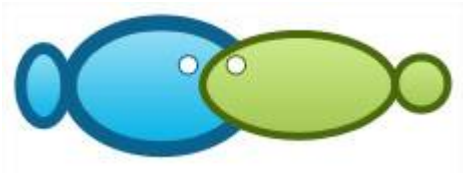

Comparison of the physicochemical quality of tuna bone gelatin extracted using aren vinegar with commercial gelatin

Asri S. Naiu, Nikmawatisusanti Yusuf, Sri R. Kalaka

Faculty of Fisheries and Marine Sciences, Universitas Negeri Gorontalo, Gorontalo 96128, Indonesia. Corresponding author: A. S. Naiu, asri.silvana@ung.ac.id

Abstract. Gelatin is a protein derivative of collagen that is denatured through a heating process preceded by a pre-treatment process using a base or acid. Acid solutions commonly used in pre-treatment are synthetic acids such as hydrochloric acid, acetic acid and citric acid. However, in this study, natural acid from the fermentation of nira water, namely aren vinegar, was used. This study aims to characterize the physiochemical properties of tuna bone gelatin soaked in aren vinegar for 25 days (G1) and 35 days (G2) and compare it with commercial fish bone gelatin (CG). The data obtained were analyzed by the Independent T-Test at a 95% confidence level. The results showed that differences in soaking time affected the yield, color degree, viscosity, moisture content, ash, fat, protein and amino acid profile of gelatin. The yields of G1 and G2 were 7.42% and 5.20% respectively, protein were 72.31% and 81.74% respectively, lower than CG with 92.34%, and viscosity was 8 mPaS and 11.83 mPaS respectively, higher than the CG viscosity of 4 mPaS. G1 and G2 met the moisture content requirements based on GMIA (2019) in the range of 8-13%, but not CG. The average amino acid composition of G1 was lower than G2 and CG and had the lowest brightness level. The pH of G1 and G2 was not significantly different and each was significantly different from CG.
Key Words: demineralization, fishbone, gelatin, immersion.

Introduction.Gelatin is the result of heat-treated collagen conversion, a polypeptide biopolymer found in animal skin and bone tissue. The use of gelatin in various industrial fields as an emulsifier, thickener, stabilizer, adhesive and film coating base material causes the demand for gelatin never decreases (Silva et al 2014; Junianto et al 2021). The advantages of reversible characteristics of gelatin are more favorable than theirreversible hydrocolloid materials of cellulose. The good quality of gelatin as an additive and active substance is one of the success factors in an industrial product (Williams & Phillips 2009). Therefore, research related to gelatin production continues to be carried out to keep up with the diverse industrial demands. Nowadays, research on gelatin, especially halal-labeled gelatin extracted from fish bones and skin has been widely conducted. The availability of waste from the processing of tuna loin and fish canning industries is very supportive of the sustainability of gelatin production. Shyni et al (2014) stated that the biggest part of the fish that ends up as waste in fish processing is skin and bones. Furthermore, Wangtueai et al (2016) mentioned that the fish by-products have a high potential to be used as raw material for gelatin production, as they are rich in collagen.

The gelatin production process generally involves several steps, namely degreasing, demineralization or pre-treatment using acid or base, extraction/conversion of collagen into gelatin, drying and refining. Some of these stages are modified by the researchers according to the type of raw materials and equipment available. Research related to the formulation and characterization of fish bone gelatin using synthetic acids and classified as a strong acid has been widely carried out, including by Hapsari et al (2017) and Pertiwi et al (2018) who used citric acid, Masrukan et al (2016) using hydrochloric acid, Hidayat et al (2016) using phosphoric acid and papain enzyme, and Tinrat & Sila-Asna (2017) using sodium hydroxide base followed by phosphoric acid.

According to Sultana et al (2018) the preparation of type A gelatin is conducted by soaking fish bones in an acid solution using hydrochloric acid, sulfuric acid and phosphoric acid. However, the use of synthetic acids and those classified as strong acids is potentially dangerous because their use in large quantities can pollute the environment and pose a risk to personal safety if not used carefully. In addition, the yield of gelatin obtained is relatively low, as reported by Junianto et al (2021), that higher concentration of hydrochloric acid in pre-treatment further reduced the yield of gelatin.

Previous research that has been done by Zulkifli et al (2013) and Naiu et al (2015) that used aren vinegar was still limited to the study of the effect of aren vinegar concentration on the physical and chemical properties of gelatin. However, this current research focused on the effect of soaking time in aren vinegar on the physicochemical properties as well as the amino acid profile of gelatin. The use of aren vinegar as a fish bone soaking agent is a way of utilizing nira water produced from the fruit of the enau tree (*Arenga pinnata*). Before use, the juice was fermented to produce an acidic compound called aren vinegar. Aren vinegar is a weak natural acid, organic acid that is more environmentally friendly and non-toxic. However, even though it is classified as a weak acid, Istiqlaal (2018) reports that the ash content of gelatin from soaking fish bones using palm vinegar, one of the natural vinegars fermented from legen water from the lontar tree (*Borassus flabellifer* Lynn) was not significantly different from the ash content of gelatin produced from soaking with strong hydrochloric acid.

According to Gómez-Estaca et al (2009) the physicochemical characteristics of gelatin are greatly influenced by raw material, species, tissue type, animal age, collagen type, collagen characteristics and manufacturing method. The characteristics of tuna bone gelatin that passed the pre-treatment using hydrochloric acid reported by Masrukan et al (2016) still produces brownish yellow gelatin, 80.90% protein content, 2.73% fat content, and fairly high ash content of 8.12%. Color degree of the gelatin reported by Sukkwai et al (2011) as L*, a*, b* values were 32.58, -1.67, and 6.14, respectively and produced 14.85 to 24.43% protein content. However, in subsequent studies that still used aren vinegar with the treatment of the ratio of aren vinegar to fish bone 3:1 to 7:1 at the pre-treatment stage, Zulkifli et al (2013) found that gelatin yield was low, i.e 2.81 to 6.09% and Naiu et al (2015) reported protein levels ranging from 69.50 to 75.20% and relatively high fat levels, namely 9.23 to 13.33%. The length of pre-treatment time done by Zulkifli et al (2013) and Naiu et al (2015) was 14 days.

Based on these results, this study was carried out by soaking fish bones for 25 days and 35 days in aren vinegar with a ratio of vinegar to bone of 5:1. The treatment data will be compared with the quality of commercial fish bone gelatin. Thus, the purpose of this study is to characterize the physical and chemical properties of tuna bone gelatin extracted using aren vinegar at different soaking times and compare each treatment with commercial fish bone gelatin. The results of this study are expected to add data related to the characteristics of gelatin that goes through the demineralization process (pre-treatment) using natural acids and can answer whether traditional vinegar can replace synthetic acids as hydrolyzers in the demineralization stage in the gelatin manufacturing process.

Material and Method. This study was conducted in Laboratory of Fishery and Marine Science Faculty, Universitas Negeri Gorontalo, Gorontalo and Laboratory of Agency for Applying Quality and Diversification in Fishery Products, Gorontalo in June-July 2023.

Materials. The materials used in this research include aren vinegar obtained from the plantation of Huangobotu Village, Bone-Bolango Regency, tuna bones from several Fish Processing Units (UPI) and Gorontalo central market, distilled water (Pudak scientific), methylenered indicator, $CuSO_4.H_{52}OK_2SO_4$, H_2SO_4 concentrated, NaOH (Pudak scientific), HCl 37% (Merck).

Gelatin manufacturing process. The gelatin manufacturing process refers to Naiu & Yusuf (2018) which was modified. Tuna bones obtained from the tuna loin processing unit and Gorontalo center market were cleaned of blood, sand and other physical objects,

then soaked in hot water of 80ºC which was allowed to cool for up to 30 minutes. The remaining flesh was removed from the bones, which were then reduced in size to between 1 and 2 cm. The next stage was to soak the fish bones in aren vinegar in a ratio of 1:5. The length of soaking, which is the treatment in this study, namely 25 days and 35 days was carried out until relatively soft bones (ossein) were formed. The ossein was then washed under running water to remove the remnants of the acidic solution until the pH was close to neutral. Ossein was extracted in distilled water in a ratio of 1:5 at 80ºC for 6 hours to form a thick gelatin solution which was then filtered using calico cloth. The filter results were dried in an oven (Memmert) at 55ºC to dry to form gelatin sheets. The gelatin was then pulverized to form powder. The gelatin obtained was characterized for its physical and chemical properties.

Calculation of gelatin yield*.* Gelatin yield is 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 characteristics of gelatin*.* The physical properties of gelatin include pH value, viscosity, and color degree.

pH value. Gelatin pH was measured using a pH meter (Hanna) following 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 could be read on the LCD screen of the pH meter.

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

Color degrees. The color test refers to Hunter Lab (2008) performed according to the Hunter Colorimeter method. The colorimeter (Colorflex, USA) was turned on with the L,a,b system, calibrated, white color was selected, and the calibration results were saved. The sample was attached to the tip of the receptor until the light came on and the results obtained were recorded.

Chemical characteristics of gelatin*.* The chemical composition of gelatin was analyzed following AOAC (1990) procedures; using an oven (Memmert) to moisture content, a furnace (Neycraft JFF 2000, Germany) for ash content, a kjeldahl (Gerhardt KB 8, Germany) for protein content, and a soxhlet (Gopal, Ind) for fat content. The amino acid composition was determined using the HPLC (UFLC Shimadzu CBM-20A, Shimadzu Corporation, Japan).

Statistical analysis*.* The experiment was conducted by soaking fish bones in aren vinegar for 25 days (G1) and 35 days (G2). Commercial fish bone gelatin (CG) was also tested and compared with the experimental gelatin. The data obtained were analyzed by Independent T-Test at 95% confidence level.

Results. The amount of gelatin obtained is an indicator of the effectiveness of the hydrolyzing agent and the efficiency of the process performed. The length of demineralization (pre-treatment) is one of the gelatin manufacturing processes that can affect the yield. The measured gelatin yield are treatments G1 (25-day soaking) and G2 (35-day soaking) which can be seen in Figure 1.

The T-Test on both gelatin samples shows a significant difference ($p < 0.05$) in yield. Gelatin obtained after pre-treatment for 25 days (G1) in aren vinegar is significantly higher than G2.

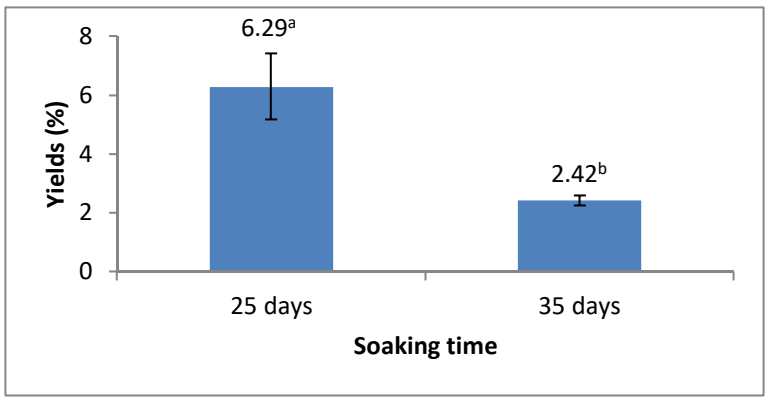

Figure 1. Yield of gelatin from pre-treatment in aren vinegar for 25 days and 35 days.

pH value. pH measurements were carried out on all three gelatin samples, namely G1, G2, and CG (Commercial Gelatin). Gelatin pH is considered to affect other properties, namely gel strength and viscosity. The pH value of the research gelatin and commercial fish bone gelatin can be seen in Figure 2

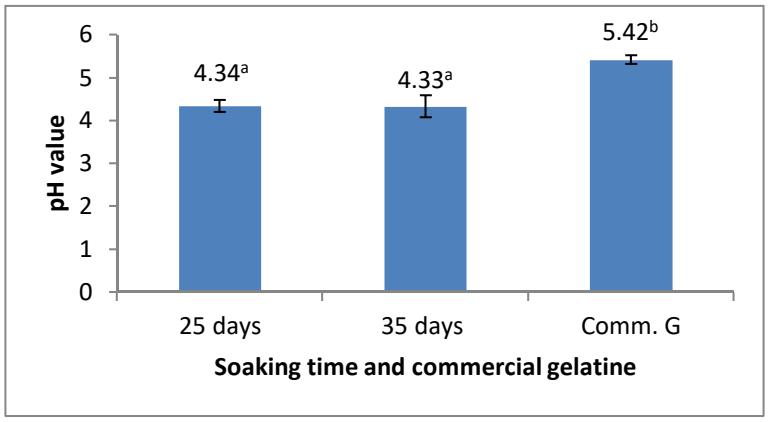

Figure 2. pH value of the gelatin pre-treated in aren vinegar for 25 days and 35 days and commercial gelatin.

The T-test conducted on treatments G1 and G2 shows no significant difference ($p > 0.05$) between them on pH value, but each of these two treatments was significantly different ($p < 0.05$) from commercial fish bone gelatin

Viscosity. Viscosity is a statement of the resistance of molecular flow in solution. Viscosity testing in this study was tested on samples G1, G2 and commercial gelatin which can be seen in Figure 3.

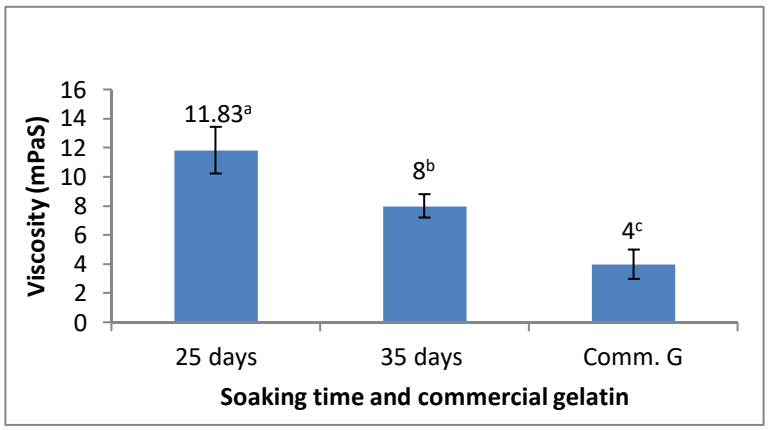

Figure 3. Viscosity of gelatin pre-treated in aren vinegar for 25 days and 35 days and commercial gelatin.

Based on the T-Test, treatments G1 and G2 provide significant differences ($p < 0.05$) on viscosity, as well as between each of these treatments and commercial gelatin.

Color degrees. The degree of gelatin color was observed based on the system L (Lightness level), a* (red/green), and b* (yellow/blue). The color of gelatin pre-treated with aren vinegar for 25 days (G1), 35 days (G2) and commercial gelatin (CG) is shown in Table 1.

Table 1

Degree of color of gelatin pre-treated with aren vinegar for 25 days (G1), 35 days (G2) and commercial gelatin (CG)

Color	*G1*	*G2*	*CG*
L	40.7 ± 0.25^{a}	46.1 ± 0.82^{b}	58.8 ± 0.95^{c}
a*	12 ± 0.10^{a}	9.3 ± 0.45^{b}	4.5 ± 0.12^{c}
b*	37.1 ± 0.64^{a}	33.5 ± 0.59^{b}	28.4 ± 0.15^{c}

The numbers in the Table are the average values of three replicate experiments. Different superscript letters in one row indicate significant differences between treatments at the 95% confidence level.

Chemical characteristics of gelatin. The proximate chemical characteristics of gelatin tested included protein content, fat content, moisture content and ash content. In addition, amino acid profiles were also tested on samples G1, G2, and CG. Proximate chemical data of the three samples are shown in Table 2.

Table 2

Proximate chemical data of gelatin pre-treated with aren vinegar for 25 days (G1), 35 days (G2) and commercial gelatin (CG)

Treatment	*Protein*	*Fat*	*Moisture*	*Ash*
G1	72.31 ± 0.55^{a}	16.5 ± 0.51^{a}	6.82 ± 0.58^{a}	3.18 ± 0.24^{a}
G2	81.74 ± 0.79^{b}	3.23 ± 0.52^{b}	10.77 ± 0.73^{b}	1.76 ± 0.07^{b}
CG	92.34 ± 0.03^{c}	0.36 ± 0.02^{c}	6.41 ± 0.91^{a}	0.08 ± 0.03^{c}

The numbers in the Table are the mean of three experimental replicates. Different superscript letters in each column indicate significant differences between treatments at the 95% confidence level.

Amino acid composition. The amino acid composition of gelatin from treatments G1, G2 and CG can be seen in Table 3.

Table 3

Amino acid composition of gelatin pre-treated with aren vinegar for 25 days (G1), 35 days (G2) and commercial gelatin (CG)

Amino acid	*Total (g/100g)*		
	G1	*G2*	*CG*
Aspartic acid	4.78	4.45	7.17
Glutamic acid	6.17	8.47	9.25
Serine	1.12	1.53	1.68
Glycine	1.56	1.98	2.34
Histidine*	1.3	1.72	1.95
Arginine	1.13	1.56	1.64
Threonine*	1.07	1.29	1.6
Alanine	1.18	1.53	1.77
Proline	2.22	2.8	3.33
Tyrosine	1.56	1.95	2.34
Valine*	1.47	1.65	1.61
Methionine*	1.24	1.68	1.86
Cysteine	1.65	2.29	2.47
Isoleucine*	1.8	2.49	2.7
Leucine*	3.87	3.81	5.81
Phenylalanine*	1.16	1.45	1.74
Lysine*	2.85	2.9	2.85

*Essential amino acid.

Table 3 shows that gelatin G2 contains a higher total amount of amino acids than G1, and the amino acid composition of these two samples is on average lower than commercial gelatin (CG).

Discussion. The low yield of gelatin after a 35-day soak is likely due to the bones being exposed to the acidic solution for an extended period of time, resulting in more hydrogen bonds between the collagen helical strands breaking. This causes an increased amount of collagen to dissolve in water during the neutralization phase. As a result, the yield produced is less. According to Junianto et al (2021) collagen in acidic solution undergoes changes that begin with breaking peptide bonds to shorten the chains, the breaking of a number of side bonds between chains, and changes in chain configuration during the extraction process in a hot atmosphere. Jamilah & Harvinder (2002) stated that the strong acid will increase the amount of dissolved collagen and it will also be lost during washing which affects the amount of yield.

The gelatin yield of treatment G1 is higher than the results obtained by Zulkifli et al (2013) which also used aren vinegar within 14 days of soaking, but the yield of treatment G2 was measured to be lower. Panjaitan (2017) reported that the yield of tuna bone gelatin obtained from pre-treatment with hydrochloric acid decreases from 5.03 to 0.22% as the acid concentration in the soaking solution increases from 3 to 11%. Gelatin production from catfish bones with pre-treatment using 1% citric acid for 48 hours of soaking obtained by Pertiwi et al (2018) produced a yield of 6.14% which was higher than the dumbo catfish bone gelatin from 41 hours of soaking in 5.8% citric acid solution reported by Iqbal et al (2015). These results prove that gelatin yield can be influenced by various factors, namely the type of raw material, type of acid, acid concentration and soaking time.

pH value. pH of all gelatin samples tested as shown in Figure 2 are categorized as acidic. The acidic nature of this gelatin is due to the production process carried out using the acid method (type A) which at the pre-treatment stage uses aren vinegar. Naiu & Yusuf (2018) reported that aren vinegar contains 5.79% acetic acid meeting the requirements of SNI 01-4371-1996, which is at least 4% (BSN 1996). The soaking time treatment that gave insignificant results was thought to be due to the incomplete washing of ossein in both treatments that still left a lot of acid. The pH value of the research gelatin and commercial gelatin is in accordance with the type A gelatin required for edible film materials according to GMIA (2019) and meets the requirements of SNI 06-3735-1995 in the pH range of 4.5-6.5 (BSN 1995). Atma & Taufik (2021) who extracted gelatin using acid from citrus fruit for 48 hours produced gelatin with a pH of 4.42 and research conducted by Hidayat et al (2016) who extracted gelatin using the strong acid of 6% phosphoric acid produced a slightly lower pH of 4.23.

Viscosity. Viscosity of gelatin as shown in Figure 3 describes that the longer the soaking period, the lower the viscosity, indicating a thinner gelatin solution. This is presumably because the G2 treatment gelatin has a higher moisture content causing it to be thinner. The organic acids in aren vinegar help provide acid ions (H^+) that play a role in breaking the peptide bonds in collagen. The breaking of peptide bonds will release water molecules, so the longer the soaking time, the more water molecules will be formed. Ward & Courts (1977) also explained that moisture content can affect viscosity. The low moisture content of gelatin causes the ability to bind water to form a gel to be higher and the amount of water bound by gelatin makes the solution (gel) thicker. In addition, the high fat and ash content of G1 gelatin resulted in a higher viscosity of G1. When compared to commercial gelatin with a water content that is not significantly different from G1, but a significantly lower viscosity, it possibly due to the very low ash and fat content in this commercial gelatin. As stated in GMIA (2019) that one of the factors that influence the gelatin solutions is the ash content and molecular weight in the solution, the more molecules dissolved, the more molecular weight increases, causing higher viscosity.

The average viscosity value of gelatin in current study is higher than that obtained by Pertiwi et al (2018) who conducted pre-treatment using citric acid to produce a viscosity of 3.83 cPs, Hidayat et al (2016) soaking tilapia bones in phosphoric acid obtained a gelatin viscosity of 4.13 cPs and soaking in papain enzyme produced a viscosity of 7.57 cPs, and Naiu & Yusuf (2018) who immersed tuna bones in aren vinegar for 14 days produced gelatin viscosities of 2.83 to 4.3 cPs. The high viscosity of gelatin solutions is due to the type of acid and bone that was used, it is also because of differences in viscosity test methods. The viscosity test in this study is the same as that carried out by Istiqlaal (2018) which also used a temperature of 40°C, lower than the other studies above which used a temperature of 60°C, obtained a higher gelatin viscosity, which amounted to 20.17 cPs. Wasswa et al (2007) mentioned that the value of viscosity depends on the temperature, with a significant decrease in viscosity above 40°C. GMIA (2019) stated that the properties of gelatin solution are influenced by various factors, namely ash content, pH, temperature, manufacturing method, and gelatin concentration in solution.

Color degrees. The color of the research gelatin and commercial gelatin in Table 1 is considered less bright because it is quite far from the number 100 which indicates the lightest/brightest color. The longer soaking time significantly increases the L value (lightness), but decreased the a* and b* values and each of these treatments is also significantly different from commercial gelatin.

Based on the a* and b* values, the G1 color is more inclined towards reddish and yellowish compared to G2 and CG. This may be due to the high fat content in G1 gelatin causing it to oxidized easily during the drying process. The results of fat oxidation make the color become more brownish yellow. This is in line with Hematyar et al (2018) who stated that brown pigments in fish may be produced by lipid-protein interactions. In this case, lipid peroxide can interact with active types of proteins, resulting in the conversion of the pale or colourless precursor into brown pigments. Gelatin G1 and G2 from the research results are also in line with Firdausiah et al (2021) who reported that gelatin made from tuna bones is brownish in color. The brightness (L) value of fish bone gelatin from this study and commercial fish bone gelatin tested is much lower than the color of the Alaska Pollock fish bone gelatin reported by Mi et al (2019) which is close to the L value of 100, i.e 98.99, but almost close to the color of tuna bone gelatin reported by Masrukan et al (2016) with an L value of 55. This difference in gelatin color can be caused by differences in raw materials, as listed in GMIA (2019) that the color of gelatin depends on the raw material and the manufacturing process. It is further stated that the color of the gelatin does not affect the properties of the gelatin and does not reduce its usefulness.

Protein content. Gelatin is the result of the conversion of collagen protein that is hydrolyzed in a hot atmosphere so that the presence of protein in gelatin is absolute and becomes the most dominating component. The protein content of gelatin pre-treated with aren vinegar for 25 days (G1), 35 days (G2) and commercial gelatin (CG) is presented in Table 2.

Based on the results of the T-Test, treatments G1 and G2 are significantly different ($p < 0.05$) on protein content, as well as each of these treatments with commercial gelatin. The protein content is higher in G2 because the longer the soaking time, the more H^+ ions from the acid that break the hydrogen bonds and peptide bonds that make up collagen. Bodanszky (1993) explained that 0.25 M weak acetic acid is able to hydrolyze peptide bonds. When soaking with acid, the tropocollagen molecule will split into three strands because of the breaking of hydrogen bonds between the helices by H ions$^+$. The increasing volume of vinegar during soaking, the number of available H^+ ions is also greater, causing more collagen to break down, and when heated, it changes its structure into gelatin. However, the low protein content of tuna bone gelatin from soaking in aren vinegar compared to commercial gelatin is thought to be due to the type of acid used, perhaps also due to the influence of the raw materials and the age of the raw materials.

The G2 protein level is slightly lower than that reported by Firdausiah et al (2021), who soaked tuna bones in 6% hydrochloric acid for two days, resulting in a gelatin protein content of 82.85%, but higher than catfish bone gelatin studied by Atma & Taufik (2021), which was 58.47%, Hidayat et al (2016) who hydrolysed tilapia bones using 6% phosphoric acid, obtained a gelatin protein content of 63.25%, and Arshad et al (2021) who extracted a protein gelatin from *S. fimbriata* bones using 1 M HCl, was 59.1%.

Fat content. The gelatin fat content shown in Table 2 is significantly higher in treatment G1, presumably because the soaking time was not long enough so that there were still many remnants of fat from the degreasing stage that had not been lifted to the surface of the soaking solution. The longer the bone soaking time, the more fat that rises to the surface of the solution, making it easier to remove. The fat content of G1 and G2 samples is relatively high and does not meet the requirements of GMIA (2019). This may be due to the fact that the fish raw materials used in this study were adult fish, as indicated by the size of the large bones. The raw bone material used is thought to come from tuna fish measuring 60 to 80 kg. Apart from that, the fat content in fish bones is thought to influence the quality of gelatin. Murthy et al (2014) reported that *Albacore* tuna bones powder contains relatively high fat in the range between 13.39 and 17.26%. When compared to commercial fish bone gelatin (CG) with a fat content close to 0%, this is likely due to different types of raw material, pre-treatment process and extraction methods. Likewise, the results obtained by Firdausiah et al (2021) who degreased the bones in boiling water, produced gelatin with a very low fat content of 0.02%. The fat content of the G2 treatment research can be reduced to 3.23% when compared to that reported by Naiu et al (2015) who also soaked fish bones in aren vinegar, but with a shorter time, namely 14 days, resulting in high fat in the range of 9.23-13.33%.

Moisture content. The T-Test shows that the moisture content of G2 gelatin is significantly higher than G1 and CG ($p < 0.05$). This is probably because the longer the soaking, the more collagen peptide bonds are broken, releasing water molecules, and the more fat that comes out of the bone tissue. In the process of heating ossein, the gelatin converted from collagen reabsorbs the water in the solution replacing the fat that comes out. The water adsorbed by gelatin during heating is free water that is not strongly bound and which evaporates during the drying process in the oven method of moisture content test. According to Duxbury (2005) water expressed as moisture content, is free water contained in intercellular spaces and pores contained in the materials as well as water that is weakly bound because it is absorbed on the surface of macromolecular colloids such as proteins.

The moisture content of G1, G2 and CG gelatins is recorded to be higher than that reported by Rosmawati et al (2021) who examined gelatin from catfish bones with a moisture content of 3.5% and almost the same as that of mackerel gelatin hydrolysed with organic acids studied by Khiari et al (2011), namely between 7 and 9%. The moisture content of the gelatin tested in this study meets the requirements of BSN (1995) with a maximum content of 16% and specifically G2 gelatin meets the requirements for gelatin for photography needs, which is between 10.5 and 12.5% (GMIA 2019).

Ash content. The T-Test results in Table 2 show a significant difference ($p < 0.05$) in ash content. The longer the soaking, the longer the bones are exposed to aren vinegar, because every two days the acid solution is discarded and replaced with a new acid solution. Fermented aren vinegar contains various kinds of organic acids in the carboxylic acid group. During soaking, organic acids react with mineral components contained in tuna bones by substitution reaction. The carboxyl group that acts as anion (COO^-) bind to minerals in the bones, such as calcium (Ca^{2+}). The longer of soaking time (treatment G2), the more COO anions$^-$ bind to minerals, so that the ash content is recorded to be lower. According to Ismangil & Hanudin (2005), the nature of organic acids is determined by carboxyl group (COO^-) which will form complex bonds with metals and minerals such

as Fe, Al, Ca and Mg and the reactivity of organic acids with minerals is influenced by the concentration of organic acids.

The ash content of gelatin obtained in this study is lower than that reported by Janpet et al (2022) on gelatin from bigeye snapper fish bones which was 5.89%. Wangtueai & Noomhorm (2009) stated that the ash content in the gelatin should not exceed 2%. According to Islam et al (2021), low-quality gelatin from sturgeon head had an ash content of 17-19%. The gelatin ash content of the three research samples met the requirements of SNI-06-3735-1995 (BSN 1995) with a maximum value of 3.25%.

Amino acid composition. Table 3 shows that the dominant amino acid in all three gelatin samples is glutamic acid followed by aspartic acid and leucine. Glycine and proline, which characterize gelatin protein, are 7th and 5th in G1, 8th and 5th in G2, and 8th and 4th in CG, respectively. This indicates that the gelatin samples tested in the study are not dominated by these two amino acids. However, glycine and proline are measured higher in CG and G2 compared to G1. According to Derkach et al (2020), fish skin and bone gelatin is characterized by a lack of proline and hydroxyproline which are responsible for the formation of collagen-like triple helices. Table 3 also shows that all three gelatin samples contain cysteine, a type of sulfur-containing amino acid that is not commonly found in gelatin. The presence of cysteine illustrates that the gelatins tested may still contain stromal proteins that are poorly soluble in water and salt. Bougatef et al (2012) stated that cysteine is an amino acid in stromal proteins, such as elastin that is highly insoluble and stable in saline solutions.

The amino acid composition of glycine, proline, and alanine of G1, G2, and CG were detected to be very low compared to that reported by Silva et al (2014) on gelatin samples from cobia (sea snakehead) fish skin, which amounted to 307 residues/1000, 111 residues/1000 and 106 residues/1000, respectively. Likewise, the amino acids glycine, proline and alanine in tuna bone gelatin studied by Mi et al (2019) were higher than the gelatin samples from this study. Silva et al (2014) mentioned that the high amino acid content in gelatin can contribute to higher rheological properties by encouraging triple helix formation and stabilization of the gelatin molecule.

Conclusions. There are differences in the quality characteristics of tuna bone gelatin soaked in aren vinegar for 25 days and 35 days, and both are also different from commercial fish bone gelatine. The soaking process has no effect on the pH value of the gelatin, but the longer soaking increases the protein content, moisture content and average amino acid composition, although it decreases the gelatin yield. The fat content and viscosity have been corrected to be closer to SNI and GMIA standards. The viscosity of gelatin from the 35-day soaking treatment can be applied for photography needs. Commercial gelatin meets SNI and GMIA standards, except for moisture content.

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

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Authors:
Asri Silvana Naiu, Faculty of Fisheries and Marine Sciences, Universitas Negeri Gorontalo, Gorontalo 96128, Indonesia, e-mail: asri.silvana@ung.ac.id
Nikmawatisusanti Yusuf, Faculty of Fisheries and Marine Sciences, Universitas Negeri Gorontalo, Gorontalo 96128, Indonesia, e-mail: nikmawatisusanti.yusuf@ung.ac.id
Sri Rahayu Kalaka, Faculty of Fisheries and Marine Sciences, Universitas Negeri Gorontalo, Gorontalo 96128, Indonesia, e-mail: rahayukalaka24@gmail.com

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