

Viscosity of gelatin. Viscosity is the flow of molecules in a solution in water, simple organic liquids and suspensions and aqueous emulsions (de Man 1999). The viscosity value of the extracted gelatin is shown in Figure 2.

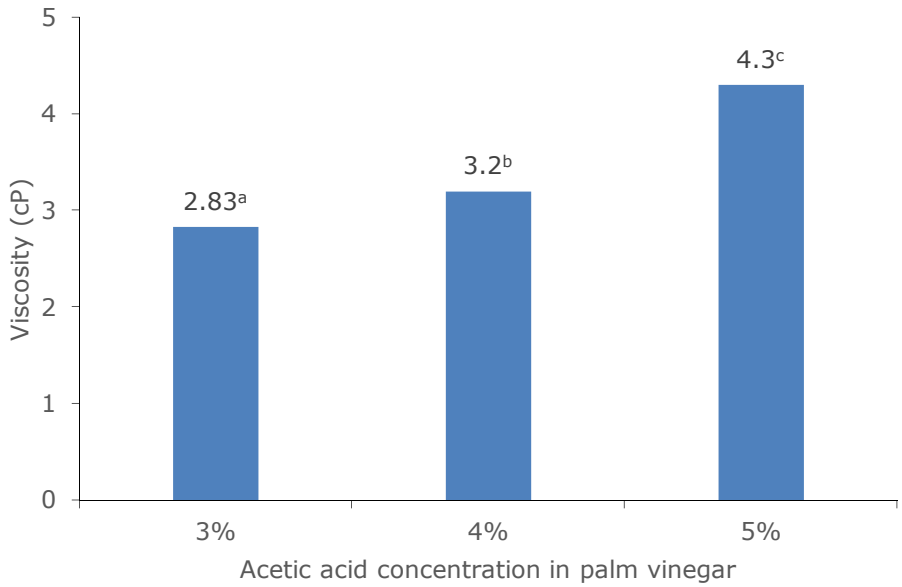

Figure 2. Viscosity of gelatin of tuna bone (note: different letters show significant differences between treatments).

Based on Figure 2, the viscosity value of extracted fish bone gelatin is in the range of 2.83-4.30 cPs. The variance analysis result showed that the different concentrations of palm vinegar used in extracting of tuna bone gelatin have a real influence on the viscosity value of the gelatin produced. Further test results show that the three concentrations were significantly different in the viscosity of the gelatin produced.

Acidity value (pH). The pH value of the gelatin ranged between 4.57 and 4.83 as shown in Figure 3. Tests illustrated that the concentration of palm vinegar used does not significantly affect the pH value of the gelatin produced. The pH value met the quality of gelatin based on GMIA (2019) standards of 3.8-6.0.

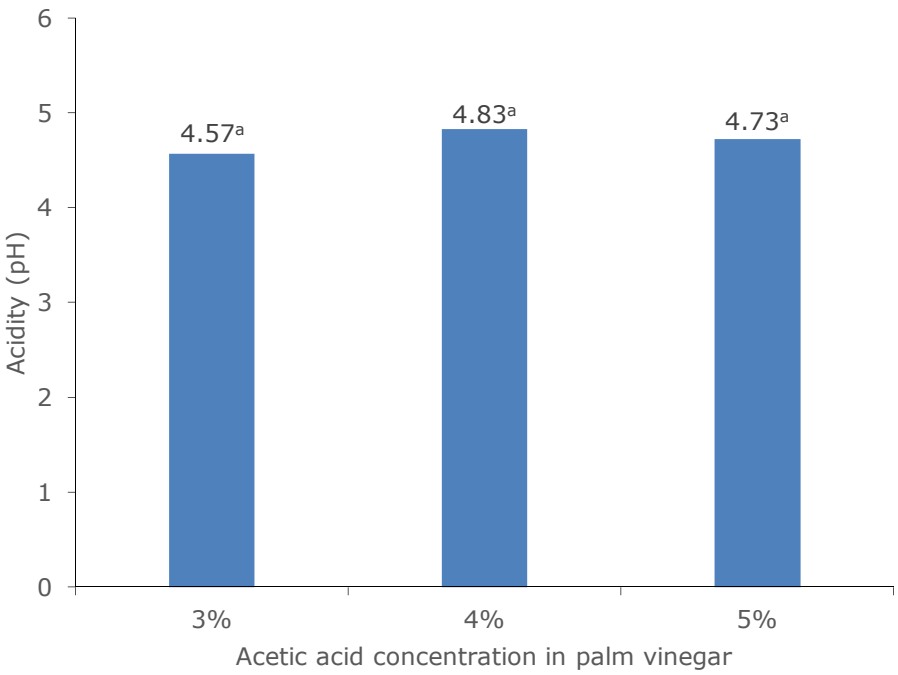

Figure 3. pH of gelatin of tuna bone (note: different letters show significant differences between treatments).

Chemical characterization of gelatin

Water content. The water content variation in the extracted gelatin ranged from 12.53 to 14.32%, as shown in Figure 4.

The water content analysis of gelatin extracted using palm vinegar yielded results from 12.53 to 14.32%. This shows that the water content of the gelatin produced in the present study is in line with the quality standards outlined in SNI 06-3735, 1995 (BSN 1995). According to these standards, the acceptable water content for gelatin is 16%.

Based on the analysis of variance, the concentration of palm vinegar used in soaking fish bones had a significant effect on the water content of gelatin. The Duncan test result showed significant differences in water content value for tuna bone gelatin, as shown in Figure 4.

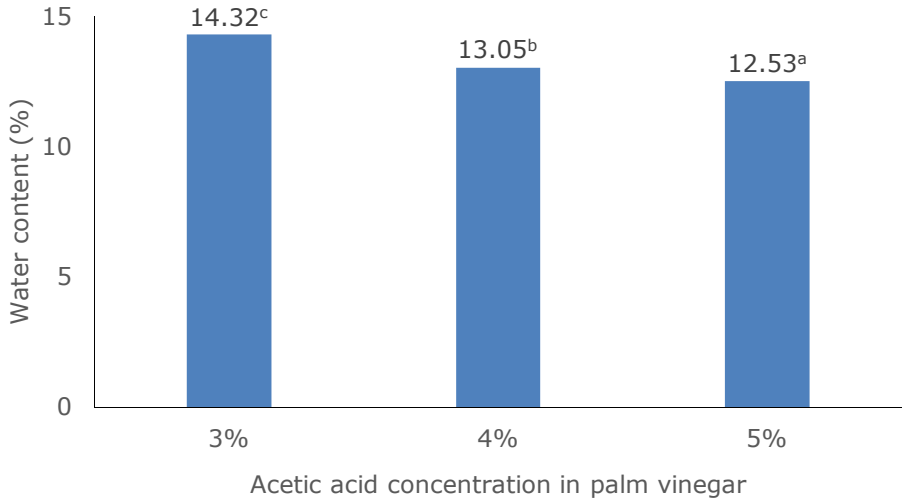

Figure 4. The water content of tuna bones gelatin (note: different letters show significant differences between treatments).

Ash content. The ash content variation in the extracted gelatin ranged from 5.69% to 6.62%, as shown in Figure 5.

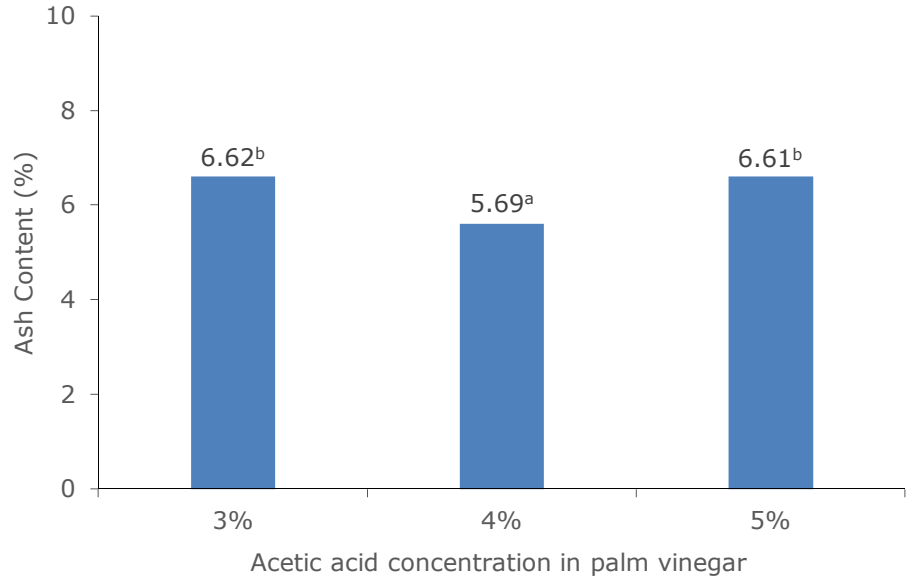

Figure 5. Ash content of tuna bones gelatin (note: different letters show significant differences between treatments).

Statistic tests showed that the concentration of palm vinegar in the extraction process significantly affects the gelatin ash levels. Subsequently, the results of the Duncan test showed a significant difference in gelatin ash content between soaking in palm vinegar with 4% acetic acid compared to the 3% and 5% levels.

Protein content. The protein content of the extracted gelatin ranged from 70.83 to 73.83%, as shown in Figure 6.

The results of statistical tests showed that the acetic acid concentration significantly affected the gelatin protein levels. As the concentration of acetic acid in palm vinegar increases, there is a corresponding rise in the protein content of gelatin.

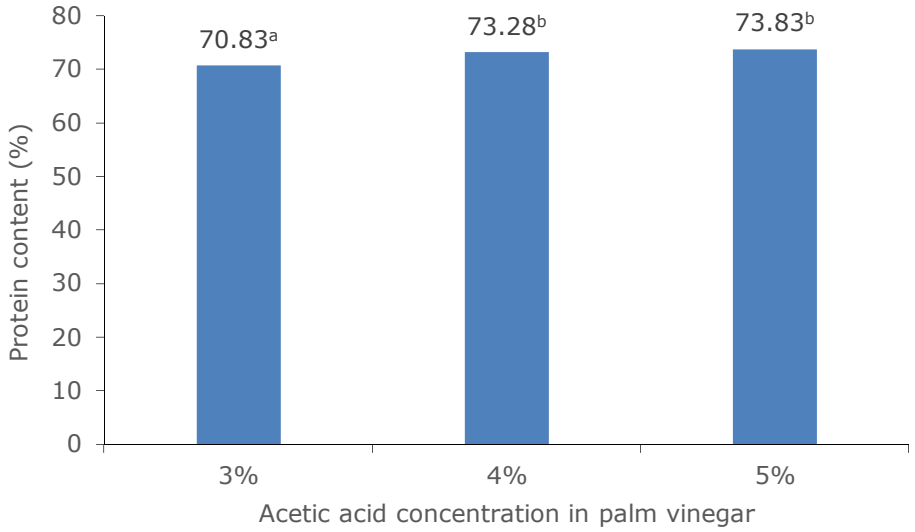

Figure 6. Protein content of tuna bones gelatin (note: different letters show significant differences between treatments).

Fat content. The fat content of gelatin varied between 1.78 and 3.24%, as shown in Figure 7. Based on statistical tests, the acetic acid concentration significantly affected the gelatin fat. Specifically, the extraction treatment using 4% acetic acid from vinegar produced the lowest gelatin fat content at 1.78%.

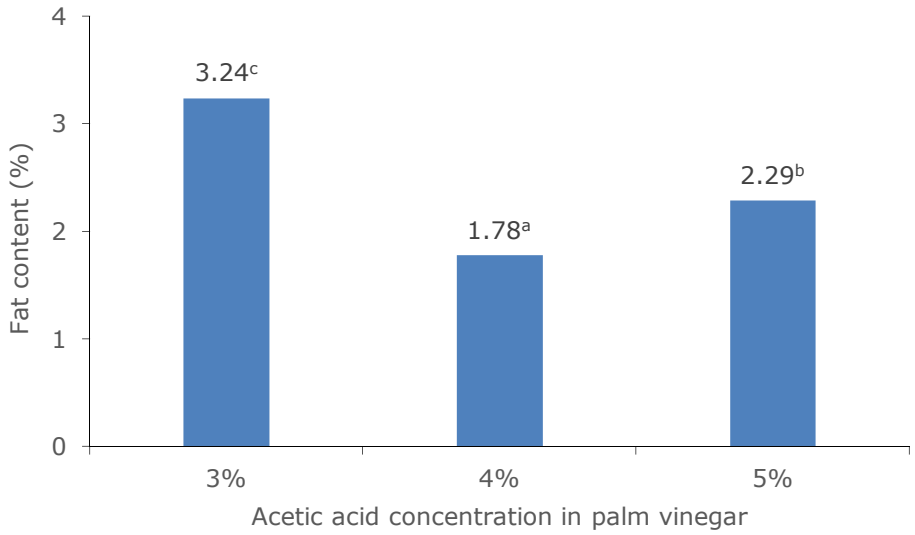

Figure 7. Fat content of tuna bones gelatin (note: different letters show significant differences between treatments).

Discussion. The analysis results showed a significant influence on the physical and chemical properties of gelatin extracted from tuna bones, when subjected to different concentrations of palm vinegar (3%, 4%, 5%) during the soaking process.

Yield value. The differences in yield observed at varying concentrations of palm vinegar are linked to the ability of acetate to hydrolyze proteins in fish bones. However, the significant 10% yield observed at a 5% palm vinegar concentration is due to the hydrolyzing effect of palm vinegar on the proteins found in fish bones, complemented by the presence of additional organic acid compounds such as lactic, formic, and propionic acids. These organic acids contributed to the generation of acid ions (H^+), and played a significant role in breaking hydrocarbon bonds between collagen during the soaking process. In addition, it enabled the production and separation of more hydrogen bonds in tropocollagen. Another factor that influenced gelatin yield was the pH of palm vinegar.

The test results showed that the yield value obtained was higher than the one reported by Zulkifli et al (2014) on the extraction of gelatin from tuna bones using different treatments. This was obtained by adjusting the volume of palm vinegar as a soaking agent and the weight of tuna bones (3:1, 5:1, 7:1) for 14 days, to yield values ranging from 2.81 to 6.095%.

Jhon & Courts (1970) as cited by Fahrul (2005) stated that higher acid concentration and prolonged soaking time led the breakdown of hydrophobic bonds, known as critical stabilizers in the collagen triple helix. This breakdown resulted in the formation of α, β, μ components, making the conversion into gelatin easier and more useful. Additionally, Courts (1977) as cited in Nurilmala et al (2006), stated that gelatin yield was influenced by pH, extraction temperature, and acid concentration. During soaking, the acid acts on the collagen helix bonds in the bone matrix through its ions. The more acidic the solvent, the lower the pH. This causes more collagen helices to break down.

The gelatin yield in this study when compared with alternative acids, showed a higher average value, ranging from 5.95 to 10%. For example, Nurilmala et al (2006) conducted a similar study using 5% hydrochloric acid, which yielded 5.33% of tuna fish bone gelatin. Fatimah & Jannah (2008) used 5% citric acid from milkfish bones to obtain

a yield of 5.14%. Additionally, Karlina & Atmaja (2010) used 5% acetic acid to produce a 1.91% yield of gelatin from stingray bones.

Viscosity. The viscosity value obtained showed that an increase in the concentration of the acid solution led to higher viscosity. This is in line with the study conducted by Poppe (1997) that higher solvent concentrations used in gelatin extraction are associated with increased viscosity value.

The test results in Figure 2 showed that the concentration of palm vinegar used significantly influenced the viscosity of the extracted gelatin. This means that higher palm vinegar concentrations are associated with increased viscosity values in the extracted gelatin. These viscosity values are in line with the results of Amiruldin (2007), where HCl was used in the tuna bone soaking process, to obtain viscosities ranging from 3.23 to 5.57 cP.

The viscosity of the gelatin solution mainly depends on the degree of hydrodynamics between the gelatin molecules, influenced by factors such as pH, temperature and concentration. Furthermore, viscosity decreases exponentially with temperatures above 40°C, while the lowest was observed at the isoelectric point in terms of pH and the concentration of the gelatin solution (Ward & Courts 1977). Additionally, the ash content and molecular weight in the solution are contributing factors, as a higher number of dissolved molecules led to an increase in molecular weight, resulting in greater viscosity (GMIA 2019).

Acidity value (pH). The results of the tests shown in Figure 3 illustrated a consistent pH value of the extracted gelatin, which is approximately 5 and within the range of weak acidity. This stability was attributed to the bone-soaking process using palm vinegar, causing the resulting gelatin to have an acidic pH value. Additionally, washing the bones after soaking in acid did not effectively remove the remaining acid levels containing ossein.

The pH of the gelatin was determined by dissolving its products in distilled water. The resulting pH value is in line with the study conducted by Amiruldin (2007), where HCl was used in the gelatin extraction process from tuna fish bones, to obtain a pH range of 4.15 to 5.54. Furthermore, the pH value obtained in this study falls within the standard range for type A gelatin, namely 3.80 to 6.00 (GMIA 2019).

Hinterwaldner (1977) observed the relationship between gelatin pH and the extraction process and reported that immersion in acids tends to produce gelatin with a low pH. The pH of gelatin is influenced by the extraction material, for example when extracted from fish using an acid and a base produces type A (acidic), and B gelatin (alkaline), respectively (Nurilmala et al 2017). Consequently, ossein washing in gelatin production plays an important role in neutralizing pH with the aim of removing residual acid to prevent further ossein breakdown, characterized by a neutral pH. Failure to optimize washing may result in excess acid remaining in the hollow space of the ossein, leading to the production of gelatin with a low pH that fails to meet the recommended standards (Kusumawati et al 2008).

Water content. The analysis results in Figure 4 showed that the higher the concentration of palm vinegar used, the lower the moisture content in the extracted gelatin. This was attributed to the strong nature of the acid in hydrolyzing protein, which led to the release of both hydrogen bonds and water from the fish bone. Fahrul (2005) stated that several factors, including acid concentration and soaking time, influence the water content of produced gelatin. Furthermore, a shorter soaking time led to minimal water absorption rate, while maximum soaking levels caused the converted gelatin to bind more, thereby increasing the water content of the material, which is lost during the drying process. The gelatin produced in this study was dried at 50°C for 3 days.

Ash content. Based on the analysis results shown in Figure 5, the ash content did not indicate significant variations. The ash content at the 4% acetic acid level (5.69%) was lower than the 3% and 5% treatments. This difference is likely due to the thorough

demineralization achieved with the 4% acetic acid treatment, which effectively binds mineral ash in tuna fish bones to the acid from sugar palm vinegar. According to Ismangil & Hanudin (2005), the dissolution of minerals in organic acids was determined by the concentration and reactivity rates. Higher acid concentrations increased the number of protons that attacked the mineral bonds, which was influenced by the carboxyl group in the acid.

The level of gelatin ash obtained exceeded the quality standard set by BSN (1995), which stipulates a maximum limit of 3.25%. This high ash content was attributed to the type of acid used in the immersion process. Ismangil & Hanudin (2005) stated that the ability of acetic acid to release only one proton to bind minerals contributed to the higher ash content observed in the gelatin.

Protein content. The results of the statistical tests showed that the concentration of acetic acid had a significant effect on gelatin protein. Figure 6 shows that the protein value in gelatin extracted with a 4% palm vinegar concentration is not significantly different from that obtained with 5% palm vinegar. However, a significant difference was observed compared to the protein value resulting from soaking in 3% palm vinegar.

The protein content in gelatin shows an upward trend with increase in the concentrations of palm vinegar due to the rise in the number of acid molecules in the solution, which led to a higher molecular density and increased collisions between acid and calcium phosphate molecules in the bones. The heightened interaction increased the effectiveness of palm vinegar in binding minerals in the bones, which led to a more significant liberation and conversion of collagen into gelatin. Fatimah & Jannah (2008) stated that the protein content in gelatin increases as the concentration of acid rises. The protein content in gelatin from tuna bone is lower than the commercial one, because the acid group consists of weak organic acids, incapable of breaking the hydrogen bonds between collagen molecules. Kusumawati et al (2008) stated that commercial gelatin typically has a protein level of 79.40%, which is lower the research by Pranoto et al (2011), were it was extracted from yellowfin tuna skin with protein levels of 81.63%.

The protein content observed in this study was lower compared to the investigation conducted by Astawan & Aviana (2003) where acetic acid was used, reporting a protein content of 86%. Furthermore, Naiu et al (2023) used a commercial palm vinegar solution to obtain a protein value of 81.74% in the extract.

Collagen proteins, as precursors of gelatin, are sensitive to both alkaline and acidic solutions. This is because exposure to these conditions can disrupt the hydrogen bonds, leading to the expansion of the collagen helix and conversion into gelatin. Almatsier (2002) stated that collagen, which is initially insoluble in water, is converted to gelatin when heated in water, diluted acid, or alkaline solutions. According to Katili (2009), among collagen fibers, hydrogen bonds are immensely sensitive to heat, acid, and bases.

Fat content. Based on statistical tests, the acetic acid concentration significantly affected the gelatin fat content. Treatment with 4% acetic acid in palm vinegar produced the lowest fat in gelatin. Consequently, the fat content obtained in this study was relatively lower than that reported by Naiu et al (2023), who used commercial palm vinegar with the same soaking time, resulting in a fat content of 3.25%.

The outcome is due to the process of renovation facilitated by palm vinegar during immersion, to obtain its maximum efficacy. During this process, various chemical compounds, including minerals and fats that bind to proteins (lipoprotein), are released, leading to the low-fat content in the gelatin. Furthermore, the fat content was influenced by the fat separation process formed after extraction. In this study, optimal fat separation occurred at the 4% vinegar concentration. Fahrul (2005) stated that gelatin fat content is dependent on the entire process from bone cleaning to the extraction and filtration stages. Proper treatments at each stage reduced the fat in the raw material, which led low-fat content in the final product.

Conclusions. Tuna bone waste could be a viable raw material for gelatin production. The physicochemical characteristics of gelatin extracted using acetic acid from palm vinegar included a yield that ranged from 5 to 10%, viscosity between 2.83 to 4.30 cPs, and a pH between 4.57 and 4.83. Additionally, the gelatin showed water content levels between 12.53 and 14.32%, ash of 5.69 to 6.62%, protein ranging from 70.82 to 73.85%, and fat between 1.78 and 3.24%.

Conflict of interest. The authors declare that there is no conflict of interest.

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