EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

*Ecological Engineering & Environmental Technology*, 2025, 26(1), 1–7
https://doi.org/10.12912/27197050/194795
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Received: 2024.10.12
Accepted: 2024.11.19
Published: 2024.12.01

# Influence of extraction time on collagen yield and proximate composition from yellowfin tuna (*Thunnus albacares*) bones: Insights from industrial waste valorization

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## ABSTRACT

The valorization of tuna bones from industrial waste as a source of halal collagen presents a promising alternative to land-based collagen sources, such as bovine and porcine collagen. This study aims to evaluate the effect of extraction duration on the quality of collagen produced, focusing on yield, proximate composition (moisture, ash, fat, and protein content), functional group analysis, and collagen morphology. Collagen was extracted using the acid-soluble collagen (ASC) method, which involved pretreatment with NaOH to remove non-collagenous proteins, isopropyl alcohol to remove fat, and hydrolysis using acetic acid ($CH_3COOH$). The extraction durations tested were 24, 48, 72, and 96 hours, with a solution-to-sample ratio of 1:10 using 0.75 M $CH_3COOH$. Results showed that extraction for 96 hours yielded the highest collagen at 4.70%. FTIR analysis confirmed the presence of functional groups Amide I, II, III, A, and B, while SEM analysis revealed collagen morphology as small, rounded particles with fine pores and clearly visible collagen fibers. The moisture content decreased with longer extraction times, from 12.8% at 24 hours to 4.2% at 96 hours, while protein content increased, reaching 85.2% at 96 hours. The fat content was reduced to 0.2%, and ash content minimized to 0.8%. The proximate composition met the Indonesian National Standard (SNI 8076:2014), indicating that tuna bone collagen is a viable and good-quality source of halal collagen derived from industrial waste.

**Keywords:** by-product valorization, environmental chemistry, industrial waste management, marine biotechnology, marine chemistry, waste utilization, yellowfin tuna bone.

## INTRODUCTION

Indonesia, as the largest archipelago in the world with over 17,000 islands, holds vast potential in its marine resources (Rochwulaningsih et al., 2019). The extensive marine territory offers significant opportunities in the fisheries sector (Sidik et al., 2023). However, despite Indonesia's high fishery production, the utilization of its by-products, especially fish waste, remains suboptimal (Atma et al., 2024; Cahyana et al., 2024). Among the abundant marine species is the yellowfin tuna (*Thunnus albacares*), a valuable fish often harvested for its meat, which is widely used in canning and freezing industries (Prajaputra et al., 2024). Unfortunately, large quantities of tuna bones from industrial waste remain underutilized, despite their potential for value-added applications, particularly in collagen extraction (Cutajar et al., 2022).

One promising application of tuna by-products, particularly its bones, is in collagen extraction (Oslan et al., 2022). Collagen, a structural protein found primarily in the connective tissues of vertebrates, plays a critical role in medical, pharmaceutical, and cosmetic industries due to

its bioactive properties (Sionkowska et al., 2020). Collagen from fish, known as marine collagen, offers a viable alternative to traditional mammalian sources, such as bovine and porcine collagen, which are associated with issues like zoonotic disease transmission, including Bovine Spongiform Encephalopathy (mad cow disease) and avian influenza (Senadheera, et al., 2020; Prajaputra et al., 2024; Isnaini et al., 2024). Moreover, porcine collagen presents a challenge for halal certification, making marine collagen an attractive, halal-compliant alternative (Duasa et al., 2022; Coppola et al., 2020; Isnaini et al., 2024).

Fish bones, particularly from yellowfin tuna, contain up to 30% collagen, rich in amino acids like glycine, proline, hydroxyproline, and arginine (Nurilmala et al., 2020), making them a promising and sustainable source of collagen. Despite the availability of tuna bones as industrial waste, the processing of these by-products into valuable biomaterials such as collagen has not been fully explored. Given the growing demand for halal collagen in global markets, optimizing extraction methods from marine sources represents a critical opportunity. Conventional sources of collagen from terrestrial animals have inherent drawbacks, not only related to disease risks but also to environmental sustainability and religious dietary restrictions. While research on collagen extraction from fish has been growing, the specific optimization of extraction conditions, especially in terms of extraction time using organic acids like acetic acid, has not been sufficiently addressed. Studies have predominantly focused on broad extraction techniques or alternative fish species, leaving a significant gap in understanding the impact of extraction parameters on collagen yield and quality from yellowfin tuna bones. This study aims to fill this gap by investigating the effect of varying extraction times using acetic acid on the yield and proximate composition of collagen derived from yellowfin tuna bones.

## MATERIALS AND METHODS

### Materials

The yellowfin tuna bones were sourced from PT Yakin Pasifik Tuna in Banda Aceh, Indonesia. After being thoroughly cleaned, the bones were sliced into small pieces and stored at -25 °C until use. Additional materials, such as distilled water, glacial acetic acid ($CH_3COOH$), isopropyl alcohol, and sodium hydroxide (NaOH), were provided by the Research Center for Marine Sciences and Fisheries at Universitas Syiah Kuala.

### Collagen preparation from the bone of yellowfin tuna

The schematic procedure of collagen extraction from tuna bone is presented in Figure 1. Collagen was prepared using the procedure previously reported by Prajaputra et al. (2024), with some modifications. Pre-treatment tuna bone was used to remove fat and non-collagenous proteins from cleaned fish bones. The bones were pre-treated by soaking them in 0.1 M NaOH at a 1:10 (w/v) ratio for 6 hours, with the solvent replaced every 2 hours while stirring. After thoroughly washing the residue with distilled water, the fat was removed by soaking

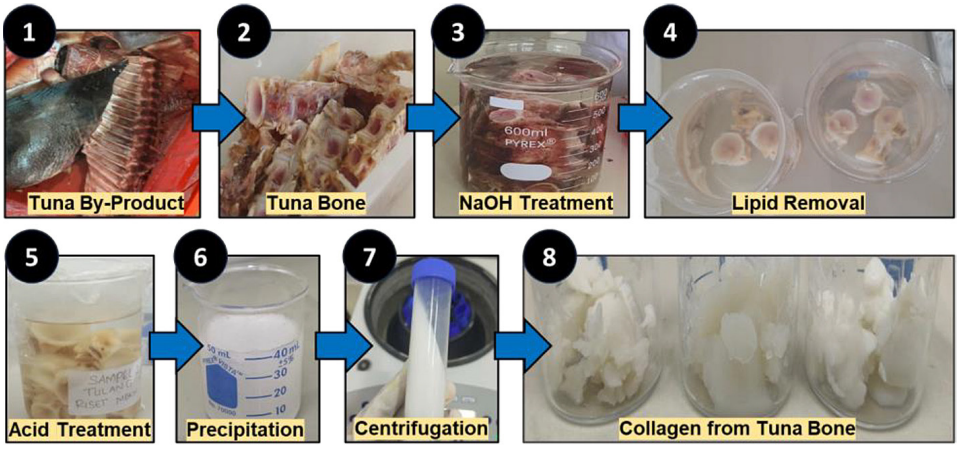


**Figure 1.** The schematic procedure of collagen extraction from yellowfin tuna bone

the bones in 10% isopropyl alcohol at 11 °C for 24 hours, with the solution being replaced daily. The residue was then thoroughly washed again with distilled water.

Collagen extraction was performed over durations of 24, 48, 72, and 96 hours, with a solvent-to-sample ratio of 1:10 (w/v) using 0.75 M acetic acid. The mixture was stored at 11 °C for 24 hours. The supernatant was then precipitated with 0.5 M NaOH at 11 °C until the pH reached 7, followed by storage at 11 °C for another 24 hours. The precipitates were centrifuged for 3 minutes at 7.000 rpm. After dissolving the pellets in 0.5 M $CH_3COOH$ at a 1:1 (v/v) ratio, the mixture underwent dialysis for 24 hours using a 14 kDa membrane in distilled water. The dialyzed solution was lyophilized to obtain dry collagen. The resulting collagen was analyzed for yield, proximate composition (moisture, ash, protein, and fat), and characteristics using FTIR and SEM. The collagen yield (%) was determined using the following formula.

$$\textit{Collagen yield}\ (\%) = \left(\frac{\textit{Weight of dried collagen}\ (\text{g})}{\textit{Weight of initial dry tuna bone}\ (\text{g})}\right) \times 100 \quad (1)$$

For proximate analysis, the moisture, ash, fat, and protein contents in the raw bone and the extracted collagen were determined using methods outlined by AOAC (2006).

### Data analysis

For collagen extraction and proximate analysis, all experiments were conducted in triplicate. The data were presented as the standard deviation of the mean. Data analysis was carried out using SPSS statistics version 16.0, and variable differences were determined using Duncan's tests at a 0.05 significance level.

## RESULTS AND DISCUSSION

### Yield and proximate analysis of collagen

The collagen yield was calculated by comparing the dry weight of the collagen to the initial weight of the sample. The extraction yield of collagen is shown in Figure 2. The highest yield of dried collagen, 2.35 g, was achieved after 96 hours of extraction using 0.75 M acetic acid, while the lowest yield, 1.05 g, was obtained after 24 hours of extraction. The study demonstrated that the collagen yield from yellowfin tuna bones increased linearly with longer immersion times in acetic acid.

Table 1 presents the collagen yield obtained from yellowfin tuna bones at different extraction times (24, 48, 72, and 96 hours). The results show a significant increase in collagen yield with longer extraction times. At 24 and 48 hours, the

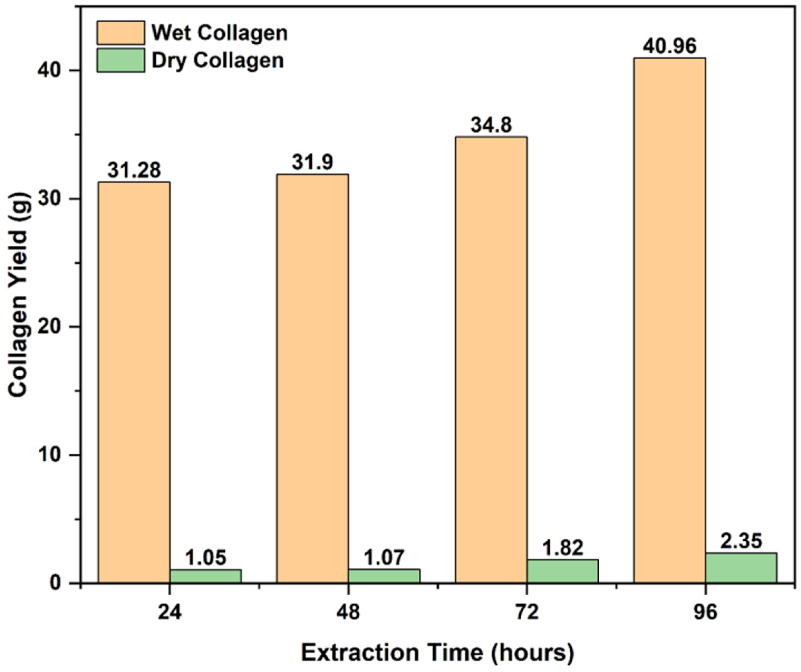


**Figure 2.** The yield of wet and dry collagen from tuna bone

**Table 1.** The yield of collagen from tuna bone at different extraction times

| Marine sources | Part | Extraction time (h) | Yield (%) |
|---|---|---|---|
| Yellowfin tuna (*Thunnus albacares*) | Bone | 24 | 2.10 ± 0.28[a] |
| | | 48 | 2.14 ± 0.62[a] |
| | | 72 | 3.64 ± 0.22[b] |
| | | 96 | 4.70 ± 0.54[c] |

**Note:** values are given as the mean ± standard deviation from triplicate determinations ($n = 3$). Different superscript letters within the same column denote significant differences ($p < 0.05$).

collagen yield was relatively low, at 2.10% and 2.14%, respectively, with no significant difference between these two durations ($p > 0.05$). However, a substantial increase in yield was observed at 72 hours, reaching 3.64%, which was significantly higher than the previous extraction times ($p < 0.05$). The highest yield, 4.70%, was recorded after 96 hours of extraction, which was also significantly greater compared to the other time points ($p < 0.05$). This indicates that extending the extraction duration can enhance collagen recovery from yellowfin tuna bones, with 96 hours yielding the best results.

In this study, the collagen yield reached 4.70%, which was significantly greater than the yields from skipjack tuna bones (3.57%), catfish (0.28%), unicorn fish (0.40%), and lizardfish (1.73%) ($p < 0.05$), as indicated in Table 2. Several factors contribute to the higher collagen content in yellowfin tuna bones compared to those of other species. First, yellowfin tuna bones are larger and more structurally complex, offering more collagen-rich tissue. Additionally, yellowfin tuna are active, long-distance swimmers, requiring robust bones and muscles with increased collagen for greater strength and flexibility.

## FTIR analysis

The FT-IR spectra of commercial collagen and collagen extracted from yellowfin tuna bone, shown in Figure 3, reveal similar IR spectra for both samples. Both types of collagens displayed characteristic peaks associated with Amide I, II, III, as well as Amide A and B. The Amide A absorption feature, typically linked to N-H stretching vibration, usually appears within the wave number range of 3400–3440 $cm^{-1}$. In this study, the Amide A absorption peak for collagen extracted from tuna bone was observed at 3340 $cm^{-1}$. This shift to a lower frequency indicates the formation of hydrogen bonds involving the N-H group in the peptide. The Amide B peaks, indicating asymmetrical stretching of $CH_2$, were detected at 2932 $cm^{-1}$.

The characteristic absorption wave number for the Amide I bond, which falls within the range of 1600–1700 $cm^{-1}$ due to the C=O stretching vibration in the polypeptide backbone of proteins, is sensitive to changes in the protein's secondary structure. This makes it useful in protein secondary structure analysis. For collagen extracted from tuna bone, the Amide I absorption peak was observed at 1657 $cm^{-1}$. The Amide II peak, typically resulting from a combination of N-H in-plane bending and C-N stretching vibration, was detected at 1545 $cm^{-1}$. Amide III bands were identified at a wave number of 1240 $cm^{-1}$. This peak is complex, involving C-N stretching and N-H in-plane bending of amide linkages, as well as absorptions from $CH_2$ group wagging vibrations, the glycine backbone, and proline side-chains. The similarity in IR spectra between commercial collagen and

**Table 2.** Comparison the yield of collagen from tuna bone and othersv

| Marine sources | Part | Yield (%) | References |
|---|---|---|---|
| Yellowfin Tuna | Bone | 4.70 ± 0.54[a] | This study |
| Skipjack tuna | Bone | 3.57 ± 0.40[b] | Di et al., 2014 |
| Catfish | Bone | 0.28 ± 0.02[d] | Abbas et al., 2022 |
| Unicorn fish | Bone | 0.40 ± 0.15[d] | Fatiroi et al., 2023 |
| Lizardfish | Bone | 1.73 ± 0.08[c] | Jaziri et al., 2022 |

**Note:** values are given as the mean ± standard deviation from triplicate determinations ($n = 3$). Different superscript letters within the same column denote significant differences ($p < 0.05$).

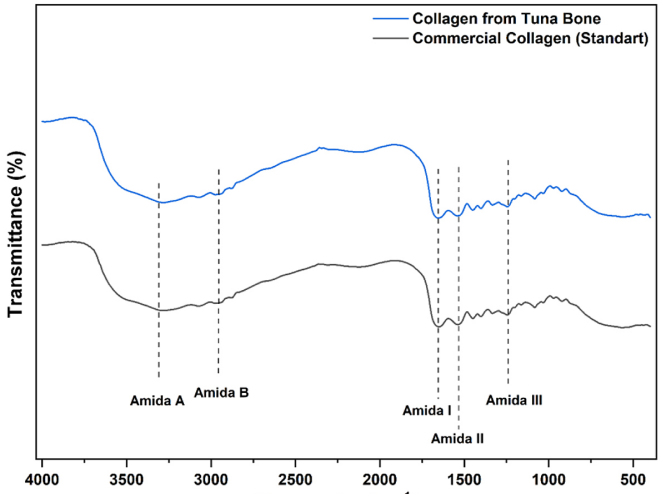


**Figure 3.** FTIR of commercial collagen and collagen extracted from tuna bone

collagen extracted from yellowfin tuna bone suggests that their structures are quite comparable.

## SEM analysis

The SEM image of collagen extracted from yellowfin tuna bones using acetic acid for 96 hours, as shown in Figure 4, reveals collagen sheets composed of fibrils and fibers, forming a dense, sheet-like network. The surface appears smooth, with some instances showing a layered structure due to the interconnecting of collagen fibers. Similar findings were reported by GÖÇER et al. (2024) and Rizk & Mostafa (2016), where SEM analyses demonstrated smooth or slightly wrinkled surfaces or sheet-like formations. Wang (2021) suggested that fish collagen, which is characterized by fibrillary, interconnected, and sheet-like film structures, holds potential as a biomaterial for applications in nutraceuticals, pharmaceuticals, and biomedicine. These uses include wound dressings, skin and bone tissue regeneration, cosmetics, cell migration, and coating materials.

## Proximate composition

Table 3 presents the proximate composition of collagen extracted from yellowfin tuna bones at four different extraction times: 24, 48, 72, and 96 hours, compared to the standards set by SNI 8076:2014 for food-grade collagen. The moisture content exhibited a marked decrease, starting at 12.8% at 24 hours and reducing to 4.2% by the 96-hour mark. This reduction was well within the SNI 8076:2014 standard of ≤ 12%. As noted by Rima (2017), the

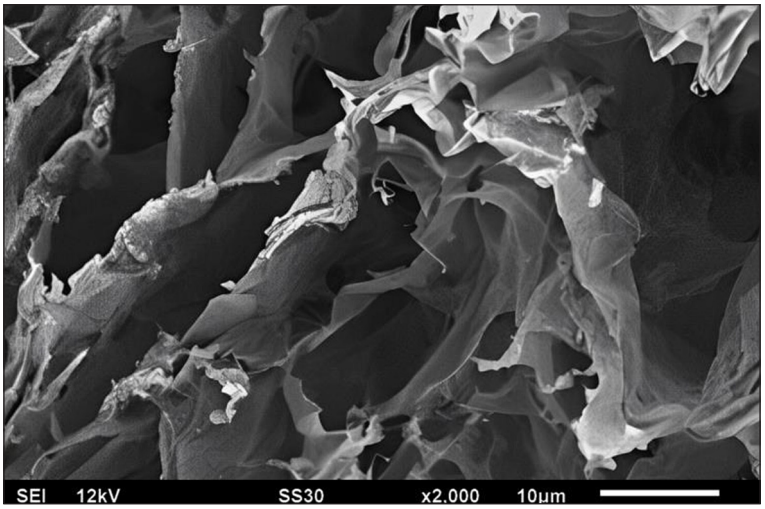


**Figure 4.** Morphological of collagen from yellowfin tuna bone

**Table 3.** Proximate composition of collagen from yellowfin tuna bone

| Parameter | Extraction time (h) | | | | SNI 8076:2014 |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | |
| Moisture content (%) | 12.8 | 10.2 | 6.6 | 4.2 | ≤ 12% |
| Protein content (%) | 75.4 | 78.6 | 82.4 | 85.2 | ≥ 75% |
| Lipid content (%) | 0.5 | 0.4 | 0.2 | 0.2 | ≤ 1% |
| Ash content (%) | 1.8 | 1.2 | 1.2 | 0.8 | < 5% |

loss of water during the drying phase and the absorption of water during soaking were critical factors influencing moisture levels in collagen. The improved quality of collagen correlated with reduced moisture content, which not only enhanced shelf life but also minimized microbial activity and mitigated unwanted enzymatic and chemical reactions that could impact the organoleptic properties of the final product (Mulyani et al., 2021).

The protein content showed a steady increase from 75.4% at 24 hours to 85.2% at 96 hours, exceeding the minimum requirement of 75% stipulated by SNI 8076:2014. This upward trend suggested that longer extraction times enhanced the yield of protein, likely due to prolonged exposure to acid, which facilitated more efficient collagen breakdown and recovery. The lipid content remained consistently low throughout the extraction periods, ranging from 0.5% at 24 hours to 0.2% at both 72 and 96 hours. This outcome satisfied the SNI requirement of ≤ 1%, indicating that the extraction process effectively eliminated fat, resulting in a quality collagen product. The relatively low lipid content in this study contrasted with previous research, which reported higher values of 2.69% for collagen derived from catfish skin (Suptijah et al., 2018) and 0.58–0.74% for collagen from tuna skin (Kusa et al., 2022). The pretreatment processes using NaOH and isopropyl alcohol contributed significantly to the fat removal, while the application of high temperatures during the drying phase further aided in reducing lipid content.

The highest ash content was observed at 1.8% during the 24-hour soaking period, progressively decreasing to 0.8% at 96 hours. The ash content reflected the mineral or inorganic components present in the collagen samples. The decrease in ash content over time signified a reduction in mineral impurities, which converted to ash during testing (Behera et al., 2018). Importantly, the ash content from all four soaking time variations complied with SNI 8076:2014, remaining below the threshold of 5%.

## CONCLUSIONS

This study successfully demonstrated the extraction of collagen from yellowfin tuna bones, revealing a significant increase in yield with extended extraction times. The highest yield of 4.70% was achieved after 96 hours of extraction using 0.75 M acetic acid, which surpassed the yields from other fish species and indicated the superior collagen content in yellowfin tuna bones. Proximate analysis showed that the extracted collagen met the SNI 8076:2014 standards, with moisture content decreasing to 4.2%, protein content increasing to 85.2%, and lipid content remaining consistently low at 0.2%. FTIR and SEM analyses confirmed the structural integrity and morphology of the extracted collagen, indicating its potential applications in various fields such as nutraceuticals, pharmaceuticals, and biomedicine. Overall, the results highlighted the viability of using yellowfin tuna bones as a valuable source of collagen, contributing to the sustainable utilization of marine resources.

### Acknowledgements

This research was supported and facilitated by Universitas Syiah Kuala and the Ministry of Education, Culture, Research, and Technology through the Lektor Kepala Research Grant Program for the 2024 fiscal year (Grant Number: 259/UN11.2.1/PG.01.03/SPK/PTNBH/ 2024).

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