Characterization of Collagen from Bone and Scales from White Sea Bass (*Lates calcarifer*)

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Abstract

Fishery by-products have become an increasingly valuable source of fish collagen as a substitute for mammalian collagen. This research aimed to analyze and compare bone and scale collagen isolated from white sea bass (Lates calcarifer) by acetic acid extraction. Bone and scale samples were deproteinized with sodium hydroxide, demineralized with EDTA, and extracted with 0.5 M acetic acid. The extraction solution was salted out with sodium chloride to precipitate collagen. The yield and various properties of the isolated collagen, including ninhydrin and Hopkins–Cole reactions, pH, moisture, UV–Vis absorption, and Fourier transform infrared (FTIR) spectra, were determined and compared with those of commercial collagen as a reference. The yields of collagen from both the scales and the bones were 0.15% and 0.18%, respectively. Both extracts tested positive for the ninhydrin reaction and negative for the Hopkins–Cole reaction. The pH values of both were 6.90 and 6.75, and the moisture content was 1.43% and 2.00% for bone and scale collagen, respectively. The UV–Vis spectra exhibited typical absorption in the region of 200–250 nm, and the characteristic Amide A, B, I, II, and III bands were recognized by FTIR analysis. These spectral features are indicative of peptides related to collagen in both extracts. Although the recovery was relatively low, the result showed that bones and scales of L. calcarifer could be used as an alternative source of marine collagen. Further modification of pretreatment and extraction parameters may be necessary for enhancing collagen extraction.

Keywords: acid extraction, fishery by-products, FTIR, marine collagen

INTRODUCTION

Collagen is a structural protein composed of three polypeptides (Gly-Pro-Hyp-rich a-chains) that form a triple helix, which is the basic molecular building block of collagen fibrils. These biochemical and structural processes explain why collagen has the potential to maintain the integrity of the extracellular matrix and function as a mediator of cell–matrix interactions (Goldberga *et al.*, 2018; Mienaltowski *et al.*, 2021). Collagen can be extracted from different animal sources, particularly mammalian skin (Noorzai *et al.*, 2019; Ahn *et al.*, 2021) and fish skin (Afifah *et al.*, 2023). Fish collagen is mainly type I collagen, which is the most abundant and structurally crucial collagen type in many tissues. It has attracted considerable interest because of its good biocompatibility, biodegradability, weak antigenicity, and abundant supply (Alves *et al.*, 2017; Carvalho *et al.*, 2018). Collagen can also be extracted from various fish processing by-products, such as skin and scales (Ampitiya *et al.*, 2022; Afifah *et al.*, 2023; Yamamoto & Tanaka, 2025), as well as from bones (Chinh *et al.*, 2019; Veronica *et al.*, 2025).

Collagen sourced from fish by-products has garnered significant attention as a viable alternative to mammalian collagens. Fish processing leads to substantial by-products in the form of heads, viscera, bones, skin, scales, fins, and meat remains, which together could represent 30–70% of the fish weight, based on species and processing procedure (Kandyliari *et al.*, 2020; Yang *et al.*, 2023). However, these by-products also contain structural proteins and high-value ingredients, which may be recovered by value-added processes to minimize waste formation and enhance the value of fishery resources (Boaventura *et al.*, 2020; Yamamoto & Tanaka, 2025). Collagen is one of the most fascinating biomolecules recovered among these various components because it is abundant in connective tissue, skin, bone, and fish scales and has a wide range of potential applications in the food, nutraceutical, cosmetic, pharmaceutical, and biomaterial industries (Alves *et al.*, 2017; Martins *et al.*, 2023; Yang *et al.*, 2023). Hence, collagen recovery from fish processing waste is not only a

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means of waste mitigation but also an upcycling process in which cheap waste biomass is converted into valuable raw materials for the sustainable development of marine resources.

Recent studies have identified the utilization of fishery processing by-products, such as skin, scales, and bones, as sources of marine collagen as becoming more attractive. These parts contain collagen with physicochemical properties that can be exploited for food, cosmetics, pharmaceuticals, and biomaterial applications (Pal & Suresh, 2017; Wu *et al.*, 2019; Waqar *et al.*, 2025). Some studies on different fish by-products have also indicated that collagen yield and properties may be affected by the source of the tissue. Collagen from the scales and bones of *Thunnus obesus* is type I collagen but exhibits variations in yield, amino acid composition, and solubility (Ahmed *et al.*, 2019). The acid extraction method is widely used to extract collagen from fishery processing industry waste. Several examples that have been reported include collagen from salmon (*Salmo salar*) and codfish (*Gadus morhua*) skin using the acid method (Alves *et al.*, 2017; Afifah *et al.*, 2023). The same method applies to needlefish skin (*Tylosurus acus melanotus*)(Ramle *et al.*, 2022), sardine scales (*Sardinella pilchardus*) (Filipe *et al.*, 2024), and sturgeon fish skin (*Huso huso*) (Atef *et al.*, 2020). Further investigations have been proposed to improve the extraction process, analyze the structural and functional properties of collagen from fish scales (Bavisetty *et al.*, 2024), and obtain collagen and bioactive collagen peptides from different fish resources (Chotphruethipong *et al.*, 2021; Liu *et al.*, 2025).

Although collagen from fish scales, bones, and skin has been widely investigated as an alternative source of marine collagen, its characteristics may vary depending on the tissue of origin. Recent studies have demonstrated the potential of *Lates calcarifer* processing by-products, particularly scales, as collagen sources. From these scales, acid- and pepsin-soluble collagens have been successfully extracted and characterized (Bavisetty *et al.*, 2024), and scale-derived collagen peptides have been investigated for value-added applications (Yang *et al.*, 2023). Similarly, collagen extracted from *L. calcarifer* skin-processing offcuts has been identified as type I collagen with a preserved triple-helical structure (Ampitiya *et al.*, 2022). However, comparative information on the characteristics of collagen from the bones and scales of *L. calcarifer* remains limited, particularly regarding the yield, amino acid-related reactions, physicochemical properties, UV–Vis absorption, and functional groups. Accordingly, this study aimed to characterize and compare collagen extracted from the bones and scales of *L. calcarifer* using an acetic acid extraction method based on extraction yield, ninhydrin and Hopkins–Cole reactions, pH, moisture content, UV–Vis absorption, and FTIR analysis. The findings are expected to provide comparative information on the characteristics of collagen from these two fish-processing by-products and their potential as alternative sources of marine collagen.

MATERI DAN METODE

The samples used in this study were the bones and scales of *L. calcarifer* obtained from a local fish market. The chemicals used included acetic acid (CH_3COOH), sodium hydroxide (NaOH), sodium chloride (NaCl), distilled water, and ethylene diamine tetraacetic acid (EDTA), all of which were of analytical grade. Additional reagents included ninhydrin solution (0.2–2% in ethanol, pro-analysis grade), phosphate buffer solution (pH 7), oxalic acid, magnesium powder, and concentrated sulfuric acid (H_2SO_4), all of which were of analytical grade. Commercial fish collagen was used as the reference sample. The equipment employed in this study included test tubes, drop pipettes, measuring cylinders, volumetric flasks, volumetric pipettes, a water bath, a centrifuge, an oven, and a UV–Vis spectrophotometer for collagen absorption spectra analysis. The samples analyzed comprised collagen solutions extracted from the bones and scales of *L. calcarifer and* commercial collagen, which was used as a reference.

Bone and scale samples of *L. calcarifer* were extracted and characterized for collagen content. Collagen extraction was performed using the acetic acid method, followed by several

characterization tests, including the ninhydrin test, Hopkins–Cole test, pH determination, water content analysis, UV spectrophotometry, and Fourier-transform infrared spectroscopy (FTIR).

Before extraction, the bones and scales of *L. calcarifer* were thoroughly rinsed with clean water to remove residual flesh and surface impurities. After cleaning, the samples were dried and weighed, yielding approximately 100 g of each material. Samples that were not processed immediately were stored in a freezer to prevent degradation. Before collagen extraction, the samples were deproteinized and demineralized to remove non-collagenous proteins and mineral components.

Deproteinization was performed by immersing the bone and scale samples in 0.1 M NaOH at a ratio of 1:10 (w/v) for 6 h, following the method described by Ramli *et al.* (2019). After treatment, the samples were rinsed three times with 200 mL of distilled water until clear. Demineralization was then performed to eliminate inorganic minerals by soaking the samples in 0.5 M EDTA at a 1:10 (w/v) ratio for 24 h at room temperature, as described by Pamungkas *et al.* (2018). Subsequently, the samples were filtered and rinsed three times with 200 mL distilled water until the pH approached neutrality.

The collagen extraction process was performed by immersing the demineralized samples in 0.5 M acetic acid (CH_3COOH) at a ratio of 1:10 (w/v) for 48 h at a low temperature (Ahmed *et al.*, 2019). After extraction, the mixture was filtered to obtain a supernatant containing dissolved collagen. The collagen extract was then treated with 2.6 M NaCl and allowed to stand for 24 h to induce collagen precipitation. Subsequently, the precipitated collagen was separated by centrifugation at 12,000 rpm for 30 min to remove any remaining solvent. Finally, the obtained collagen was dried in an oven at 40°C to yield dry collagen powder.

The obtained dry collagen was subsequently used to determine the yield, amino acid content, collagen quality, and absorption spectrum in ultraviolet (UV) and infrared (IR) regions. The amino acid compositions of bone and scale collagen were analyzed using the Ninhydrin and Hopkins–Cole tests, with commercial collagen serving as a comparison sample. The collagen yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{Total\ weight\ of\ dry\ collagen}{Total\ weight\ of\ dry\ sample} \text{ x } 100$$

The ninhydrin test was done on collagen samples to detect the presence of free amino groups. Each dry collagen sample was treated with 0.1 M NaOH and 1% ninhydrin solution, heated, and the resulting color change was noted. A positive reaction was indicated by the appearance of blue, purple, or pale yellow coloration, which proved the presence of amino acids in the sample. The Hopkins–Cole test was carried out for detection of tryptophan-type amino acids in the bone and scale collagen samples of *L. calcarifer*. The test was done by adding 1% oxalic acid, Mg powder, and concentrated sulfuric acid (H_2SO_4) to the sample. A purple ring at the interface of the two liquid layers showed a positive result for tryptophan (Subroto *et al.*, 2020).

The pH and water content results were used to evaluate the quality of bone collagen and scales. The pH of the collagen solution was determined by a digital pH meter as described by Ramli *et al.* (2019). The pH value indicates the acidity and potential solubility of collagen samples. The pH and water content test results were compared with collagen standards in accordance with SNI 2014. The water content was determined using a *drying oven* at 105°C until the sample weight was constant and calculated using the following formula:

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

Note: A = weight of the dried sample and container (g), B = weight of the sample and container before drying (g), C = weight of the empty dried container

The collagen spectrum under ultraviolet light was analyzed based on the results of the UV spectrophotometer test at a wavelength of 200–400 nm to determine the characteristic maximum absorption region (200–250 nm), which is typical of collagen. The collagen spectrum against infrared light *was* observed based on the test results of *Fourier Transform Infrared* (FTIR), which was measured at wavenumbers between 4000-400 cm^{-1}. This was done to observe the characteristic absorption bands: amide I (around 1650 cm^{-1}), amide II (around 1550 cm^{-1}), and amide III (around 1240 cm^{-1}), which indicate the presence of peptide bonds and preservation of the collagen triple-helix structure (Safithri *et al.*, 2019).

RESULTS AND DISCUSSION

Bone and scale samples of *L. calcarifer* were used as collagen sources. After extraction and drying, the bone-derived collagen appeared as a light brown powder, whereas the scale-derived collagen was beige with a finer texture. The visual differences between bone and scale collagen may indicate variations in composition and collagen yield, which were further analyzed through physicochemical and spectroscopic characterization of the samples. The process of extracting collagen from *L. calcarifer* bones and scales yielded dry collagen as shown in Figure 1.

Based on yield calculations, bone collagen exhibited a higher yield than scale collagen at 0.18% and 0.15%, respectively (Table 1). The higher collagen yield from bones may be attributed to their denser structure and higher collagen content than scales. Differences in collagen yield between tissues are also influenced by factors such as the degree of mineralization, protein crosslinking, and extraction process efficiency (Ramli *et al.*, 2019; Ahmed *et al.*, 2019). These results indicate that fish bones can serve as a more efficient source of collagen than fish scales, although both by-products remain valuable for further utilization.

Table 1. Collagen yield from *L. calcarifer* bone and scale

Sample	Dry sample weight (g)	Dry collagen weight (g)	Yield (%)
Bone	700	1.32	0.18
Scale	700	1.05	0.15

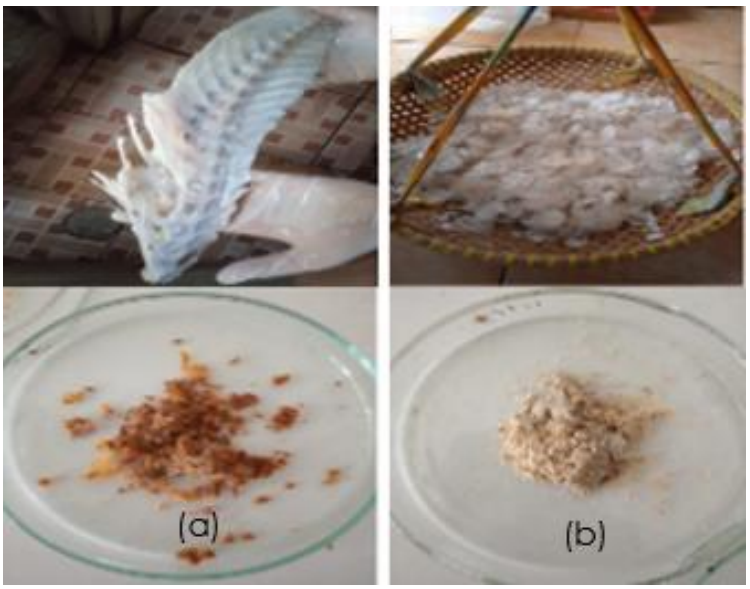


Figure 1. Appearance of the raw materials and extracted dry collagen from *L. calcarifer*: (a) bone samples and bone collagen, (b) scale samples and scale collagen

The ninhydrin test results (Table 2) showed positive reactions for collagen isolated from the bones and scales of *L. calcarifer,* confirming the presence of ninhydrin-reactive amino groups in the extracts. A typical positive ninhydrin reaction involves the synthesis of a blue-violet or purple color, termed Ruhemann's purple, due to the interaction of ninhydrin with primary amino groups. In contrast, amino acids with secondary amino groups, namely proline and hydroxyproline, tend to have yellow pigmentation (Stauß *et al.*, 2024). In this study, scale collagen had a darker purple color, whereas bone collagen had a paler purple color. The variation in color intensity could be due to differences in the levels of ninhydrin-reactive amino groups and/or types of amino compounds in the extracts. However, the intensity of color cannot be used to estimate the concentration or composition of amino acids in the shade. The commercial collagen was light yellow, which could be due to the retention of amino acids such as proline and hydroxyproline. Accordingly, the nitrogenous constituents of the marine collagen extracts demonstrated a positive ninhydrin reaction, substantiating the evidence supporting the existence of amino-based components in the collagen extracts.

The results of the Hopkins–Cole test (Table 2) showed that neither bone collagen nor scale collagen contained the amino acid tryptophan, as indicated by the absence of a purple ring in the reaction mixture. Similar results were obtained for the commercial collagen sample. The Hopkins–Cole test specifically detects the presence of tryptophan residues through the formation of a purple color resulting from the reaction between tryptophan and glyoxylic acid in concentrated sulfuric acid (Subroto *et al.*, 2020). The absence of color change in all the tested samples indicated that tryptophan was not present in *L. calcarifer* collagen, which is consistent with previous reports showing that collagen generally lacks aromatic amino acids such as tryptophan.

The results of the collagen quality tests are shown in Table 3. The pH of the bone, scale, and commercial collagen were 6.90, 6.75, and 6.23, respectively. These results showed that all collagen samples were in the range of pH permitted by Indonesian National Standard (SNI 8066:2014), i.e., the pH of collagen is ideal when in the range of 6.5-8.0. The pH values were close to neutrality, indicating that the collagen extracted had good chemical stability and low residual acid from extraction (Song *et al.*, 2019). Small differences in pH between samples might be attributed to variation in raw material and efficiency of the neutralization step in the purification.

Table 2. Qualitative tests for collagen identification

Test Type	Sample	Results	Observation
Ninhydrin	Bone collagen	Positive	Color changed from yellow to light purple
	Scale collagen	Positive	Color changed from yellow to light purple
	Commercial collagen	Positive	Color changed from yellow to light purple
Hopkins-Cole	Bone collagen	Negative	No purple ring observed
	Scale collagen	Negative	No purple ring observed
	Commercial collagen	Negative	No purple ring observed

Table 3. Physicochemical properties of collagen samples from *L. Calcarifer*

Parameter	Bone collagen	Scale collagen	Commercial collagen	Standard for collagen (SNI 8066 : 2014)
pH	6.90	6.75	6.23	6.5-8.0
Water content (%)	1.43	2.0	0.5	<12.0

Note: SNI = Indonesian National Standard

The water contents of bone, scale, and commercial collagen were 1.43, 2.0, and 0.5%, respectively, and they were all lower than the requirement of SNI 8066:2014; the standard was less than 12%. The low moisture content in the isolated collagen indicates good drying, and the collagen was more stable to microbial degradation. Collagen with a lower moisture content is beneficial for storage and further processing as it reduces the chances of enzymatic and microbial degradation. Although the commercial sample of collagen had the minimum moisture content, the bone and scale collagen also met the quality requirements, which means the processes of extraction and drying produced stable collagen with good physicochemical characteristics.

The UV–Vis absorption spectra of *L. calcarifer* bone collagen, scale collagen, and commercial collagen are shown in Figure 2. Bone collagen showed the highest absorption intensity in the 200–250 nm range, followed by scale and commercial collagen, which suggests a higher concentration or purity of collagen extracted from the bone. This wavelength region is where strong absorption occurs, which is due to peptide bonds (C=O and N–H), and is evidence of the proteinaceous nature of the extracted material in collagen molecules (Ahmed *et al.*, 2019; Dong & Dai, 2022; Mo *et al.*, 2023). Conversely, bone, scale, and commercial collagen samples did not show any prominent absorption peak near 280 nm, which means that the level of aromatic amino acid residues, especially tyrosine and tryptophan, which are the primary contributors to protein absorption in this region, is very low. Similar low or non-existent absorption at 280 nm has been reported for fish-derived collagens and is correlated with the relatively low levels of aromatic amino acids found in collagen (Ampitiya *et al.*, 2022; Chen *et al.*, 2021; Dong & Dai, 2022). The absence of absorption peaks above 280 nm means that aromatic amino acids, such as tryptophan, tyrosine, and phenylalanine, were either not present or were at such low concentrations that they did not show up, which agrees with the negative results of the Hopkins–Cole test.

Figure 3 presents the FTIR spectra of *L. calcarifer* bone collagen, scale collagen, and commercial collagen. Typical absorption peaks of collagen were detected in all samples, indicating that the usual amide functional groups to the peptide backbone of the proteins were present. The significant peaks detected were Amide A (about 3300–3400 cm^{-1}), Amide B (at 2930 cm^{-1}), Amide I (1650–1660 cm^{-1}), Amide II (1550–1560 cm^{-1}), and Amide III (1230–1240 cm^{-1}), which correspond to the expected spectrum for type I collagen (Kudo *et al.*, 2017; Chen *et al.*, 2021; Ampitiya *et al.*, 2022).

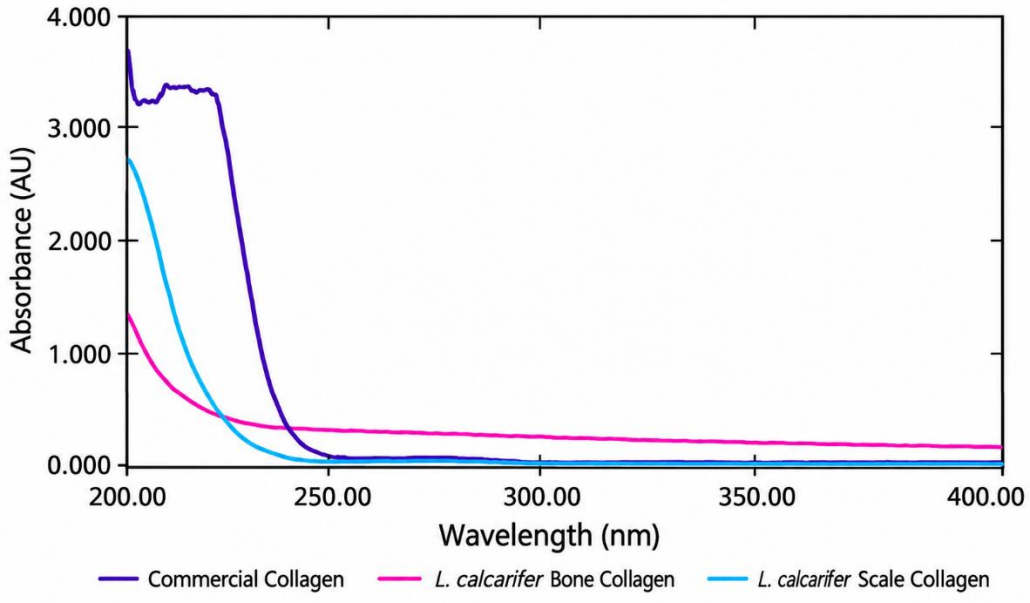


Figure 2. UV absorption spectra of bone collagen, scale collagen, and commercial collagen

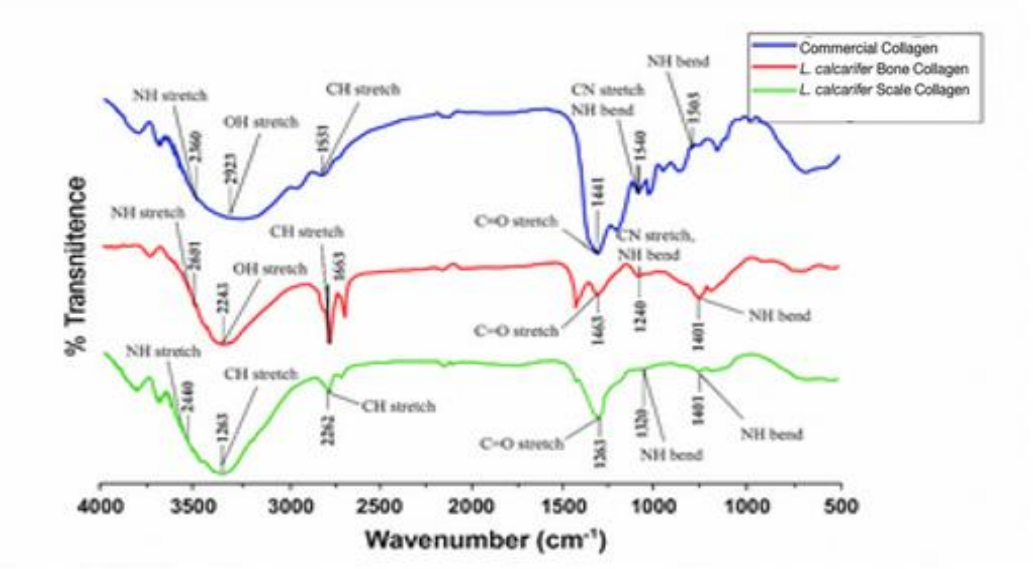


Figure 3. FTIR spectra of bone collagen, scale collagen, and commercial collagen

The Amide A band is related to N–H stretching vibrations with the formation of hydrogen bonding, and the Amide B band is related to the asymmetric CH_2 stretching. Amide I is primarily related to the C=O stretching vibrations of peptide bonds, and the secondary structure of collagen mainly affects the Amide I band, whereas Amide II is due to N–H bending and C–N stretching. Amide III is associated with C–N stretching and N–H deformation vibrations, confirming the helical nature of the collagen triple helix (Ahmed *et al.*, 2019). The characteristic peaks also present in bone and scale collagen indicate that the triple helical structure of collagen was maintained after the extraction. The slight shifts in wavenumber and intensities relative to the commercial collagen could be attributed to variations in percentage of amino acid compositions, hydrogen bonding strength, and molecular arrangement (Schwaighofer *et al.*, 2021; Kotov *et al.*, 2025). These findings indicate that the use of acetic acid to extract collagen did not cause marked changes in the primary structure of collagen and that L. calcarifer bone and scale collagen in this study maintained their structural integrity and purity. These slight differences in wavenumber among commercial collagen, bone collagen, and scale collagen may be due to differences in the peptide chain molecular environment, hydrogen bonding interactions, and composition or organization of collagen derived from different tissues. In particular, changes in the Amide A region are affected by hydrogen bonding with N–H groups. Similar variations in amide band positions have been observed among collagen samples extracted from various fish tissues and species (Ahmed *et al.*, 2019; Ampitiya *et al.*, 2022).

In general, collagen derived from bones and scales of Lates calcarifer in this research presented physicochemical and structural properties similar to those of collagens obtained from other fish by-products; however, the yield of extraction was relatively low. The extraction yields from the bone and scales amounted to 0.18% and 0.15%, respectively, which were low compared to the 0.38% acid-soluble collagen from *L. calcarifer* scales reported by Chuaychan *et al.* (2015), and markedly low in contrast to the 2.12–4.62% acid-soluble collagen yields from *L. calcarifer* scales through ultrasound-assisted pretreatment or extraction (Bavisetty *et al.*, 2024). The differences in yields in the present study may be attributed to differences in extraction methods, i.e., the traditional acetic acid extraction without enzymatic or ultrasound-assisted methods. The extent to which collagen can be recovered is also largely dependent on the source tissue composition and mineralization, conditions of pretreatment, acid concentration, time, and temperature of extraction (Gaikwad & Kim, 2024). However, the relatively low yield of isolated collagen did not seem to affect the physicochemical and structural properties of collagen. Bone and scale collagen had pH values of 6.90 and 6.75, respectively, which were in line with SNI 8066:2014, and their low moisture content

(1.43% and 2.00%) implied excellent drying and storage stability. Therefore, although the extraction yield obtained in this study was lower than that reported in several previous studies, the extracted collagen demonstrated appropriate physicochemical properties and preserved characteristic collagen structures. These results indicate that both bone and scale by-products of *L. calcarifer* have potential as alternative sources of value-added marine collagen, while further optimization of pretreatment and extraction conditions is recommended to improve collagen recovery.

CONCLUSIONS

The acetic acid-soluble collagen isolated from *Lates calcarifer* scales and bones exhibits the usual physicochemical and structural features of fish collagen. The extraction yield was 0.18% for bone and 0.15% for scales. Both collagens were ninhydrin-positive and Hopkins - Cole negative, which means there were amino-containing compounds and no tryptophan could be detected. The collagens were claimed to have a practically neutral pH of 6.90 and 6.75, low moisture content of 1.43% and 2.00% for the bone and scales, respectively, which fulfilled the requirements established in SNI 8066:2014. UV-Vis absorption of native collagen exhibited typical absorption below 250 nm, but FTIR spectra revealed characteristic Amide A, B, I, II, and III bands indicating that native collagen structure and triple helical conformation were preserved during extraction. *L. calcarifer* bone and scale by-products also provide a starting point for sourcing marine collagen with enhanced value. It is recommended to further improve the pretreatment and extraction process in order to improve collagen recovery.

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