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ARTICLE

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Collagen and Gelatin Extraction from Manyung Bone Waste as Alternative Halal Raw Materials for Food Production

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Abstract: *Collagen and gelatin are widely used in the food and pharmaceutical industries, yet their halal status remains a persistent concern for many products on the market. This study aimed to optimize the extraction and isolation of halal collagen and gelatin from Manyung fish bone waste, an abundant and low-cost Indonesian resource. Collagen was extracted at varying acid concentrations and reaction times, while gelatin was produced under eight treatment conditions varying in acid type, processing temperature, and extraction duration. Collagen and gelatin were characterized by Fourier Transform Infrared Spectroscopy (FTIR). In addition, the resulting gelatin was qualitatively examined using potassium dichromate and trinitrophenol solutions, which are commonly applied in Indonesia's pharmaceutical industry. The FTIR spectrum of collagen showed absorption peaks corresponding to amide A, amide I, and amide II, whereas amide B and amide III bands were not detected. The highest collagen yield (4.4%) was obtained using 0.75 M acetic acid with a 5-day reaction time. For gelatin, FTIR confirmed the presence of characteristic amide A, amide B, amide I, amide II, and amide III bands. The optimal gelatin extraction was achieved with treatment 8, which involved immersion in 4% HCl, followed by 4% H_3PO_4, and extraction at 80°C for 6 hours, yielding 3.74%. Overall, these findings demonstrate that Manyung fish bone waste is a promising alternative source of halal collagen and gelatin, with potential applications in both food and pharmaceutical manufacturing.*

Keywords: *collagen, food waste prevention, gelatin, halal, manyung waste bone*

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1. Introduction

Collagen and gelatin have become prominent commodities worldwide. Data shows that demand for collagen and gelatin in many industries remains high, generating a turnover of USD 4.7 billion in 2020 and growing yearly (Ahmad et al., 2024; Cao et al., 2022). Collagen sources are dominated by livestock, such as pork, bovine, and poultry (León-López et al., 2019; Shen et al., 2019). Thus, issues arise as the need for halal products continues to increase, and Muslim customers prefer products with halal labels (Bashir, 2019; Nofianti & Rofiqoh, 2019; Vizano et al., 2021). Apart from slaughtering concerns, the use of cows as a source for collagen and gelatin raises health issues due to potential biological contamination in cattle, including bovine spongiform encephalopathy or mad cow disease, transmissible spongiform encephalopathy, and foot and mouth disease (Wenz et al., 2001). Furthermore, the high amino acid content in land animals causes a faster denaturation process, so the collagen quality is relatively low (Xiaoxia Zhang et al., 2020).

On the other hand, the fisheries processing industry is plagued by the problem that 50% of its initial weight is a byproduct (Nam et al., 2020; Nurilmala et al., 2022) of 80–232 ton annual production (Garlock et al., 2020; Naylor et al., 2021). Despite its enormous benefits, fish consumption remains limited to the flesh, which is considered the only edible part. Fish byproducts are usable through processing to become raw materials for medicine, biomedicine, cosmetics and food ingredients, including collagen and gelatin (Jafari et al., 2020; Shavandi et al., 2019). Using collagen originating from aquatic animals such as fish has a high potential as a halal alternative raw material. Additionally, using fishery waste to produce collagen and gelatin can increase its economic value while addressing the environmental pollution problems it causes. Manyung bone waste can be used as a source of high economic value as an alternative halal source for collagen and gelatin. Manyung is a marine fish obtained from the coast of Indonesia, with an estimated production of 40 tons annually (Mustaruddin et al., 2024).

Fish and fish-derived products, including gelatin and collagen, are widely recognized in halal food science literature as permissible (halal) ingredients and are acceptable alternatives to mammalian-sourced gelatins (Arnamalia et al., 2022; Zakaria & Bakar, 2015). Fish gelatin is valued for its halal compatibility, immunological safety, and environmental sustainability, making it a practical substitute for porcine gelatin in food and pharmaceutical applications (Zakaria & Bakar, 2015). While jurisprudential opinions vary among Islamic schools of thought—notably between the Hanafi school and other Sunni schools regarding the permissibility of certain marine species—common edible fish species such as catfish (including manyung/*Arius thalassinus*) are generally accepted as halal raw materials for gelatin and collagen production (Duasa et al., 2022; Reza & Annissa, 2023).

Collagen can be extracted from the skin, scales, and bones (Kittiphattanabawon et al., 2005; Matinong et al., 2022). Over the past few decades, optimization of collagen extraction methods has continued to develop, through solvent or enzymatic extraction, by conventional reaction methods or ultrasound-assisted (Carpio et al., 2023; Deng et al., 2022; Gaikwad & Kim, 2024; Li et al., 2009; Lu et al., 2023; Vate et al., 2022). Hydrolysis reaction conditions are significant factors in the extraction and isolation of collagen and gelatin. Different collagen sources require unique reaction conditions. Reaction with enzymes can produce more stereoselective collagen but is less profitable. In addition, use of an enzyme will raise a halal issue related to the source, as most enzymes used for extraction are derived from pigs (Benjakul et al., 2010; Chotphruethipong et al., 2019; Nalinanon et al., 2007). Likewise, sophisticated reactor-assisted reaction methods, such as ultrasound or microwaves, are challeging to apply in small industries. Therefore, exploring sources and conditions for collagen extraction and isolation with simple, practical, and cost-effective methods is necessary.

Regarding the processing acids employed, acetic acid, hydrochloric acid, and phosphoric acid are routinely used as technical processing aids in halal gelatin extraction protocols (Al-Nimry et al., 2021; Duasa et al., 2022; Phon et al., 2023; Reza & Annissa, 2023). These inorganic acids function as pretreatment and hydrolysis agents to optimize yield and product quality, and their use in fish-derived gelatin production is compatible with halal compliance, provided proper controls are maintained to prevent cross-contamination with non-halal sources, ensure traceability of reagents, and verify the halal status of raw materials through documented management systems (Mohamad et al., 2015; Wikasitakusuma & SW, 2024). The halal integrity of the final product is primarily determined by the animal source (fish versus porcine or non-halal bovine) rather than the inorganic processing aids employed, emphasizing the importance of raw material authentication and process control in halal gelatin manufacturing (Mohamad et al., 2015; Wikasitakusuma & SW, 2024).

While existing research has explored collagen and gelatin extraction from various fish species using acid-based methods, significant knowledge gaps remain that limit the translation of these findings into standardized, halal-certified food ingredients. Previous studies have predominantly focused on single-acid extractions or compared acids across different species, extraction protocols, and characterization endpoints, making direct comparisons of acid effectiveness (da Trindade Alfaro et al., 2009; da Trindade Alfaro et al., 2014; Fawzya et al., 2024; Koli et al., 2013; Phon et al., 2023; Rodiah et al., 2018; Sanaei et al., 2013). Notably, while hydrochloric acid and acetic acid have been extensively studied in fish bone gelatin extraction from species such as tuna (*Thunnus* sp.) and mackerel (*Scomberomorus commerson*) (Rodiah et al., 2018; Xue et al., 2025), phosphoric acid remains underexplored as a primary extraction agent despite its documented role in demineralization processes. Furthermore, the literature reveals no systematic investigation of collagen and gelatin extraction from manyung bone waste, representing an unexplored raw material source with potential for halal food applications. The present study addresses these critical gaps by conducting a head-to-head comparison of hydrochloric acid, phosphoric acid, and acetic acid under standardized extraction conditions using manyung waste as feedstock. This controlled comparative approach, coupled with comprehensive physicochemical characterization and explicit integration of halal certification pathways, provides novel insights into optimal acid selection for producing food-grade halal gelatin from an underused fishery byproduct. By linking extraction chemistry directly to halal compliance documentation and food application potential, this research fills a documented void where fish gelatin is recognized as a halal alternative but systematic certification workflows and species-specific processing protocols remain poorly defined. The findings will not only expand scientific understanding of acid-mediated extraction from catfish-family bones but also deliver practical guidance for halal food manufacturers seeking certified, sustainable gelatin sources.

This study aims to obtain a simple, cost-effective method for isolating collagen and gelatin from manyung waste sources. Several research studies show that acidic conditions are preferable for collagen extraction from fish (Oslan et al., 2022; Yu et al., 2018). Acetic acid is commonly used due to its suitable acid strength and compatibility in the body. Acid concentration and extraction duration are two variables that prominently determine the accomplishment of collagen extraction (Devi et al., 2017). This study aims to optimize the acetic acid concentration between 0.5 to 1 M with reaction time ranging from 3 to 5 days.

2. Materials and Methods

Manyung bone waste (*Arius thalassinus*) was obtained from Lamongan, East Java, Indonesia. Identification of manyung was conducted at the Faculty of Science and Technology, Universitas Airlangga. Standard collagen and gelatin, and chemicals were purchased from Sigma Aldrich®.

2.1. Collagen Extraction from Manyung Bones

Samples for the extraction process were prepared by cutting the manyung bone waste into segments, then soaking them in 0.1 N NaOH solution (ratio of 1:7 w/v). The solution was replaced each day until no meat was attached. The bone was subsequently washed with water until the pH value was around 7, then reduced to remove the fish oil. The bone was dried by aeration and ground homogeneously.

Extracting and isolating collagen from the manyung bone samples was carried out at room temperature, except for the centrifuging step at 40°C. Around 100 g of powdered manyung bone was weighed and placed into a 500 mL Erlenmeyer flask. Three samples were extracted using 0.5, 0.75, and 1 M acetic acid, respectively, with three replications for each sample and extraction for 3 days and 5 days on a rotary shaker. The results were filtered to separate the residue from the extract (supernatant). The supernatant was precipitated by NaCl and left for 24 hours to obtain collagen precipitate (salting-out process). The precipitate was centrifuged at 9000 rpm for 30 minutes, then freeze-dried for 24 hours to obtain dry collagen (Ogawa et al., 2004).

2.2. Characterization of Collagen Properties

Functional groups of collagen were analyzed using the PerkinElmer Spectrum One Fourier Transform Infrared Spectrometer (FTIR; USA) at a range of 450–4000 cm^{-1}. Moisture and ash contents were determined according to the procedure described in the Farmakope Indonesia VI edition (Ministry of Health of the Republic of Indonesia, 2020). Collagen rendement was obtained from the weight ratio of dried collagen to the initial raw material.

2.3. Isolation of Gelatin from Manyung Bone Waste

Sample preparation was carried out by first washing the fish bone and immersing it in 80°C water for 1–2 minutes for easy removal of meat remnants, scales, and the outer fat layer. The degreasing step was repeated until all remaining fat and meat remnants were removed by soaking it in 80°C water for 10 minutes. Then, the manyung bone was dried in the sun and cut into 2×2 cm pieces to expand the surface area.

Extraction of gelatin from the manyung bone waste used an acid method with a completely randomized design, using 8 treatment groups with variations in the type of acid, reaction temperature, and reaction time, as shown in Figure 1.

Around 100 g of fish samples were subjected into the demineralization process by soaking the samples in the acid solution according to the experiment design depicted in Figure 1. The ratio of bone sample weight to acid solution volume was 1:5 (w/v). This demineralization process was performed to remove the remaining calcium salts and others. Ossein (soft bone) was obtained from this process, which was then washed with distilled water to a neutral pH value. In a glass beaker, ossein was added with an aquadest on a 1:3 (w/v) ratio, then placed in a waterbath set at temperatures of 70 and 80°C for 5 and 6 hours. The filtrate obtained from the extraction process was filtered into a beaker for drying in an oven at 55°C for ±24 hours. The dried gelatin was milled to obtain gelatin powder.

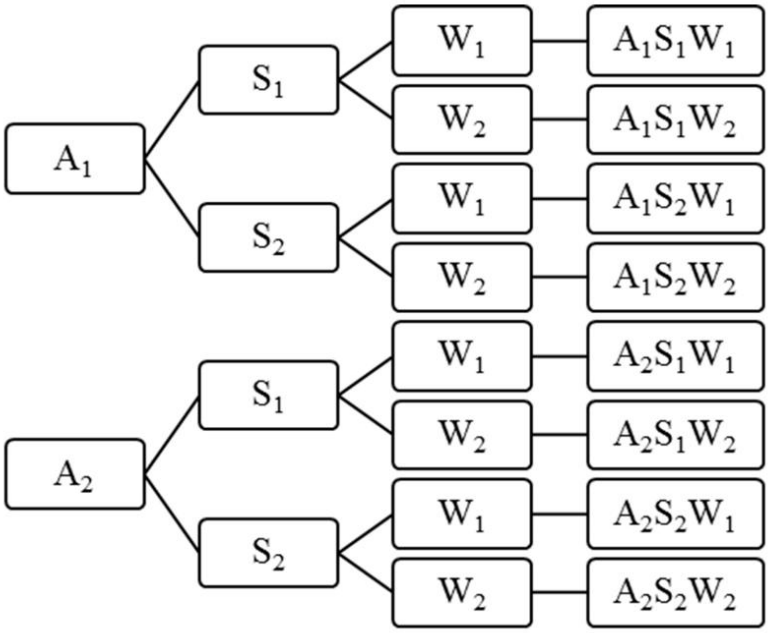

Figure 1. Design of extraction conditions with acid type, temperature, and extraction time variations. A1 = soaking with 4% HCl solution for 48 h; A2 = soaking with 4% HCl solution for 24 h followed by 4% H_3PO_4 solution for 24 h; S1 = extraction temp. of 70°C; S2 = extraction temp. of 80°C; W1 = extraction time of 5 h; and W2 = extraction time of 6 h.

2.4. Characterization of Gelatin Properties

The infrared (IR) spectra of the gelatin produced were analyzed using PerkinElmer Spectrum One Fourier Transform Infrared Spectrometer (FTIR; USA) then compared to the standard compound. Moisture and ash contents were determined according to the procedure described in the Farmakope Indonesia VI edition (Ministry of Health of the Republic of Indonesia, 2020). Protein levels were measured using a Nanodrop™ Spectrophotometer (USA). The gelatin's viscosity was measured based on a standard procedure (Gelatin Manufacturers Institute of America, 2019) that the gelatin solution with a concentration of 6.67% (w/v) was prepared in distilled water before measurement using a viscometer at a temperature of 60ºC. The value of the viscosity was expressed as centripoise (cP).

The gelatin pH was measured based on 6.67% (w/v) gelatin solution in distilled water at room temperature, based on the previous method proposed (Haug & Draget, 2009). For the gel strength or bloom value, measurement was carried out on 6.67% (w/v) gelatin solution prepared at 60°C and cooled to 10°C for 17 hours prior to measurement (Gelatin Manufacturers Institute of America, 2019). The rendement of gelatin was calculated from the weight ratio of the dried gelatin produced compared to the starting material in percentage.

2.5. Data Analysis

Analysis of Variance (ANOVA) was performed using SPSS version 22 (IBM™, USA) to identify statistically the effect of treatment conditions on collagen and gelatin rendement as well as the characteristics by the Indonesian National Standard (SNI) requirement as raw material. The level of confidence was set at 95%.

3. Results and Discussion

3.1. IR Profiles of Extracted Collagen

The IR spectra of standard collagen (Calf Skin Sigma Aldrich®) and collagen extracted from the manyung bone waste are presented in Figure 2. Figure 2 shows that collagen from manyung bone has a typical peak at 3436–3466 cm^{-1}, which is the –OH group. The characteristics of the collagen functional group from FTIR analysis are presented in Table 1. The wavenumber of amide A is around 3500–3300 cm^{-1}; when hydrogen bonds influence NH in peptides, the position shifts to the lower frequencies (Muyonga et al., 2004).

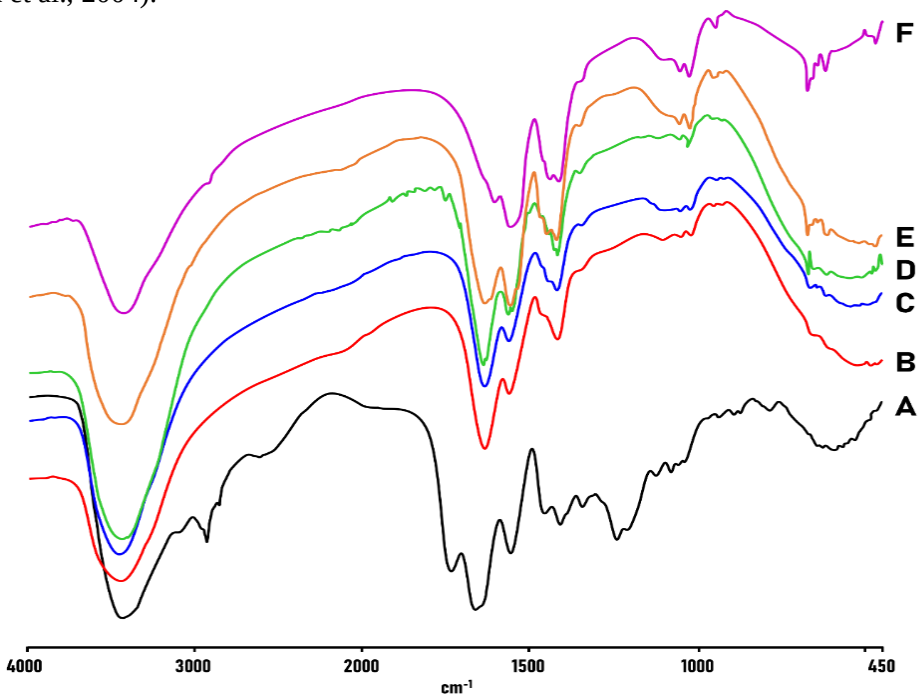

Figure 2. Identification of collagen extracted under various conditions using IR spectral profiles: (A) standard collagen and extracted collagens, (B) 0.5 M acetic acid for 3 days, (C) 0.75 M acetic acid for 3 days, (D) 0.75 M acetic acid for 5 days, (E) 1.0 M acetic acid for 3 days, and (F) 1.0 M acetic acid for 5 days.

The wavenumbers indicating amide B absorption are formed from the asymmetrical stretch of CH_2 (Kong & Yu, 2007). The absence of peaks at 2935–2915 or 2865–2845 cm^{-1} identifies no apparent amide B in collagen from manyung bones. The amide B peak in FTIR spectral profiles of collagen is sometimes observed as a distinct absorption band in the high-frequency stretching region, typically appearing between approximately 2900–3500 cm^{-1} (Lino-Sánchez et al., 2023; Upasen et al., 2019). This peak arises primarily from backbone N–H stretching vibrations of the peptide bonds, with additional contributions from C–H stretching vibrations of amino acid side chains (Upasen et al., 2019). The appearance and intensity of the amide B band are strongly influenced by the hydration state of the collagen sample, as bound water molecules contribute OH stretching absorptions in the same spectral window, creating a complex, overlapping absorption envelope (Upasen et al., 2019). The peak's visibility and characteristics depend on several factors, including sample preparation (lyophilized versus solution state), environmental conditions (humidity and temperature), and the structural integrity of the collagen triple helix (Lino-Sánchez et al., 2023). During thermal denaturation or when the triple helix unfolds, the amide B peak shifts to lower frequencies and changes shape, paralleling changes in the amide A band, making it a useful reporter for monitoring conformational changes, hydration dynamics, and structural stability in collagen-based materials (Lino-Sánchez et al., 2023; Liu et al., 2023). The variability in amide B peak prominence across different FTIR studies reflects these multiple contributing factors and the sensitivity of this spectral region to the experimental conditions and collagen structural state.

Although the wavenumber and precise vibrational assignment of this amide B band were not explicitly reported in this study, its presence is an important quality control marker indicating intact backbone functional groups and successful retention of collagen's native conformation (Hasanuddin et al., 2024; Xuening Zhang et al., 2023). The significance of the unidentified amide B band lies not in its individual mechanistic contribution, but in its role as part of the complete FTIR fingerprint that enables species-specific identification, extraction quality assessment, and prediction of functional performance in applications where moderate thermal stability and preserved triple-helix functionality are acceptable, such as in nutraceutical, cosmetic, and biomedical uses (Fawzya et al., 2024; Martins et al., 2022; Xuening Zhang et al., 2023). To elucidate the mechanistic implications of the manyung amide B band—such as linking wavenumber shifts to hydrogen-bonding states or amino acid composition—additional spectroscopic characterization with precise wavenumber reporting, complementary circular dichroism (CD) or X-ray diffraction (XRD) analysis, and detailed amino acid profiling would be required (Martins et al., 2022).

Amide I should be detected in the range of 1690–1600 cm^{-1} (Kong & Yu, 2007), while the wavenumber detected in the manyung bones is 1611–1637 cm^{-1} collagen. The wavenumber in collagen from skin is higher, which is 1640 cm^{-1}. In the FTIR spectra of collagen, the amide I band (≈1600–1700 cm^{-1}) arises mainly from the stretching vibration of the peptide carbonyl (C=O) group and is commonly used as a characteristic band to confirm collagen/protein (de Campos Vidal & Mello, 2011). The wavenumber of Amide I of gelatin from squid outer skin is from 1635 to 1632 cm^{-1} (Nagarajan et al., 2012). A wavenumber at 1633 cm^{-1} is a random coil characteristic of gelatin. This means the molecular compounds produced from the extraction process are collagen.

Table 1. Characteristics of Functional Groups of Collagen from Manyung Fish Bone

Concentration and Extraction Time	Amide A	Amide B	Amide I	Amide II	Amide III
Standard collagen	3500–3300	2935–2915	1690–1600	1575–1480	1301–1229
0.5 M (3 days)	3465	-	1634	1562	-
0.5 M (5 days)	3466	-	1634	1563	-
0.75 M (3 days)	3465	-	1631	1564	-
0.75 M (5 days)	3465	-	1637	1563	-
1 M (3 days)	3466	-	1630	1557	-
1 M (5 days)	3436	-	1611	1563	-

The presence of Amide II is shown by absorption at 1557–1563 cm^{-1}. Amida II shows a helical structure (Stani et al., 2020) and usually possesses an absorption peak of 1575–1480 cm^{-1}. This area of amide absorption II is related to CN stretching and NH bending (Kong & Yu, 2007).

3.2. Rendement, Moisture, and Ash Content of Collagen

Characterization results of the collagen extracted from the manyung bone are presented in Table 2. Rendement is a parameter that shows the effectiveness of the conversion of a raw material into a product. Collagen rendements from the manyung bone in varied extraction conditions ranged from 1.62 to 4.40%. The highest yield was obtained from the treatment group, which had 0.5 M acetic acid and an extraction time of 5 days with 4.40% rendement. Meanwhile, the treatment group of 0.5 M shows the lowest collagen yield with 3 days extraction time.

Table 2. Rendement, Moisture Content, and Ash Content of Collagen Isolated from Manyung Fish Bone

Acetic Acid Concentration and Extraction Time	Collagen Rendement (%)	Moisture Content (%)	Ash Content (%)
0.5 M (3 days)	1.62 ± 0.12	6.97 ± 0.99	39.61 ± 9.09
0.5 M (5 days)	4.40 ± 0.33	6.28 ± 1.61	37.53 ± 3.53
0.75 M (3 days)	2.33 ± 0.27	6.35 ± 1.71	35.58 ± 4.86
0.75 M (5 days)	3.59 ± 0.51	7.04 ± 1.78	40.19 ± 4.83
1 M (3 days)	2.60 ± 0.09	7.97 ± 2.42	39.95 ± 4.88
1 M (5 days)	1.70 ± 0.19	8.92 ± 2.03	35.68 ± 6.67

Collagen isolation yields from fish vary substantially depending on the tissue source, species, and extraction methodology, with reported values spanning from less than 1% to over 70% on a dry weight basis (Luc, 2018; Nalinanon et al., 2007; Shaik et al., 2023). Fish skin consistently provides the highest collagen yields, with optimized acid extraction protocols achieving 72.9% from Pangasius skin (Luc, 2018), pepsin-assisted extraction yielding 51.47% from jumbo flying squid skin (Gonapinuwala et al., 2025), and ultrasound-assisted methods reaching up to 94.88% from golden carp skin (Aberoumand,

2012). In contrast, scales typically produce lower yields ranging from 1–16% using conventional acid or enzyme methods (Al-Abadi & AI-Temimi, 2022; Hasanuddin et al., 2023). Bone tissue generally yields the least collagen, with most studies reporting 0.8–8% recovery (García-Sifuentes et al., 2021; Phon et al., 2023). The extraction method significantly influences yield outcomes: pepsin-soluble collagen extraction typically produces higher yields than acid-soluble collagen alone (Nalinanon et al., 2010; L. Wang et al., 2015), while process intensification techniques, including ultrasound, microwave irradiation, and CO_2-acidified water extraction, can substantially increase recovery efficiency (de Campos Vidal & Mello, 2011; Feng et al., 2021; Sousa et al., 2020). Additional factors affecting yield include extraction time and temperature, acid type and concentration, pretreatment procedures (particularly decalcification for scales), and species-specific biological characteristics (Blanco et al., 2019; Luc, 2018; Shaik et al., 2023). The wide variability in reported yields underscores the importance of optimizing extraction parameters for fish species and tissue types to maximize collagen recovery from marine by-products.

Moisture content affects long-term stability because it is closely related to metabolism that occurs during collagen storage, such as enzymatic, microbial, and chemical activities (rancidity and non-enzymatic reactions) that can cause changes in organoleptic properties and quality values (Xiaoxia Zhang et al., 2020). If collagen possesses a lower moisture content, its shelf life will be longer. The results show that collagen with the best value for moisture content is obtained from the treatment group with 0.5 M acetic acid and 5 days extraction time, resulting in 6.278%. The treatment group with 1 M acetic acid and 5 days extraction had the highest moisture content of 8.92%. The national standard (SNI) requirement for the moisture content of dried collagen is no more than 12%. Extracted collagens from all treatment groups conform to SNI requirements. Table 3 compares the moisture content of collagen extracted from different fish types.

Table 3. Moisture Content of Collagen Extracted from Manyung and Other Fishes

Extraction Condition: Acetic Acid Conc. (Extraction Time)	Moisture Content of Fish Bone Collagen (%)			
	Manyung	Tilapia	Milkfish	Mackerel
0.5 M (3 days)	6.97 ± 0.99	7.46	8.48	5.29
0.5 M (5 days)	6.28 ± 1.61			
0.75 M (3 days)	6.35 ± 1.71			
0.75 M (5 days)	7.04 ± 1.78			
1 M (3 days)	7.97 ± 2.42			
1 M (5 days)	8.92 ± 2.03			

Ash content is a mixture of inorganic components or minerals in a compound. Food consists of 96% organic matter and water, while the rest are mineral elements. Organic materials in the combustion process will burn, but not inorganic components, which is called ash content. Fishery products have different ash content (Ahmed et al., 2022). Table 4 shows that the collagen samples from manyung bone have lower ash content on average in all treatment groups, compared to the collagen isolated from milkfish, mackerel, and tilapia bones at values of 50.75%, 53.41%, and 54.63%, respectively (Darmanto et al., 2014). It can be concluded that the mineral content in collagen from manyung bone is lower than that of the other fishes mentioned. Ash content indicates the presence of minerals in a raw material. Dried collagen contains several minerals, such as calcium and magnesium. The mineral also can be dissolved with collagen during extraction (Dang et al., 2019; Genin et al., 2009; Stock, 2015; M. Wang et al., 2025), so the mineral level will also increase in the rendement. The mineral content can be in organic and inorganic salts form, including salt from malic acid, axillary, acetate, and pectate for organic salts, while inorganic salts are in the form of phosphate, carbonates, sulfates, nitrate (Al Hajj et al., 2024; Gulevsky, 2020). Exposure factors at high temperatures cause the mineral content in food to decrease (Al Hajj et al., 2024; León-López et al., 2019).

Table 4. Ash Content of Collagen Extracted from Manyung and Other Fishes (Darmanto et al., 2014)

Extraction Condition: Acetic Acid Conc. (Extraction Time)	Ash Content of Fish Bone Collagen (%)			
	Manyung	Tilapia	Milkfish	Mackerel
0.5 M (3 days)	39.61 ± 9.09	54.63	50.75	53.41
0.5 M (5 days)	37.53 ± 3.53			
0.75 M (3 days)	35.58 ± 4.86			
0.75 M (5 days)	40.19 ± 4.83			
1 M (3 days)	39.95 ± 4.88			
1 M (5 days)	35.68 ± 6.67			

3.3. Extraction of Gelatin

The extraction process of gelatin from manyung bone waste (*Arius thalassinus*) in this study used acid as a marinade. Acids can convert triple-helix collagen fibers into a single chain so the breakdown process is faster (Tazwir et al., 2014). This encourages the study of extracting gelatin from bone waste using an acid method. 4% HCl is used as the solution for soaking purposes because it is known to produce gelatin with good rendement and quality (Suryanti et al., 2006). In the previous study, demineralization with 5% HCl solution followed by 6% H_3PO_4 produced gelatin with high yield and good results (Hidayat et al., 2016). Other factors that influence gelatin extraction from manyung in this acid condition are temperature and time of extraction. The extraction temperatures used in this study were 70 and 80°C because at 70°C a fairly large amount of good quality gelatin is produced (Hidayat et al., 2016), whereas 80°C is the optimum temperature (Suryanti et al., 2006).

The best extraction time of gelatin is around 5-6 hours (Rahayu & Fithriyah, 2015; Suryanti et al., 2006). After 24 hours, the acid solution is removed and ossein (soft bone) forms. The ossein is then washed with water until it reaches pH 5. Ossein is neutralized to pH 5 because, generally, pH 4–5 is the isoelectric point of the components of non-collagen proteins. The isoelectric point is where the amino acids making up non-collagen proteins become bipolar and have a zero charge (Hart et al., 2012). The amino acid does not move to any electrode, so ossein is extracted when the non-collagen protein component is not extracted.

The next step is conversion of collagen to gelatin. Ossein at pH 5 is put into a glass beaker and distilled water is added with a ratio of 1:3 (w/v). The extraction process is carried out in a water bath with temperature variations of 70 and 80°C and 5 and 6 hour extraction times. The subsequent step is to dry the gelatin. The filtrate obtained from the extraction process is filtered through filter paper into an Erlenmeyer flask, then the solvent is evaporated at 60°C. Then, the gelatin liquid is heated at 55°C for ± 24 hours. The dried gelatin is ground to obtain gelatin in powder form. Gelatin extracted from manyung bone waste is a brownish-yellow powder, slightly smelly and hygroscopic.

3.4. Gelatin Identification by FTIR

Characterization of gelatin from manyung with FTIR shows typical functional groups of amides A, B, I, II, and III present (Figure 3 and Figure 4). Amide A indicates NH stretching at 3478-3310 cm-^{1} (Muyonga et al., 2004), and the gelatin from manyung bone is found at a range of 3421 to 3467cm^{-1}. The standard gelatin from Sigma Aldrich® (pig skin) shows an absorption peak at 3433 cm^{-1}. The NH absorption peaks sharply and narrowly because of OH groups derived from hydroxyproline (Santoso et al., 2015).

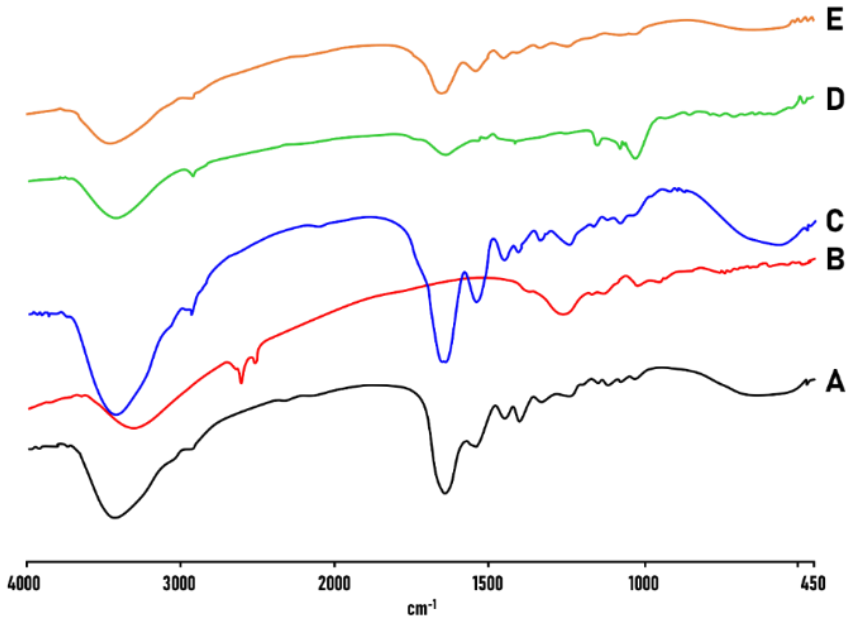

Figure 3. Identification of gelatin using IR spectral profiles (A) standard ngelatin from Sigma Aldrich® (pig skin) and extracted gelatins from treatment group: (B) 1, (C) 2, (D) 3, and (E) 4.

Amide I in gelatin from manyung bone is indicated by absorption at 1636 to 1654 cm^{-1}. This value is similar to amide I absorption detected in standard gelatin from Sigma Aldrich® (pig skin) at 1646 cm^{-1}. The components of α-helix are shown in the absorption peak around 1690–1600 cm^{-1} (Kong &

Yu, 2007), so absorption of amide I in the gelatin samples indicates the presence of α-helix components. The amide I wavenumber has a double stretch of carbonyl groups, C=O, NH bending, and CN, resulting in absorption peaks at 1690–1600 cm^{-1} (Muyonga et al., 2004). The amide II group is detected in the 1575–1480 cm^{-1} absorption area. Amide II shows CN stretching and NH bending (Kong & Yu, 2007). Amide II group in gelatin from the manyung bone is detected at 1540 to 1559 cm^{-1}, with similar values also presented by standard gelatin from Sigma Aldrich® (pig skin) at 1552 cm^{-1}. The amide III group is detected at around 1301–1229 cm^{-1}. Amida III shows CN stretching and NH bending (Kong & Yu, 2007), which is found at 1243 cm^{-1} for standard gelatin from Sigma Aldrich® (pig skin). Amida III in gelatin from manyung bone is observed at 1237 to 1262 cm^{-1}, but several gelatin samples could not be read on the amida III group. The same situation occurs in gelatin from Nile perch bone extracted at low temperature (50°C), which does not show an absorption peak for amide III. Amide III absorption is related to a triple helix structure (Muyonga et al., 2004).

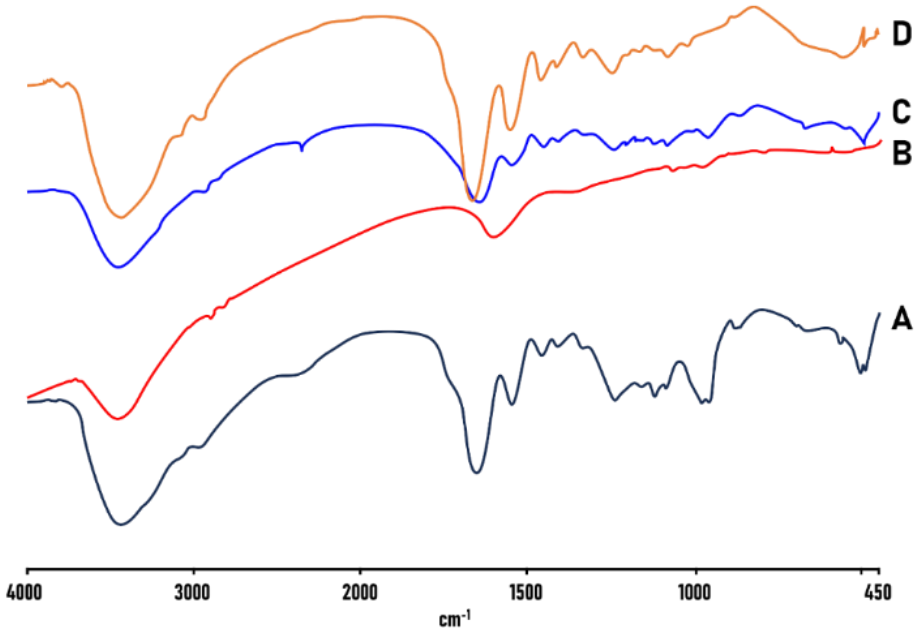

Figure 4. Identification of extracted gelatin using IR spectral profiles from each treatment group: (A) 5, (B) 6, (C) 7, and (D) 8.

3.5. Identification of Gelatin with Trinitrophenol Solution and Dichromate Solution

Identification of gelatin extracted from manyung bone waste in all treatment groups using solutions of trinitrophenol and potassium dichromate is presented in Figure 5 and Figure 6, respectively. The results are presented in Pharmacopoeia Indonesia edition VI (Ministry of Health of the Republic of Indonesia, 2020). The gelatin solution added with the two reagents forms yellow precipitation. The same result is also indicated by the standard gelatin from Sigma Aldrich® (pig skin), meaning that gelatin from fish bone waste was positively contained.

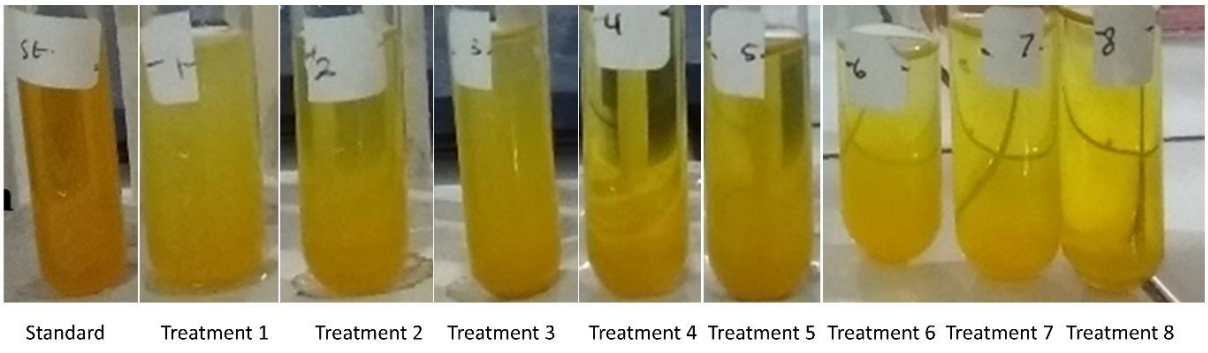

Figure 5. Identification of standard gelatin from Sigma Aldrich® and gelatin samples extracted from manyung bone with potassium dichromate solution.

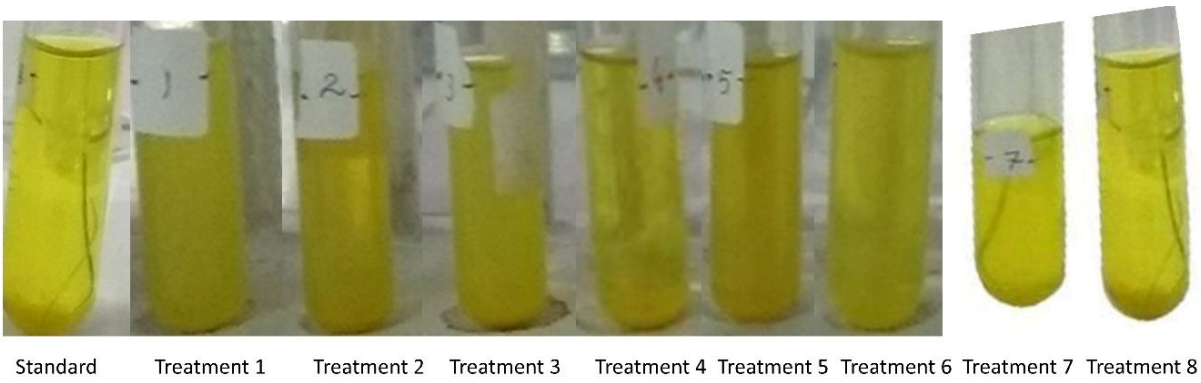

Figure 6. Identification of standard gelatin from Sigma Aldrich® and gelatin samples extracted from manyung bone using trinitrophenol solution.

3.6. Physicochemical Properties of Gelatin

From 100 g of manyung bone waste, gelatin is extracted with a rendement percentage ranging from 1.32 ± 0.50 to 3.74 ± 1.88 (Table 5). The highest yield value is obtained from treatment group 8, with immersion in 4% HCl solution followed by 4% H_3PO_4 solution, extraction tempature of 80°C, and extraction time of 6 hours. The smallest yield is given by treatment group 2, with immersion in 4% HCl solution, extraction temperature 80°C, and extraction time of 6 hours. Yield value is one of the important parameters in making gelatin. Based on statistical analysis with Kruskal Wallis, the p 0.008 < α (0.05) value indicates significant differences between the treatment groups on yield value.

Table 5. Rendement, Moisture Content, and Ash Content of Gellatin Isolated from Manyung Bone

Treatment Group	Average Rendement ± SD (%)	Average Water Content ± SD (%)	Average Ash Content ± SD (%)	Average pH ± SD (%)
1	1.47 ± 0.58	10.03 ± 1.10	2.56 ± 0.19	5.31 ± 0.09
2	1.32 ± 0.50	13.85 ± 2.03	3.24 ± 1.49	5.11 ± 0.02
3	1.41 ± 0.60	9.91 ± 5.04	3.50 ± 0.52	5.08 ± 0.05
4	2.02 ± 0.10	13.04 ± 2.14	4.79 ± 0.90	5.14 ± 0.03
5	1.65 ± 0.27	10.69 ± 2.22	3.21 ± 0.21	5.03 ± 0.05
6	2.90 ± 0.15	11.41 ± 3.82	3.26 ± 0.26	4.77 ± 0.11
7	2.55 ± 0.48	9.83 ± 5.37	4.60 ± 1.48	4.91 ± 0.17
8	3.74 ± 1.88	14.83 ± 1.32	3.32 ± 0.96	5.03 ± 0.03

The demineralization process using a combination of 4% HCl solution and 4% H_3PO_4 solution, resulting in higher rendement than using 4% HCl solution alone, can be attributed to the presence of Ca^{2+} in gelatin, so it reacts with the PO_4^{3+} in the solution, resulting in the deposition of calcium as $(Ca)_3(PO_4)_2$. Strong acids have the advantage of being able to decompose collagen fibers better and faster without affecting the quality of the gelatin produced (Fasya et al., 2018).

Extraction at 80ºC gives a higher yield with a value range of 1.41 ± 0.60% to 3.74 ± 1.88% compared to 1.32 ± 0.50% to 2.90 ± 0.15% yield value obtained from extraction at 70ºC. The extraction temperature can affect the yield produced by leading to more conversion of collagen to gelatin (Suryanti et al., 2006). Extraction time affects the amount of yield produced. The longer the extraction time, the higher the gelatin yield. In this study, extraction for 6 hours results in rendement of about 1.32 ± 0.50% to 3.74 ± 1.88%, notably higher than for 5 hours extraction, which has a value of 1.41 ± 0.60% to 2.55 ± 0.48%. This higher gelatin rendement is expected due to the dissolution of other minerals, such as calcium and salt, which are still present in the ossein, thus increasing the gelatinous ash content and decreasing the gelatin quality (Suryanti et al., 2006).

Gelatin isolation yields from fish exhibit substantial variability ranging from less than 1% to over 75% on a dry weight basis, influenced primarily by tissue source, species characteristics, and extraction methodology (da Trindade Alfaro et al., 2014; Koli et al., 2013; Sanaei et al., 2013). Fish skin consistently produces the highest gelatin yields, with ultra-high pressure-assisted extraction achieving 75.03% from unspecified fish skin (da Trindade Alfaro et al., 2014), optimized acid pretreatment yielding approximately 80.8% recovery from silver carp skin (Guan et al., 2022), and catfish bone remarkably producing 60.54% under aggressive hydrochloric acid pretreatment (3.35% HCl, 14.5 hours) followed by hot water extraction (Sanaei et al., 2013). Scales typically generate moderate yields ranging

from 4% to 47%, with ultrasound-assisted extraction significantly improving recovery efficiency—bighead carp scales yielded 30.94–46.67% with ultrasound compared to 19.2–36.4% using conventional water bath methods (Koli et al., 2013). Bone gelatin yields span a wide range from 0.35% (bigeye snapper hot water extraction) to 60.54% (catfish bone with optimized HCl pretreatment), demonstrating the importance of pretreatment intensity (Janpet et al., 2022; Sanaei et al., 2013).

The parameters to determine the characteristics of gelatin are moisture content, ash content, and pH. The moisture content of gelatin from manyung bone waste is measured at a range of 9.83 ± 5.37% to 14.83 ± 1.32%, which fulfills SNI 1995 requirements at a maximum of 16%. Based on statistical analysis with ANOVA (One Way), a p value of 0.442 > α (0.05) is obtained, indicating no significant differences between groups for treatment of water content. Ash content shows the amount of inorganic material contained in gelatin, which is measured at a range of 2.56 ± 0.19% to 4.79 ± 0.90%. According to SNI (1995), the maximum value of ash content is 3.25%. While some samples in this study exceed the maximum value required, statistical analysis with ANOVA (One Way) obtains a p-value of 0.108 > α (0.05), which indicates no significant difference between groups for treatment of ash content.

Gelatin's pH value determines the gel's strength, as the higher the pH value, the lower the strength of the gel, and vice versa (Nurilmala et al., 2022). The pH value is expected to be close to neutral so it can be widely used (Nurilmala et al., 2022). In this study, the pH value of gelatin obtained from manyung bone is between 4.77 ± 0.11 in treatment group 6 and 5.31 ± 0.09 in group 1. These values conform to the requirement standards of type A gelatin (Gelatin Manufacturers Institute of America, 2019), which ranges from 3.8 to 5.5. pH value depends on the washing process after demineralization. A good washing process leads to better removal of acid moieties trapped in the ossein, so the pH value will be close to neutral. Based on statistical analysis with the Kruskal Wallis, the p-value 0.005 < α (0.05) shows a significant difference between treatment groups towards pH value.

The results of this research demonstrate favorable extraction outcomes and robust halal compliance characteristics that position these materials as viable alternatives to mammalian-derived gelatin for Muslim consumers. Fish gelatin is fundamentally considered halal in Islamic jurisprudence and offers distinct advantages over mammalian sources: it eliminates risks of bovine spongiform encephalopathy and other zoonotic diseases, provides traceability through DNA-based and spectroscopic authentication methods (FTIR, electrophoresis, chromatography combined with multivariate analysis), and sustainably uses abundant aquaculture by-products (Rahmayanti et al., 2025; Reza & Annissa, 2023; Riyanto et al., 2023). Identification conducted in this research is scientifically validated, economically viable, and religiously compliant alternatives that meet functional performance requirements and halal certification standards for food, pharmaceutical, and biomedical applications.

The isolation of halal collagen and gelatin through acid-mediated extraction represents a critical technical and regulatory junction where processing methodology must align with Islamic dietary law (Shariah) principles and certification requirements. In the conventional gelatin industry, acid pretreatment—classified as type A processing—employs mineral and organic acids to solubilize collagen from raw materials, with hydrochloric acid, acetic acid, and phosphoric acid as the primary extraction agents (Rakhmanova et al., 2018). Within the halal certification framework, the permissibility of these acids hinges not on their chemical identity per se, but on three fundamental criteria: the halal status of the source material, the absence of cross-contamination with haram (forbidden) substances, and comprehensive traceability throughout the supply chain (Uddin et al., 2021). Hydrochloric acid used in this study effectively demineralizes bone matrices and hydrolyzes collagen while maintaining compliance with halal standards, provided the source fish is from a permissible species and processing equipment is adequately segregated from non-halal operations. Similarly, phosphoric acid has been employed experimentally in halal fishbone gelatin extraction at various concentrations, yielding products with acceptable organoleptic and physicochemical properties when accompanied by proper documentation of reagent purity and supplier guarantees. Acetic acid, as an organic acid alternative, offers theoretical advantages in halal processing due to its natural occurrence and milder extraction conditions. The regulation issued by the Ministry of Religious Affairs of the Republic of Indonesia No. 1360 of 2021 explicitly states that those acids are included in the list of ingredients exempt from the obligation to be halal certified. The halal certification process for acid-extracted gelatin mandates rigorous documentation, including raw material origin certificates, slaughter compliance attestations for terrestrial animals, reagent specifications confirming the absence of haram-derived additives, validated cleaning protocols to prevent cross-contamination, and Hazard Analysis Critical Control Point monitoring at key extraction stages. Importantly, Shariah jurisprudence principles such as *istihalah* (chemical transformation) and *taharah* (purity) are invoked when evaluating the permissibility of

extraction processes, though scholarly consensus generally holds that mere chemical transformation does not render pork-derived gelatin halal, thereby reinforcing the primacy of source material selection over processing chemistry (Duasa et al., 2022). Consequently, the use of hydrochloric acid, phosphoric acid, or acetic acid in halal gelatin isolation is technically permissible and practically employed, provided manufacturers implement robust quality management systems, maintain comprehensive audit trails, and secure formal halal certification from recognized authorities through documented compliance with technical specifications and Shariah requirements (Samsudin et al., 2024).

4. Conclusion

Collagen and gelatin can be isolated from manyung bone, each showing FTIR spectra identical to the standard. Collagen extracted from manyung bone using 0.5 M acetic acid and 5 days extraction time results in the highest yield of 4.40 ± 0.33% and the lowest water content of 6.28 ± 1.61%, which conform to SNI requirements. The ash content of collagen from manyung bone is observed to be around 37.53 ± 3.53%. The ideal extraction conditions for gelatin are: for the demineralization process, immersion in 4% HCl solution for 24 hours followed by 4% H_3PO_4 for 24 hours, extraction temperature of 80ºC, and extraction time of 6 hours. This results in the highest rendement of 3.74 ± 1.88%, with moisture content of 11.70 ± 3.31% and ash content of 3.56 ± 1.04%.

CRediT Authorship Contribution Statement

Kholis Amalia Nofianti: Writing – original draft, Formal analysis, Review & Editing; **Nia Safitri**: Formal analysis, Methodology; **Khomsya Ninteen Januar:** Formal analysis, Methodology; **Sugijanto:** Writing – original draft, Review & Editing; **Noor Erma Nasution:** Writing – original draft, Review & editing; **Diajeng Putri Paramita:** Review & editing; **Norhayati:** Review & editing; **Luqmanul Hakim:** Review & editing.

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