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Characterization of Collagen Isolated from Tuna (Thunnus sp.) Skin Originating from Bitung and Vietnam Using FTIR and SDS-PAGE in Comparison with Standard Collagen

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ABSTRACT

Tuna (Thunnus sp.) processing generates large volumes of skin as a by-product that remains underutilized, whereas fish skin is a promising raw material for collagen. This study aimed to characterize collagen isolated from tuna skin originating from two sources, Bitung (North Sulawesi, Indonesia) and Vietnam, and to compare it with standard collagen in order to confirm its suitability. Collagen was isolated from the skins and obtained as a powder, then analyzed using Fourier Transform Infrared (FTIR) spectroscopy to identify functional groups and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) to determine the molecular weight profile. FTIR spectra of all samples exhibited the characteristic collagen amide bands, namely Amide A (~3282-3416 cm-1), Amide B, Amide I (~1630-1647 cm-1), Amide II (~1514-1551 cm-1), and Amide III (~1240 cm-1), confirming a preserved triple-helix structure. A lipid ester band near 1742 cm-1 appeared in the raw skin samples but was absent in the collagen powder and the standard, indicating effective purification. The band positions of the isolated collagen were consistent with those of the standard collagen. SDS-PAGE revealed protein bands within the range of the 5-245 kDa marker, corresponding to collagen chains. The results indicate that collagen isolated from both Bitung and Vietnam tuna skins is comparable to standard collagen and is suitable for further utilization.

INTRODUCTION

Tuna (Thunnus sp.) is one of the most important fishery commodities in Indonesia and a major export product, with processing carried out on a large scale in production centres such as Bitung, North Sulawesi. Tuna processing, however, produces substantial amounts of by-products, particularly skin, bones, and heads, which are often discarded or sold at very low value (Nagai & Suzuki, 2000). Fish skin alone may account for a significant portion of the processing waste, and its accumulation raises both economic and environmental concerns. Converting this by-product into higher-value products is therefore in line with the principles of sustainable and zero-waste fisheries.

Fish skin is a promising raw material for collagen because it is rich in structural protein and is free from the risk of transmissible diseases associated with mammalian sources (Kittiphattanabawon et al., 2005). Collagen is widely used in the food, pharmaceutical, cosmetic, and biomedical industries as a gelling agent, emulsifier, carrier, and biomaterial (Silvipriya et

al., 2015). Growing consumer preference for non-mammalian and halal-compliant collagen has further increased the demand for collagen derived from aquatic sources, making fish skin collagen an attractive alternative to bovine and porcine collagen.

Before collagen from a new source can be utilized, its identity and quality must be verified through characterization. Fourier Transform Infrared (FTIR) spectroscopy is commonly used to identify the functional groups of collagen and to assess the integrity of its triple-helical structure through the characteristic Amide A, B, I, II, and III absorption bands (Muyonga et al., 2004; Doyle et al., 1975). Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) complements this by resolving the protein sub-units according to molecular weight, allowing the collagen type to be inferred from the presence of α- and β-chains (Laemmli, 1970; Sinthusamran et al., 2013).

Although collagen has been characterized from the skin of various fish species, comparative information on collagen isolated from tuna skin of different geographical origins, benchmarked against a standard collagen, is still limited. Such a comparison is important to confirm that the isolated product is genuine collagen with quality comparable to a reference material. Therefore, this study aimed to characterize collagen isolated from tuna (Thunnus sp.) skin originating from Bitung and Vietnam using FTIR and SDS-PAGE, and to compare its characteristics with those of standard collagen.

METHOD

Time, Location, and Materials

Analyses were conducted in May 2026. FTIR spectra were recorded at the Laboratory of the Faculty of Pharmacy, Universitas Airlangga, while SDS-PAGE analysis was performed at the Institute of Tropical Disease (ITD), Universitas Airlangga. The main materials were tuna (Thunnus sp.) skins obtained from two sources, namely Bitung (North Sulawesi, Indonesia) and Vietnam. A commercial standard collagen was used as a comparison (reference) material.

Collagen Isolation

Collagen was isolated from cleaned tuna skins from each source and subsequently obtained in powder form. The collagen powder produced from the tuna skins was used as the test sample and was analyzed together with the raw skin samples and the standard collagen.

FTIR Analysis

Functional groups of the samples were identified using a Bruker FTIR spectrophotometer operated with OPUS 8.1.29 software. Spectra were acquired in transmittance mode over the wavenumber range of approximately 4000-400 cm-1 at a resolution of 4 cm-1, using Happ-Genzel apodization with three scans per measurement. The absorption peaks obtained for each sample (tuna skin from Bitung, tuna skin from Vietnam, collagen powder, and standard collagen) were recorded and assigned to their corresponding functional groups, focusing on the characteristic collagen bands of Amide A, Amide B, Amide I, Amide II, and Amide III. Band positions of the isolated collagen were compared with those of the standard collagen to evaluate their conformity.

SDS-PAGE Analysis

The molecular weight profile of the samples was determined by SDS-PAGE. Protein was first isolated using RIPA lysis buffer. Separating and stacking gels were prepared, and each sample was mixed with loading buffer before being loaded onto the gel. Electrophoresis was run at 100 volts for 90 minutes. A Prestained Protein Ladder with an extra-broad molecular weight range (5-245 kDa) was used as the marker. After the run, the resulting protein bands were compared with the marker to estimate the molecular weight of the sub-units and to identify the collagen chains.

Data Analysis

Data were analysed descriptively. FTIR spectra were interpreted by comparing the positions of the absorption bands among samples and with reference values reported in the

literature, while the SDS-PAGE banding pattern was interpreted by comparison with the molecular weight marker. The characteristics of the collagen isolated from the two tuna skin sources were then compared with those of the standard collagen to determine their suitability.

RESULT AND DISCUSSION

FTIR Spectra and Functional Groups

The FTIR spectra of all samples displayed the absorption bands characteristic of collagen. The positions of the main functional groups for the tuna skin from Bitung, tuna skin from Vietnam, the isolated collagen powder, and the standard collagen are summarized in Table 1.

Table 1. FTIR absorption bands (cm-1) and functional group assignments of the samples

Functional group (assignment)	Tuna skin (Bitung)	Tuna skin (Vietnam)	Collagen powder	Standard collagen
Amide A (N–H stretching)	3282.44	3292.49	3301.45	3415.93
Amide B / C–H stretching	2926.27	2960.58	3086.91	3082.25
CH2 stretching	2854.71	2853.26	2960.50	2958.80
Lipid ester (C=O)	1742.45	1742.54	–	–
Amide I (C=O stretching)	1635.33	1634.90	1630.29	1647.21
Amide II (N–H bend, C–N)	1551.32	1550.58	1514.63	1541.12
CH2 bending	1456.23	1455.90	1442.12	1450.47
Amide III (C–N, N–H)	1241.11	1240.63	1232.97	1244.09

Note: (–) indicates that the band was not detected.

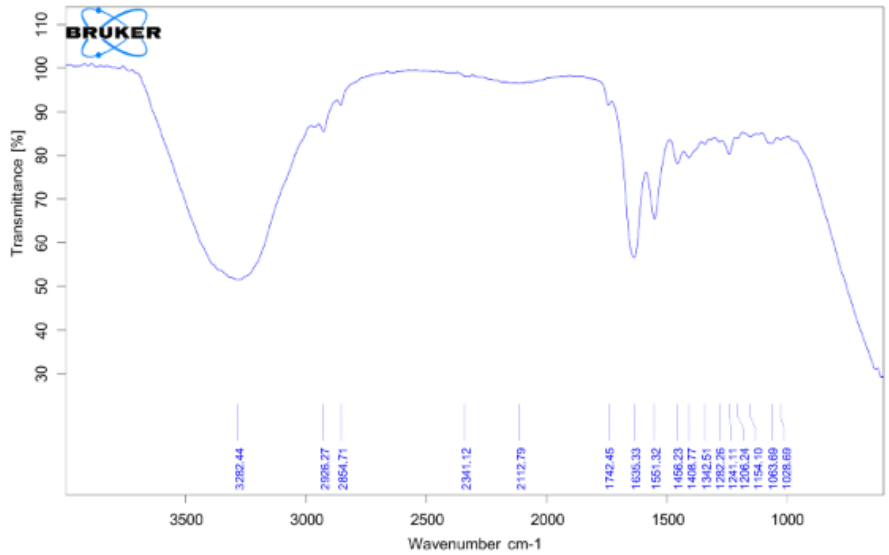
The Amide A band, which arises from N–H stretching vibrations, appeared at 3282.44 and 3292.49 cm-1 for the Bitung and Vietnam skins respectively, at 3301.45 cm-1 for the collagen powder, and at 3415.93 cm-1 for the standard collagen. The lower Amide A wavenumbers observed in the tuna skin and collagen powder samples indicate stronger hydrogen bonding of the N–H groups within the collagen structure, whereas the higher value in the standard reflects comparatively freer N–H groups (Muyonga et al., 2004). The Amide B band associated with C–H and asymmetric CH2 stretching was also detected in all samples.

The Amide I band, originating mainly from C=O stretching along the polypeptide backbone, is the most sensitive indicator of the secondary structure of collagen. It was observed at 1635.33 cm-1 (Bitung), 1634.90 cm-1 (Vietnam), 1630.29 cm-1 (collagen powder), and 1647.21 cm-1 (standard). The position of this band within the 1630-1660 cm-1 range confirms the presence of a triple-helical structure in all samples (Payne & Veis, 1988). The Amide II band, resulting from N–H bending coupled with C–N stretching, and the Amide III band near 1240 cm-1 were likewise present in every sample. The clear appearance of the Amide III band at 1241.11, 1240.63, 1232.97, and 1244.09 cm-1 indicates that the triple-helical conformation of the collagen was preserved and not denatured into gelatin (Doyle et al., 1975; Kittiphattanabawon et al., 2005).

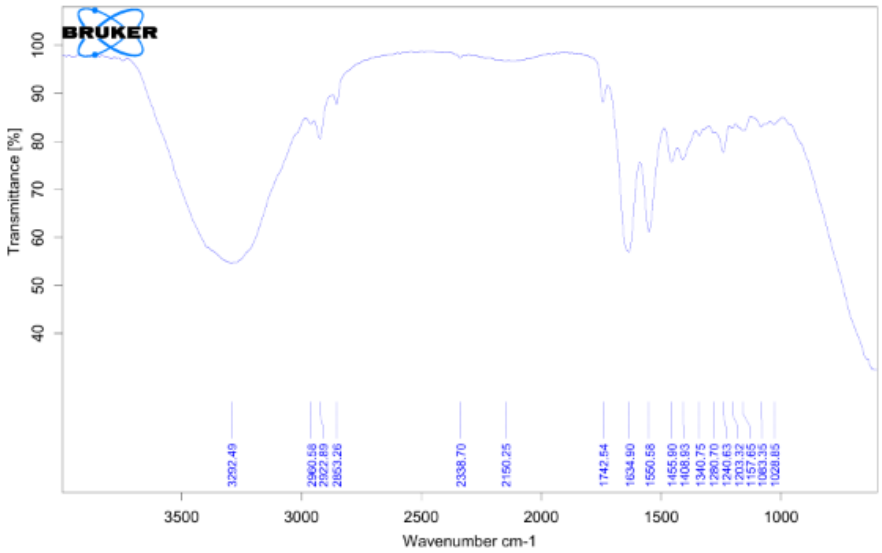
A notable difference between the samples was the lipid ester band at approximately 1742 cm-1, which appeared in the raw tuna skin samples from Bitung (1742.45 cm-1) and Vietnam (1742.54 cm-1) but was absent in both the collagen powder and the standard collagen. This band is attributed to the C=O stretching of lipid esters and reflects residual fat still present in the raw skin. Its disappearance in the collagen powder indicates that the isolation process effectively removed the lipid fraction, yielding a purer collagen comparable to the standard (Nagai & Suzuki, 2000).

Overall, the band positions of the collagen powder isolated from the tuna skins were consistent with those of the standard collagen across the Amide A, B, I, II, and III regions. The similarity of these characteristic bands confirms that the isolated material is genuine collagen and that collagen from both the Bitung and Vietnam tuna skins conforms to the standard collagen. The complete FTIR spectra of the four samples are presented in Figure 1.

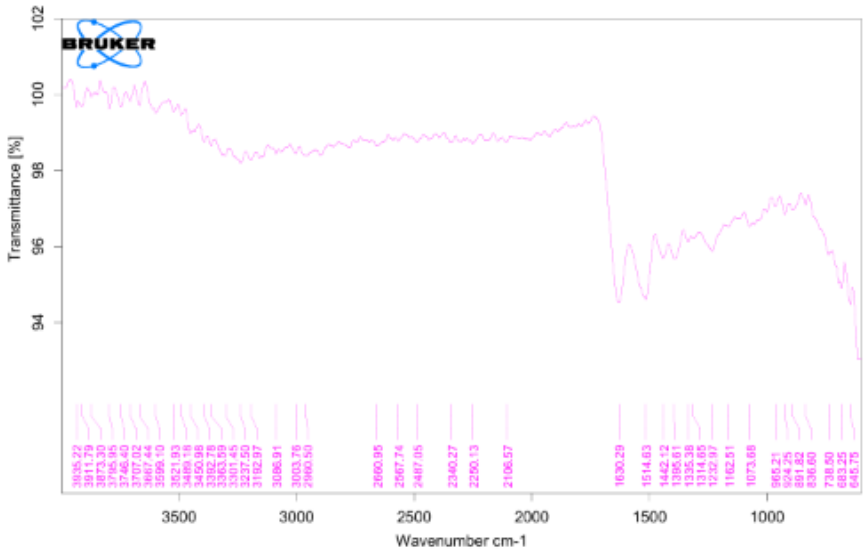
(a) Tuna skin from Bitung



(b) Tuna skin from Vietnam



(c) Collagen powder



(d) Standard collagen

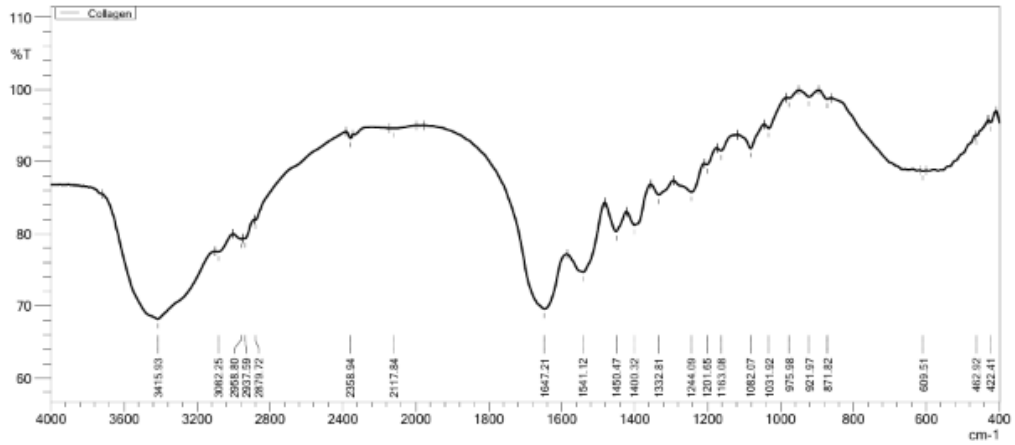


Figure 1. FTIR spectra of the samples (Transmittance [%] versus wavenumber, cm-1, over ~4000-400 cm-1): (a) tuna skin from Bitung, (b) tuna skin from Vietnam, (c) collagen powder, and (d) standard collagen.

Molecular Weight Profile by SDS-PAGE

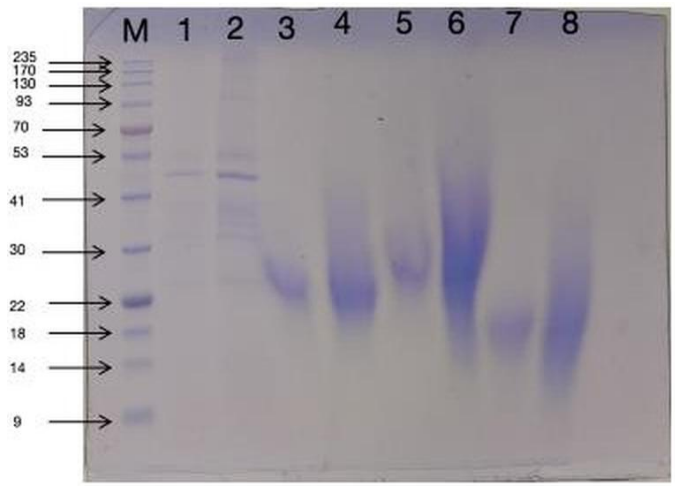


Figure 2. SDS-PAGE profile of the samples. M = prestained protein marker (5-245 kDa); lanes 1-8 correspond to the analysed samples.

The banding pattern of the isolated collagen samples corresponded to that expected for collagen chains and was comparable among the different sources, supporting the conclusion drawn from the FTIR data that collagen from the Bitung and Vietnam tuna skins is comparable to the standard collagen. Minor differences in band intensity between samples may be related to differences in the concentration and purity of the extracted protein.

Conformity of the Isolated Collagen with the Standard

Taken together, the FTIR and SDS-PAGE results consistently indicate that the collagen isolated from tuna (Thunnus sp.) skin, both from Bitung and from Vietnam, exhibits the characteristic functional groups and molecular weight profile of collagen and is comparable to the standard collagen. The preserved Amide bands, the removal of residual lipids after isolation, and the protein banding pattern collectively confirm the suitability of tuna skin from both origins as a raw material for collagen production. These findings reinforce the potential of tuna processing by-products as a sustainable source of collagen for food, pharmaceutical, and cosmetic applications.

CONCLUSION

Collagen isolated from tuna (Thunnus sp.) skin originating from Bitung and Vietnam was successfully characterized using FTIR and SDS-PAGE. The FTIR spectra of all samples showed the characteristic collagen Amide A, B, I, II, and III bands, indicating a preserved triple-helical structure, while the lipid ester band present in the raw skins disappeared in the collagen powder, confirming effective purification. The band positions of the isolated collagen were consistent with those of the standard collagen, and the SDS-PAGE banding pattern corresponded to collagen chains within the 5-245 kDa marker range. It can be concluded that collagen from both tuna skin sources is comparable to standard collagen and is suitable for further utilization.

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