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Extraction of Collagen from Kedukang Fish Bone (*Hexanematichthys sagor*) at Various Concentrations of Acetic Acid Solution

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ABSTRACT

The bones of kedukang fish, a by-product of jambal roti production in Pangandaran, have the potential to be a source of collagen. Collagen extraction can be done chemically using acetic acid solution. The extraction process is declared effective if it produces optimal collagen yield. This study aims to determine the best acetic acid concentration in producing optimal collagen yield. The research was conducted from December 2024 to February 2025 at the Tropical Marine Fisheries Laboratory and Central Laboratory of Padjadjaran University and the Center for Testing and Application of Fishery Product Quality (BP2MHP) Semarang. The method used was experimental method, namely the difference in acetic acid concentration in collagen extraction with three treatments (0.5 M; 0.75 M; and 1 M). Parameters observed included yield, physicochemical characteristics and functional groups of collagen from the best treatment. Based on the results, 1 M acetic acid concentration treatment was the best treatment with a yield value of 34.62%±1.31. The characteristics of collagen produced include moisture content of 9.507%, ash content of 2.60%, protein content of 64.91% and pH 6.94. FTIR results showed collagen identified Amide A at the absorption peak of 3404 cm^{-1}, Amide I at the absorption peak of 1698.40 cm^{-1} and 1636.98 cm^{-1}, Amide II at the absorption peak of 1546.40 cm^{-1} and Amide III at the absorption peak of 1230.36 cm^{-1}.

INTRODUCTION

Pangandaran Regency, located geographically at 108°30'-108°40' E and 7°40'20"-7°50'20" S, is a newly autonomous region established under Law No. 21 of 2012 in the southern part of West Java Province. It covers an area of 101,092 hectares with a coastline stretching 91 km. The region relies on two main sectors: marine tourism and capture fisheries, both of which significantly contribute to the local economy and create business opportunities for the community (Kartika *et al.*, 2020; Nurhayati *et al.*, 2013). According to Andhikawati and Permana (2023), the substantial fisheries potential in Pangandaran is accompanied by the growth of

fishery product processing industries in the form of Micro, Small, and Medium Enterprises (MSMEs). One of Pangandaran's signature processed products is "jambal roti," a salted fermented fish product primarily made from kedukang fish (*Hexanematichthys sagor*) (Junianto *et al*., 2024; Maulid & Abrian, 2020).

The rising demand for jambal roti, both as a local delicacy and a tourist souvenir, has expanded its processing industry at both household and industrial scales (Junianto *et al*., 2023). However, the processing practices in Pangandaran often lack standardized waste management, resulting in underutilization of by-products (Kamsiah & Ponirah, 2021). Jambal roti production generates substantial solid waste such as fish heads, viscera, and bones (Andhikawati & Permana, 2024), with approximately 50% of the raw material's weight turning into waste (Junianto *et al*., 2024). On average, fishery MSMEs in Pangandaran produce around 20 kg of waste per month (Andhikawati & Permana, 2023), and from the total fish weight, about 75% is discarded, including 30% in the form of bones and skin (Pan *et al*., 2010; Songchotikunpan *et al*., 2008; Martinez *et al*., 2015). Therefore, utilizing fish bone waste is crucial to prevent environmental pollution and to harness its potential as a valuable resource, such as collagen (Simamora *et al*., 2019).

Collagen is an insoluble protein found in bones, skin, ligaments, tendons, and the extracellular matrix, comprising about 30% of total body protein (Raman & Gopakumar, 2018). It is widely used across cosmetics, food, and pharmaceutical industries (Duan *et al*., 2009). Most collagen currently comes from terrestrial animals like cattle, pigs, and poultry, but these sources pose halal concerns and risks of zoonotic diseases such as Bovine Spongiform Encephalopathy (BSE), swine flu, foot-and-mouth disease, and avian flu (Sadowska *et al*., 2003; Liu *et al*., 2015). As an alternative, fish-derived collagen is increasingly favored due to its safety, ethical acceptability, and broad market acceptance (Jongjareonrak *et al*., 2005).

Fish bone-derived collagen is classified as type I collagen, recognized for its excellent properties (Jafari *et al*., 2020; Rahantan & Lalopua, 2024). Extraction methods include enzymatic and chemical approaches (Lin *et al*., 2010), with chemical methods dominating due to their simplicity (Kartika, 2016). Acid-based extraction, particularly using acetic acid, is more straightforward and yields higher extraction efficiency compared to hydrochloric or citric acids (Kasim, 2013; Oktaviani *et al*., 2023). The carboxyl group (-COOH) in acetic acid facilitates the disruption of hydrogen bonds and cross-links within collagen, enhancing its solubility (Rahantan & Lalopua, 2024). Importantly, the concentration of acetic acid significantly affects the yield and quality of extracted collagen. Yield serves as a key indicator to assess the effectiveness and efficiency of extraction methods, where higher yields reflect more efficient processes (Febriansyah *et al*., 2019).

Previous studies have demonstrated varying results depending on acid concentration: Tawisna (2022) reported the highest yield (5.07%) from sea cucumber collagen using 1 M acetic acid, along with superior chemical properties;

Lilis *et al*. (2024) achieved a 9.368% yield from gourami fish bone collagen extracted with 0.75 M acetic acid, confirming type I collagen via amide group identification; Putra *et al*. (2013) observed a 5.96% yield from black tilapia skin using 0.75 M acetic acid. Although collagen extraction from various fish species has been explored, no specific study has evaluated the extraction of collagen from kedukang fish bones using different acetic acid concentrations.

Therefore, this research aims to determine the optimal acetic acid concentration for extracting collagen from kedukang fish bones, based on yield, and to analyze the physicochemical properties and functional groups of the highest-yielding treatment. This study is expected to contribute to the development of sustainable fishery waste utilization technology.

LITERATURE REVIEW

Collagen has an important role in the cosmetic, food, and pharmaceutical industries, with widespread applications in anti-aging products, edible food casing, and burn medicine (Setyowati and Setyani, 2015; Duan *et al*., 2009). However, collagen production in Indonesia is still low, with dependence on imports reaching 182,753 tons in 2020 (Utami *et al*., 2024). Most of the imported collagen comes from terrestrial sources, which raises concerns related to its halalness and potential risk of zoonotic diseases such as BSE, swine flu, foot-and-mouth disease, and avian flu (Liu *et al*., 2015). Collagen from fish, particularly from kedukang fish bones, offers a safer and ethical alternative and has wide market acceptance.

Collagen extraction can be done by chemical or enzymatic approaches. Enzymatic methods, although effective, require complex settings related to pH, temperature, and time, so they are often difficult to implement on an industrial scale (Nurjanah and Nurhayati, 2021). In contrast, chemical extraction, especially using acids, is more commonly chosen due to its ease of implementation and stable and efficient results (Lin *et al*., 2010; Meng *et al*., 2019). One of the frequently used acids is acetic acid, which has the ability to remove hydrogen bonds and crosslinks in the collagen structure, thereby increasing its solubility and resulting in more efficient extraction (Kasim, 2013).

Research conducted by Kasim (2013) showed that acetic acid with a concentration of 0.5 N was more effective than citric acid and hydrochloric acid in extracting collagen from tuna skin, with a higher yield (1.2%). On the other hand, research by Nurhayati (2013) showed that the use of 0.5 M acetic acid concentration produced collagen with higher amino acid content and denaturation temperature compared to the higher concentration (1.5 M). Therefore, for this study, acetic acid with various concentrations (0.5 M, 0.75 M, and 1 M) was chosen to determine the optimal concentration that produces high collagen yield and suitable quality.

Further research by Ariyanti *et al*. (2018) on the extraction of collagen from clam shells using 0.75 M acetic acid showed better results compared to the lower

concentration (0.25 M), with higher yields of 2.03% and 1.96%. Similarly, Putra *et al*. (2013) reported that the extraction of collagen from black tilapia skin with 0.75 M acetic acid produced the highest yield (5.96%), indicating that higher acid concentrations often provide more optimal results. Lilis *et al*. (2024) also confirmed that the extraction of collagen from carp bones with 0.75 M acetic acid concentration gave a higher yield (9.368%) compared to 0.5 M (1.178%).

These results provide strong evidence that acetic acid concentration has a significant influence on the yield of collagen obtained, with higher concentrations generally producing more optimal results in terms of yield and chemical quality of collagen. This study aims to further explore the potential of collagen extraction from kedukang fish bones using various concentrations of acetic acid, to determine the conditions that produce collagen with the best yield and chemical characteristics.

METHOD

This study uses an experimental method with a Completely Randomized Design (CRD). The treatments given are variations in acetic acid concentrations (0.5 M, 0.75 M, and 1 M), each repeated 3 times.

Place and Time

This research was conducted from December 2024 to February 2025. The collagen production was carried out in the Tropical Fisheries and Marine Laboratory, Universitas Padjadjaran PSDKU Pangandaran. The chemical testing, including moisture content, ash content, and protein content, was conducted at the Fisheries Quality Testing Laboratory (BPMHP) Semarang. The FT-IR spectroscopy analysis of collagen functional groups was carried out in the Central Laboratory of Universitas Padjadjaran.

Materials and Equipment

The materials used in this study include:

- 180 grams of dried Kedukang fish bones, obtained from the Jambal Roti production facility in Pangandaran.
- Acetic acid (CH_3COOH) 98%, sodium hydroxide (NaOH) CP 99%, and distilled water, obtained from Rofa Laboratory Centre, Bandung.

The equipment used includes: Knife, chopping board, Pyrex measuring glasses (25 mL and 1000 mL), Pyrex beaker glass (500 mL), Pyrex Erlenmeyer flask (1000 mL), glass jars, plastic containers, Whatman no. 42 filter paper (90 mm diameter), filter, glass funnel, spoon, weighing containers, Ohaus SPX 220 digital balance (precision 0.01 grams), Osuka analytical digital balance (precision 0.0001 grams), Ez9902 pH meter, Hanna Instruments 190M magnetic stirrer, Hettich EBA200 centrifuge, 15 mL centrifuge tubes, Memmert UF450 oven, 30 mL porcelain dishes, petri dishes, aluminum foil, and labels.

Collagen Production Procedure

The collagen extraction method in this study refers to the modified procedures of Fabella *et al*. (2018) and Qutrinnada *et al*. (2022), with the following steps:

a. Sample Preparation

Fish bones are cleaned thoroughly to remove any remaining meat, then dried. After drying, the bones are cut into approximately 1x1 cm pieces using a knife. The bones are then pre-treated with NaOH to remove non-collagen proteins and fats (degreasing). The bones are placed in a beaker and soaked in 0.1 M NaOH solution at a sample-to-solution ratio of 1:7 (w/v) for 3x24 hours at room temperature, with the NaOH solution being replaced three times. After soaking, the bones are filtered and washed with distilled water until a neutral pH is reached.

b. Collagen Extraction

At the extraction stage, there are nine trials, each consisting of three different treatments, repeated three times. For each trial, 20 grams of fish bones are placed in glass jars and soaked in acetic acid solution (0.5 M, 0.75 M, 1 M) at a sample-to-solution ratio of 1:6 (w/v) for 3x24 hours at room temperature. The extracted collagen is then filtered to separate the residue from the extract. The extract (supernatant) is precipitated by adding 1 N NaOH for 24 hours to obtain collagen precipitate. Subsequently, the precipitate and supernatant are centrifuged at 6000 rpm for 20 minutes. The wet collagen obtained from the centrifugation process is transferred to a petri dish and dried in an oven at 50°C for 2x24 hours.

c. Yield Calculation

Collagen yield is calculated based on the weight of dry collagen produced compared to the initial weight of the bone material, following the method of Paudi *et al*. (2020). The yield is calculated using the following equation:

$$\text{Collagen Yield (\%)} = \frac{\text{Dry collagen weight (g)}}{\text{Bone material weight (g)}} \text{ x } 100\% \qquad (1)$$

Physicochemical Characteristics of Collagen

pH (Acidity)

The pH of the collagen is measured using the method of Romadhon *et al*. (2019). One gram of collagen is dissolved in 100 mL of distilled water (1:100 w/v) until completely dissolved, and the pH is measured using a pH meter until stable readings are obtained.

Moisture Content

Moisture content is analyzed using the SNI 2354.2:2015 method (BSN, 2015). The sample is heated in a vacuum oven at 95–100°C with a pressure of no more than

100 mmHg for 5 hours. The weight loss due to water evaporation is used to calculate the moisture content, using the formula:

$$\text{Moisture content (\%)} = \frac{B-C}{B-A} \text{ x } 100\% \quad (2)$$

Information:

- A = Weight of empty dish (g)
- B = Weight of dish + initial sample (g)
- C = Weight of dish + dried sample (g)

Ash Content

Ash content is analyzed according to the SNI 2354.1:2010 method (BSN, 2010). The sample is dried and then burned in a furnace at 550 ± 5°C for 16–24 hours until white ash remains. Ash content is calculated using the formula:

$$\text{Ash content (\%)} = \frac{B-A}{\text{Sample weight (g)}} \text{ x } 100\% \quad (3)$$

Information:

- A = Weight of empty porcelain dish
- B = Weight of dish with ash

Protein Content

Protein content is analyzed using the Kjeldahl method based on SNI 01-2354.4-2006 (BSN, 2006). The sample is digested with sulfuric acid and catalyst, distilled, and the result is titrated using 0.2 N HCl. Protein content is calculated using the formula:

$$\text{Protein content (\%)} = \frac{(Va-Vb)\text{HCL x N HCL x } 14{,}007 \text{ x } 6{,}25 \text{ x } 100\%}{W \text{ x } 1000} \quad (4)$$

Information:

- Va = Volume of HCl for titration of the sample (mL)
- Vb = Volume of HCl for titration of the blank (mL)
- N = Normality of the HCl standard used
- W = Weight of the sample (g)

Functional Groups of Collagen

The identification of functional groups is done using FT-IR spectroscopy, based on Nofita *et al*. (2023). The FT-IR spectrum is obtained using the Thermo Scientific Summit instrument with the ATR technique in the 4000–400 cm^{-1} wave number range. The peaks in the spectrum are analyzed to identify collagen functional groups such as amide A, I, II, and III.

Data Analysis

Collagen yield data are analyzed statistically using ANOVA. If significant differences are found (Fhitung > Ftabel at the 5% significance level), Duncan's test is performed to determine the differences between treatments. The physicochemical characteristics and functional groups of collagen from the best

treatment are analyzed descriptively and compared to SNI 8076:2020 and related references.

RESULT AND DISCUSSION

Collagen Yield of Kedukang Fish Bone

Yield is the ratio of the weight of dry collagen produced to the weight of bone raw materials used. According to Suptijah *et al*. (2018), yield shows the proportion of raw materials that can be utilized, so it is an important parameter in determining the economic aspects and efficiency of using a material or product. The data of the yield calculation of kedukang fish bone collagen is presented in Figure 1.

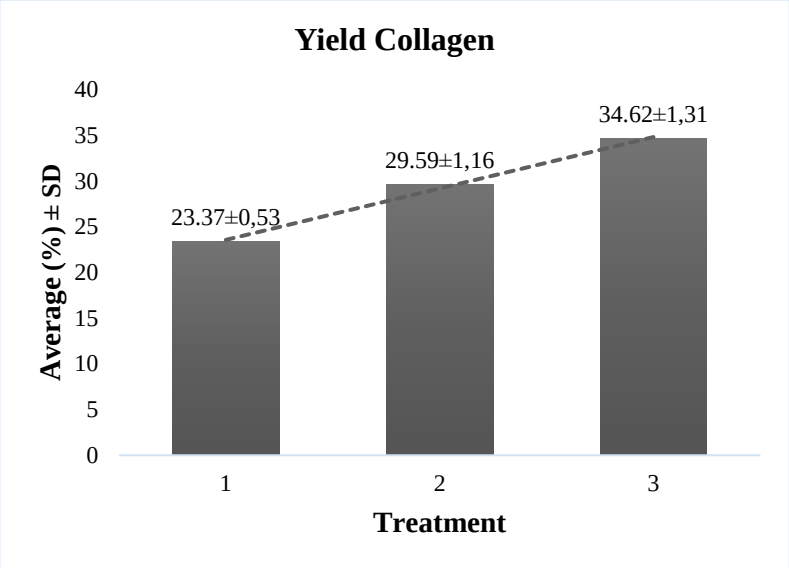


Figure 1. Data of Collagen Yield of Kedukang Fish Bone
Source: Processed data (2025)

Based on the calculations that have been carried out, there is an increase in yield as the concentration of acid given increases. The largest yield was obtained from the use of 1 M acetic acid concentration at 34.62%, followed by 0.75 M concentration at 29.59%, and the smallest yield was at 0.5 M concentration at 23.37%.

The results of statistical analysis using the Anova test showed that the treatment of different concentrations of acetic acid solution had a significant effect on the yield of kedukang fish bone collagen, where Fhitung > Ftabel (85.91 > 5.14). Furthermore, Duncan's further test was conducted to determine the best treatment at the 95% confidence interval. The results of the further test are presented in Table 1.

Table 1. Collagen Yield of Kedukang Fish Bone

Treatment	Concentration Variation	Yield ± SD
1	0,5 M	23,37%±0,53[a]
2	0,75 M	29,59%±1,16[b]
3	1 M	34,62%±1,31[c]

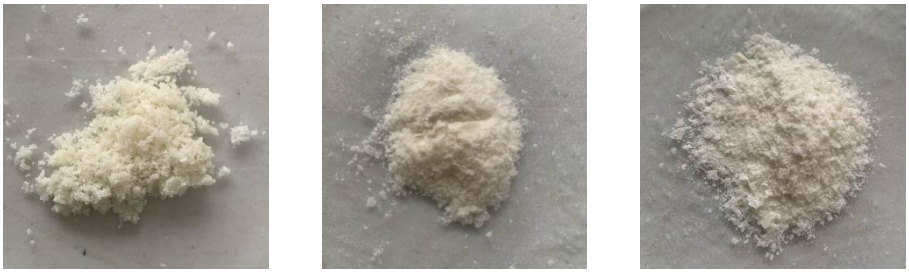
Description : Numbers followed by different letters (a, b, c) indicate significantly different results according to Duncan's test at the 95% confidence level. significantly different according to Duncan's test at 95% confidence level. Numbers with the same letter are not significantly different.

Based on the results of Duncan's New Multiple Range Test (DNMRT), the results showed significant differences between treatments. The P3 treatment, which is the use of 1 M acetic acid concentration, produced the highest yield and was significantly different compared to P2 and P1 (Table 1). Thus, the concentration of 1 M acetic acid can be stated as the best treatment in the extraction process of kedukang fish bone collagen to produce optimal yield.

Figure 1 shows that the higher the concentration of acetic acid used, the more optimal the collagen produced. This is in accordance with the statement of Ariyanti *et al*. (2018), that a higher concentration of acetic acid can open the collagen structure more effectively, thus increasing the amount of collagen extracted. The yield of collagen from kedukang fish bones was higher than the yield of tilapia fish bone collagen, which was 0.53% (Romadhon *et al*., 2019), tuna fish bones by 21% (Rahmawati, 2020), and tuna fish bones by 21.05% (Hanivia, 2022). This difference is caused by variations in the concentration of acetic acid used. According to Kittiphattanabawon *et al*. (2005) stated that the higher the concentration of acetic acid, the more H^+ ions are produced. H^+ ions from acetic acid function to disrupt hydrogen bonds and electrostatic interactions between collagen chains, which causes the collagen structure to be more easily detached from the tissue matrix (Muyonga *et al*., 2004).

The difference in yield obtained can also be influenced by the characteristics of the raw materials. The protein content of kedukang fish bones reached 26.23%, higher than the protein content of tilapia bones at 20.85% (Haris, 2008), tuna at 17.17% (Agustin, 2016), and tuna at 26.02% (Nurilmala, 2006). In addition, other factors such as differences in pretreatment and extraction conditions, including acid concentration and washing procedures, also affect yield results (Astiana *et al*., 2016). This is in line with the statement of Ratnasari *et al*. (2013) that collagen yield is influenced by the raw materials, acid concentration, pH conditions, as well as the amount of collagen lost during the pretreatment process. In addition to producing

the highest yield, collagen extracted with 1 M acetic acid concentration also showed a better physical appearance compared to other treatments (Figure 2).



(a) (b) (c)

Figure 2. Collagen appearance with different concentrations of acetic acid: (a) 0.5 M; (b) 0.75 M; and (c) 1 M.

Based on visual observation, collagen from 1 M treatment (c) appeared as a bright white powder. This result is in accordance with the quality standard of collagen according to SNI 8076:2020, namely collagen has a white to transparent white color and neutral odor. In addition, the collagen in treatment 1 M (c) also did not show any pungent odor, thus fulfilling the required sensory criteria.

Physicochemical Characteristics of Collagen from the Best Treatment

The characteristics of collagen were tested to determine the quality of collagen produced. This test includes chemical testing namely water content, ash content and protein content. Physical testing is pH and functional group analysis of collagen to determine the structural and functional properties of collagen. The physicochemical characteristics of collagen in the best treatment are presented in Table 2.

Tabel 2. Physicochemical Characteristics of Collagen from the Best Treatment

No	Characteristics Physicochemical	Result	SNI 8076:2020 Coarse Collagen
1	Degree of Acidity (pH)	6,94	6-7
2	Water Content	9,507%	Max. 14%
3	Ash Content	2,60%	Max. 1%
4	Protein Content	64,91%	Min. 75%
5	Collagen Function Groups	Amida A, I, II dan III	-

Source: Processed data (2025)

Degree of Acidity (pH)

The results showed that the pH of kedukang fish bone collagen with 1 M acetic acid solvent has neutral properties with pH 6.94 ± 0.02. This value is higher than the pH of red snapper bone collagen extracted with 0.19 M acetic acid which is

6.34 (Kamaluddin, 2024). The neutral pH of collagen is thought to be from the neutralization and precipitation process using NaOH solution after the collagen extraction process. The neutralization process also refers to the research of Qutrinnada *et al*. (2022), where the pH measurement results of tuna bone collagen with the addition of NaCl has a pH of 4.65 which is lower than the addition of NaOH solvent which is 6.88. This difference is caused by the absence of a neutralization process during isolation with NaCl, while the addition of NaOH neutralization is carried out until the pH is close to 7 (Qutrinnada *et al*., 2022). According to Alhana *et al*. (2015) neutralization plays a role in determining the final pH of collagen by reducing the residual acid or base remaining after soaking. Based on the research, the pH of kedukang fish bone collagen obtained is in accordance with the collagen standard, which is in the range of 6.00-7.00 (BSN, 2020).

Moisture Content

Moisture content testing has an important role in the product as it can affect the metabolic activities during storage. The result of water content in kedukang fish bone collagen is 9.507%. This result is almost similar to several studies, namely the water content of yellowfin tuna bone collagen of 9.18% (Handaratri & Hudha 2021) and manyung fish bone collagen of 8.92% (Safitri, 2019). According to Panjaitan (2017) moisture content is one of the important parameters because it can affect its acceptability, freshness, and durability. High water content can encourage the growth of microorganisms that accelerate collagen breakdown (Winarno 1997). In this study, the moisture content of kedukang fish bone collagen was still within the limit set by the Indonesian National Standard (SNI) for collagen, which is no more than 14% (SNI 8076:2020). The low moisture content in this study is thought to be caused by the drying process using an oven at 50ºC. According to Rahmawati (2020) the water content in collagen easily evaporates when dried in an oven. This is in line with the opinion of Winarno (1995), which states that the higher the drying temperature, the faster the evaporation process, so that the moisture content in the material becomes lower.

Ash Content

Ash content testing aims to measure the amount of minerals in a material. Minerals commonly found in collagen include calcium phosphate, calcium carbonate, and magnesium phosphate (Trilaksani *et al*., 2006). According to Junianto *et al*. (2006), during the extraction process, these minerals can dissolve with collagen, so collagen also contains minerals. The ash content of kedukang fish bone collagen was 2.60%. The ash content in this study is lower than the ash content of manyung fish bone collagen of 39.95% (Safitri, 2019), milkfish bone collagen, mackerel and tilapia respectively (50.75%; 53.41%; 54.63%) (Darmanto *et al*., 2012). This shows that the mineral content in kedukang fish bone collagen is less.

The range of ash content obtained does not meet the standards set by the Indonesian National Standard (SNI) for collagen, which exceeds 1% (SNI 8076:2020). The high ash content raises questions about the type of minerals contained in it. Therefore, further research is needed to identify the mineral composition. If the dominant mineral is calcium, it could potentially be utilized in the medical field (Aisyah *et al*., 2012).

Protein Content

Most of the protein in fish bones consists of collagen. Collagen is the main protein in body tissues that can be found in skin, connective tissue, and bones, and functions as a structural protein of the body (Raman & Gopakumar 2018). According to Fitri *et al*. (2016), fish bones generally contain proteins that fall into the category of stromal proteins. Amino acids lysine and arginine are binding proteins that play an important role in calcium absorption in bones (Kusumaningrum *et al*., 2016). The results showed that the protein content of kedukang fish bone collagen was 64.91%. This value is higher than the protein content of red snapper bone collagen which is 47.36% (Kamaluddin, 2024) and combined collagen (head, bone and tail) of 53.94% (Rahantan & Lalpoua 2024). However, the protein content produced did not meet the quality standard of collagen SNI 8076:2020 which requires protein content above 75%. The low protein content is thought to be due to the use of a high concentration of acetic acid. This is supported by the statement of Chamidah and Elita (2002), if the concentration of acetic acid is too high in the extraction process, the hydrogen bonds in collagen will be broken excessively, so that some amino acids are released and carried away in the washing water, resulting in lower protein levels. In addition to the extraction method, collagen protein content is also influenced by biological factors such as environmental temperature, food type, age, and fish habitat (Rima, 2017).

Functional Groups of Collagen

FTIR spectroscopy is a commonly used analytical technique to identify functional groups in a compound. Each frequency of light, including infrared, has a specific wave number. If an organic compound absorbs a certain IR frequency, vibrations will occur in its molecule (Nagarajan *et al*., 2012). In this study, FTIR analysis was performed to confirm that the detected compounds or functional groups are the constituent components of collagen based on the structure of collagen functional groups. Spectra of functional groups of kedukang fish bone collagen are presented in Figure 3.

The FTIR spectrum results of kedukang fish bone collagen show several characteristic absorption peaks consistent with the general collagen spectrum. The absorption band at 3404 cm^{-1} indicates N–H stretching vibration, which is characteristic of Amide A in proteins. According to Muyonga *et al*. (2004), the Amide

A absorption region ranges from 3440–3400 cm^{-1}, indicating the presence of N–H stretching vibrations and hydrogen bonding. The presence of amine groups plays a role in forming the secondary structure of collagen.

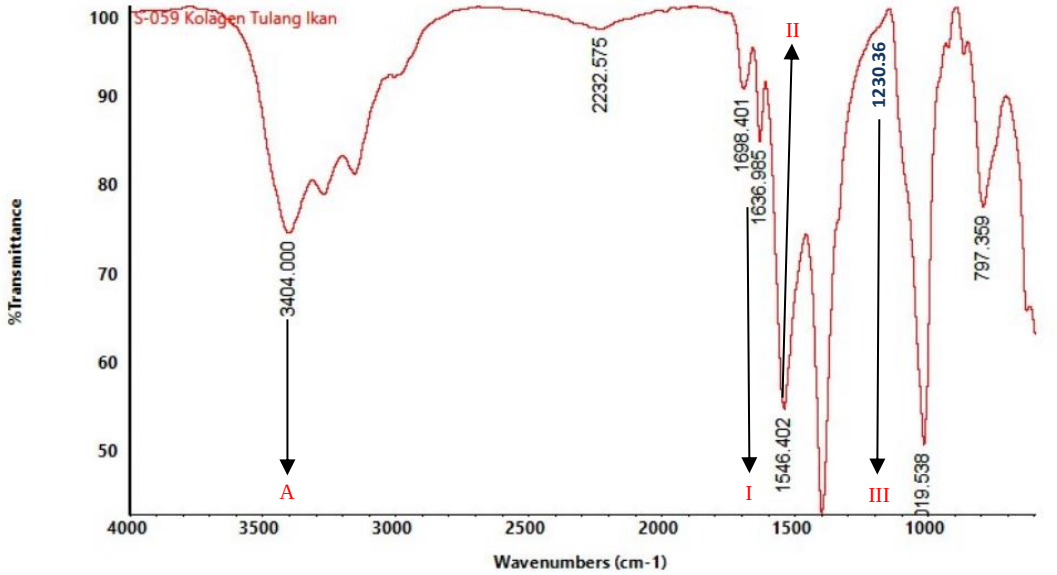


Figure 3. FTIR Spectrum of Kedukang Fish Bone Collagen

This absorption value aligns with findings from other studies, such as the Amide A peak in gamma sea cucumber collagen (3406.29 cm^{-1}) (Safithri *et al*., 2020), pangasius fish skin collagen (3407.37 cm^{-1}) (Suptijah *et al*., 2018), tuna fish skin collagen (3412 cm^{-1}) (Kolanus *et al*., 2019), and red snapper fish bone collagen (3421.17 cm^{-1}) (Ramli *et al*., 2019).

Next, the Amide I absorption peaks in kedukang fish bone collagen were detected at 1638.98 cm^{-1} and 1698.40 cm^{-1}, still within the typical collagen range of 1700–1600 cm^{-1} (Cheheltani *et al*., 2014). These values are close to findings from manyung fish bone collagen (1611.32–1637.33 cm^{-1}) (Safitri, 2019) and snakehead fish skin collagen (1635.64 cm^{-1}) (Nofita *et al*., 2023). Amide I is associated with the stretching vibration of carbonyl (C=O) groups and is the main functional group in collagen's structure (Shah & Manekar, 2012). According to Muyonga *et al*. (2004), Amide I has four protein secondary structure components: α-helix, β-sheet, β-turn, and random coil, whose spectra overlap. Based on spectrum interpretation, the absorption ranges for each structure are 1700–1660 cm^{-1} (β-turn), 1654–1650 cm^{-1} (α-helix), 1644–1640 cm^{-1} (random coil), and 1640–1620 cm^{-1} (β-sheet) (Kong & Yu, 2007). Thus, the FTIR spectrum of kedukang fish bone collagen indicates the presence of β-sheet structures at 1638.98 cm^{-1} and β-turn structures at 1698.40 cm^{-1}. This suggests that the collagen has not been denatured into gelatin. This aligns with Muyonga *et al*. (2004), who stated that one main characteristic of gelatin is the shift of peaks to the random coil absorption range due to denaturation.

The Amide II absorption peak was detected at 1546.40 cm^{-1}, which is within the 1480–1575 cm^{-1} range (Mberato *et al*., 2020). This value aligns with the Amide

II peaks in red snapper bone collagen and snakehead skin collagen, detected at 1541.12 cm^{-1} (Ramli *et al*., 2019; Nofita *et al*., 2023). Amide II is related to a combination of C–N stretching and N–H bending vibrations. Additionally, there was an extra absorption at 1403.56 cm^{-1}, similar to the Amide II absorption value in parrotfish scale collagen (1402 cm^{-1}) (Mberato *et al*., 2020). The shift of the Amide II absorption to lower wavenumbers indicates strong hydrogen bonding within the collagen structure. As explained by Prasetyo (2018), the more hydrogen bonds present, the more stable the collagen structure becomes, causing the IR spectrum to broaden and shift to lower wavenumbers.

Meanwhile, the Amide III absorption peak was detected at 1230.36 cm^{-1} with a ratio of 96.04. The Amide III absorption band lies in the range of 1229–1301 cm^{-1}, reflecting intermolecular interactions in collagen and related to C–N stretching and N–H bending (Kong & Yu, 2007). This result is close to the findings of Suptijah *et al*. (2018), who reported the Amide III absorption peak in pangasius fish skin collagen at 1239.46 cm^{-1}. According to Ramli (2019), the presence of the Amide III band can be used to identify the triple helix structure, which is a hallmark of collagen. The ratio between the Amide III band and the band around 1450 cm^{-1} approaching 1.0 indicates that the collagen's triple helix structure is maintained (Tziveleka *et al*., 2017). In this study, the obtained ratio was 1.33, whereas in parang-parang fish skin collagen, the ratio was 1.13 (Safithri, 2019). As a comparison, Goissis *et al*. (1998) reported that denatured collagen has a ratio of 0.59, indicating the loss of the triple helix structure and its transformation into gelatin.

Table 4. Results of Collagen Absorption Identification from Kedukang Fish Bone

Amida type	Absorption Range (cm^{-1})	Result	Notes
Amida A	3440-3400	3404 cm^{-1}	Vibrasi *Stretching* NH
Amida I	1700-1600	1638.98 dan 1698.40 cm^{-1}	Vibrasi *Stretching* C=O
Amida II	1575-1480	1546.40 cm^{-1}	NH *Bending*, CH *Stretching*
Amida III	1301-1229	1230.36 cm^{-1}	CH *Stretching*, NH *Bending*

Source: Processed data (2025)

CONCLUSION

Based on the results of the study, the optimal acetic acid concentration for extracting collagen from kedukang fish bone was found to be 1 M, yielding the highest recovery at 34.62±1.31%. The physical characteristics of the collagen at the optimal treatment showed a pH value of 6.94. The chemical characteristics included a moisture content of 9.51%, ash content of 2.60%, and protein content of 64.91%. FTIR analysis indicated that the extracted collagen was classified as type I collagen, as evidenced by characteristic absorption peaks at 3404 cm^{-1} (Amide A), 1698.40

cm^{-1} and 1636.98 cm^{-1} (Amide I), 1546.40 cm^{-1} (Amide II), and 1230.36 cm^{-1} (Amide III).

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