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CHARACTERIZATION OF FISH SKIN FROM DEMERSAL SPECIES AS POTENTIAL COLLAGEN SOURCES

KARAKTERISTIK KULIT IKAN DEMERSAL SEBAGAI SUMBER KOLAGEN

Mala Nurilmala[1*], Nurafi Razna Suhaima[2], Vania Mahardika[1], Agy Wirabudi Pranata[3]
[1]Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, IPB University, Jl. Agatis, IPB Dramaga Campus, Bogor 16680, Indonesia
[2]Study Program of Food Quality Assurance Supervisor, School of Vocational Studies, IPB University, Jl. Kumbang No. 14, Bogor 16128, Indonesia
[3]PT Geltech Prima Indonesia, Jl. Hanamaru No. 1, Ciapus, Kecamatan Ciomas, Bogor 16610, Indonesia
*Corresponding author: mnurilmala@apps.ipb.ac.id

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ABSTRACT

The utilization of fishery by-products, particularly fish skin, offers a promising alternative source of halal and safe collagen and gelatin. This study aims to evaluate the characteristics of fish skin from demersal species as potential collagen sources. The fish skins used in this study were from purple-spotted bigeye (*Priacanthus tayenus*), lattice monocle bream (*Scolopsis taenioptera*), and Bleeker's grouper (*Epinephelus bleekeri*). This study employed an experimental and exploratory approach. Analyses included histological observation, proximate composition, amino acid profile, fatty acid profile, and heavy metal content. Histological observations revealed that fish skin was dominated by the dermal layer with well-defined collagen fiber structures, with the thickest collagen layer observed in grouper skin. Proximate analysis indicated that the fish skin samples were characterized by high moisture content (61.05–66.99%) and protein (17.88–20.87%) (wet basis). The amino acid profile showed glycine (3.99% w/w) and alanine (3.15% w/w) as the predominant amino acids, which are characteristic of collagen proteins. Hydroxyproline analysis further confirmed collagen presence, showing an increase from 0.0739 µg/µL in raw skin to 0.4071 µg/µL in gelatin. The fatty acid profile of the mixed sample indicated total detected fatty acid content (55.43% w/w), dominated by saturated fatty acids. Heavy metal analysis showed very low concentration. These findings indicate that fish skin from purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper has strong potential as an alternative, safe, halal, and value-added source of collagen and gelatin.

Keywords: amino acids, collagen, demersal fish, fish skin, gelatin

ABSTRAK

Pemanfaatan hasil samping perikanan, khususnya kulit ikan, menawarkan alternatif sumber kolagen dan gelatin yang halal dan aman. Penelitian ini bertujuan untuk mengevaluasi karakteristik kulit ikan demersal sebagai sumber kolagen potensial. Kulit ikan yang digunakan yaitu ikan swanggi (*Priacanthus tayenus*), coklatan (*Scolopsis taenioptera*), dan kerapu (*Epinephelus bleekeri*). Penelitian ini menggunakan pendekatan eksperimental dan eksploratif. Analisis yang dilakukan meliputi pengamatan histologi, komposisi proksimat, profil asam amino, profil asam lemak, dan kandungan logam berat. Hasil pengamatan histologi menunjukkan bahwa kulit ikan didominasi oleh lapisan dermis dengan struktur serat kolagen yang jelas, dengan lapisan kolagen paling tebal ditemukan pada kulit ikan kerapu. Analisis proksimat menunjukkan bahwa kulit ikan memiliki kadar air tinggi (61,05–66,99%) dan protein (17,88–20,87%) (basis basah). Profil asam amino menunjukkan bahwa glisin (3,99% b/b) dan alanin (3,15% b/b) merupakan asam amino dominan, yang merupakan karakteristik protein kolagen. Analisis hidroksiprolin turut mengonfirmasi keberadaan kolagen, dengan kadar yang meningkat dari 0,0739 µg/µL pada kulit menjadi 0,4071 µg/µL pada gelatin. Profil asam lemak sampel mix menunjukkan kandungan lipid total yang rendah (55,43% b/b). Analisis logam berat menunjukkan kadar yang sangat rendah, dengan arsen (As) sebesar 0,01–0,02 mg/kg, sedangkan merkuri (Hg), kadmium (Cd), dan timbal (Pb) berada di bawah batas. Temuan ini menunjukkan bahwa kulit ikan swanggi, coklatan, dan kerapu memiliki potensi yang kuat sebagai sumber alternatif kolagen dan gelatin yang aman, halal, dan bernilai tambah.

Kata kunci: asam amino, gelatin, ikan demersal, kolagen, kulit ikan

INTRODUCTION

Collagen is an important commodity in various industries in Indonesia due to its extensive applications in the food, cosmetic, pharmaceutical, and healthcare sectors. Collagen is a natural protein found in animals, and the term "collagen" is derived from the ancient Greek words "kolla" and "genes", which literally mean "glue producer" (Dondero *et al.* 2025). The global market trend indicates an increasing demand for collagen. In Indonesia, collagen supply remains highly dependent on imports from countries such as China, Thailand, Brazil, Bangladesh, India, New Zealand, Japan, and Germany (BPS 2024). In addition, the primary raw materials used in conventional collagen production are generally derived from mammalian skin and bones, particularly porcine and bovine sources (Nurilmala *et al.* 2019).

The use of collagen derived from porcine and bovine sources raises several concerns. For Muslims, porcine-derived materials are classified as *haram*, while pork-derived ingredients are also prohibited under Jewish kosher dietary laws. In addition, the consumption of cattle-derived products may be restricted among many Hindus because cows are regarded as sacred in Hindu traditions. Beyond religious considerations, some consumers are also concerned about the safety of bovine-based collagen due to the potential transmission of diseases such as mad cow disease and foot-and-mouth disease, which have previously been identified in several collagen-exporting countries (Alves *et al.* 2017). Furthermore, the implementation of the Indonesian Halal Product Assurance Law Number 33 of 2014 has strengthened the urgency of providing collagen raw materials with clear halal certification, particularly for food, pharmaceutical, and cosmetic products. These conditions emphasize the necessity to explore alternative collagen sources that are halal, safe, and acceptable to diverse consumer groups.

One potential alternative source of collagen is the fishery industry by-products. In addition to fish meat, body parts such as skin, bones, scales, and heads, which are categorized as by-products, may account for approximately 30–40% of the total fish weight (Hadinoto and Idrus 2018). Along with the rapid growth of the fisheries industry in Indonesia, the availability of fishery by-products has increased substantially. Although a large proportion of these by-products is currently utilized for low-value products, such as fish meal and fish oil, their application in high-value products remains limited. Consequently, fish skin and other processing by-products have considerable potential to be further valorized as raw materials for collagen production (Nurilmala *et al.* 2019).

Indonesia's total fisheries production in 2020 reached 21,834,106.56 tons, consisting of both pelagic and demersal fish catches. Demersal fish, which inhabit and are associated with the seabed, represent one of the dominant fish groups caught in the Java Sea and possess significant economic value. Several major demersal fish species captured in Indonesian waters in 2020 included purple-spotted bigeye (*Priacanthus tayenus*) at 34,334.77 tons, lattice monocle bream (*Scolopsis taenioptera*) at 2,581.98 tons, and Bleeker's grouper (*Epinephelus bleekeri*) at 120,302.24 tons (Statistical Data and Information Center, Ministry of Marine Affairs and Fisheries 2022). The high production volume of demersal fish directly contributes to the increasing amount of fishery by-products, particularly fish skin, which has considerable potential as a collagen raw material. Based on the separation process conducted in this study, the proportion of scale-free skin obtained from scaled fish skin varied among species, reaching 54.8% in purple-spotted bigeye, 44.7% in lattice monocle bream, and 62.2% in grouper.

On the other hand, data from the Ministry of Marine Affairs and Fisheries (KKP) in 2024 showed that Indonesia has 4,776,480 marine and fisheries business operators, with the number of Fish Processing Units ("*Unit Pengolahan Ikan*"-UPI) reaching 70,052 units. However, most of these processing units have not utilized fish skin as a raw material for value-added products, thereby potentially contributing to environmental problems due to the accumulation of organic waste. Fish skin generated from processing activities is generally underutilized.

Fish skin is recognized as a promising source of collagen and has been widely reported as a raw material for gelatin and collagen production with favorable functional characteristics. Many studies have shown that fish skin is a rich source of collagen with biochemical properties that make it useful for food, medicine, and biomaterials (Devita *et al.* 2021; Stone *et al.* 2021; Nurilmala *et al.* 2022). In addition, the development of gelatin extraction technologies from fish skin continues to advance to improve product quality and process efficiency (Nurilmala *et al.* 2023, 2025). Therefore, this study utilized fish processing industry waste in the form of skins from purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper, which are categorized as

bycatch fish species. The selection of these raw materials is expected not only to provide added value to fishery waste but also to support the development of halal, safe, and sustainable gelatin sources based on local resources.

METHODS

Materials and tools

The main materials used in this study were scaled skins of purple-spotted bigeye (*Priacanthus tayenus*), lattice monocle bream (*Scolopsis taenioptera*), and Bleeker's grouper (*Epinephelus bleekeri*), which were obtained as fishery bycatch species. The fish skin samples were collected, packed under chilled conditions in closed containers, and transported to the laboratory for further preparation. Upon arrival at the laboratory, the samples were washed thoroughly under running water to remove impurities and subsequently stored at a low temperature before analysis.

Supporting materials used in this study included distilled water, sodium dodecyl sulfate (Sigma-Aldrich, USA), and double-distilled water (ddH_2O). The equipment employed consisted of an analytical balance (Adventurer, Ohaus), beakers (Pyrex), a vortex mixer, a microscope (Olympus CX23), and a spectrophotometer (Rayleigh).

Sample preparation and analysis

The raw materials were initially prepared by removing adhering impurities and fat under running water. The cleaned fish skins were subsequently subjected to chemical composition analysis (AOAC 2005), histological observation using the Masson's trichrome method (Sheehan and Hrapchak 1980) as modified by Suvik and Effendy (2012), amino acid analysis (Waters Corporation 2012), fatty acid analysis, and heavy metal analysis.

Chemical composition analysis

Chemical composition analysis (AOAC 2005) was conducted to determine the moisture, ash, fat, protein, and carbohydrate contents of purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skin raw materials.

Histological observation

Histological observation of fish skin tissue was performed using the Masson's trichrome method (Sheehan and Hrapchak 1980) as modified by Suvik and Effendy (2012). The preparation of histological slides from the scaled skin tissues of purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper involved several stages, including tissue collection (sampling), fixation using 10% neutral buffered formalin, through a graded ethanol series (70%, 80%, 90%, 95%, and absolute ethanol), clearing in xylene for 30 minutes, impregnation in paraffin for 1 h, followed by tissue embedding in paraffin. The paraffin blocks were then sectioned using a microtome (CUT 4050, microTec Laborgeräte GmbH, Germany) at a thickness of 5 µm.

The Masson's trichrome staining procedure consisted of several steps, including deparaffinization in xylol for 3 min, immersion in Bouin's fixative solution (1 h at 56 °C), staining with Weigert's iron hematoxylin solution (10 min), immersion in Biebrich scarlet-acid fuchsin solution (15 min), immersion in phosphomolybdic–phosphotungstic acid solution followed by aniline blue solution (15 min), and subsequent immersion in acetic acid solution. The slides were then dehydrated in alcohol, cleared in xylol for 1 min, and mounted using Entellan®.

Histological observations were conducted using a microscope equipped with Indomacroview software at 40× magnification with a 100 µm scale bar. The collagen fiber area was quantified using the ImageJ application.

Amino acid analysis

The amino acid composition of fish skin was determined using an Ultra Performance Liquid Chromatography (UPLC) system equipped with a fluorescence detector (FLD). A total of 0.1 g of sample was placed into a screw-cap tube, followed by the addition of 5 mL of 6 N HCl. The mixture was hydrolyzed in an oven at 110 °C for 22 h. The resulting hydrolysate was cooled to room temperature and diluted to 50 mL with distilled water in a volumetric flask. The solution was filtered using filter paper and subsequently re-filtered through a 0.2 µm membrane filter before being transferred into a sample tube. The sample tube was filled with 500 µL of the filtered sample, 40 µL of AABA (amino acid standard), and 460 µL of distilled water. The mixture was vortexed and subjected to derivatization. Derivatization was carried out in a microvial containing 10 µL of sample or standard solution and 70 µL of borate buffer, followed by vortexing for 10 s. The mixture was allowed to stand at room temperature for 1 min and then heated at 55 °C for 10 min. The derivatized sample was transferred into a vial

for injection into the UPLC system.

Hydroxyproline content analysis

The analysis procedure was initiated by weighing 10 mg of the sample, which was then placed into a heat-resistant sample tube and homogenized using a tissue grinder pestle. The sample was subsequently dissolved in 100 µL of dH_2O and mixed with 100 µL of concentrated HCl (~12 N). The sample tube was tightly sealed and wrapped with aluminum foil, followed by hydrolysis at 120 °C for 3 h. After hydrolysis, the sample was vortexed and centrifuged at 10,000 × g for 3 min, and the supernatant was transferred into a well plate. For hydroxyproline determination, a standard solution series of 0.1 mg/mL was first prepared by diluting 10 µL of hydroxyproline standard solution with 90 µL of dH_2O. Subsequently, 0 (blank), 2, 4, 6, 8, and 10 µL of the 0.1 mg/mL standard solution were transferred into the well plate to obtain hydroxyproline standards of 0, 0.2, 0.4, 0.6, 0.8, and 1 µg/well, respectively.

The assay consisted of a mixture of T-chloramine/oxidation buffer and DMAB reagent. A total of 100 µL of T-chloramine/oxidation buffer assay solution was prepared by mixing 6 µL of T-chloramine with 94 µL of oxidation buffer. The mixture was added to each well containing the samples and hydroxyproline standards, followed by incubation at room temperature for 5 min. Subsequently, 100 µL of DMAB reagent assay was prepared by mixing 50 µL of DMAB concentrate with 50 µL of perchloric acid/isopropanol solution, which was then added to each well containing the T-chloramine/oxidation buffer assay. The samples and hydroxyproline standards were incubated in a drying oven at 60 °C for 90 min and subsequently analyzed at an absorbance of 560 nm using a microplate reader or ELISA reader (Thermo Fisher Scientific, MS, USA). The analysis was performed in duplicate. The hydroxyproline concentration of the raw material was calculated using the following formula:

$$C = \frac{Sa}{Sv}$$

Description:
C = Hydroxyproline concentration in the sample
Sa = Amount of hydroxyproline obtained from the standard curve (µg)
Sv = Volume of sample added into the reaction well (µL)

Fatty acid profile analysis

The fatty acid profile of fish skin was analyzed using chromatography according to the procedure conducted at the Integrated Laboratory of IPB University, Bogor. Fatty acid composition was determined as fatty acid methyl esters (FAME) using a GC QP-2010 gas chromatograph (Shimadzu, Japan). The formation of FAME from the samples was preceded by hydrolysis, followed by esterification.

Approximately 20–30 mg of sample was placed into a Teflon-capped tube, followed by the addition of 1 mL of 0.5 N NaOH (Merck, Germany), and heated in a water bath for 20 min. Subsequently, 2 mL of 16% BF_3 and 5 mg/mL of internal standard were added, and the mixture was reheated for another 20 min. The sample was then cooled, followed by the addition of 2 mL of saturated NaCl solution (Merck, Germany) and 1 mL of hexane (Merck, Germany). The hexane layer was transferred using a dropper pipette into a tube containing 0.1 g of anhydrous Na_2SO_4 (Merck, Germany) and allowed to stand for 15 min.

A total of 5 mL of the liquid phase containing the FAME standard mixture was injected into the gas chromatography column. The operational conditions of the gas chromatograph were as follows: capillary column (cyanopropyl methyl siloxane), injector temperature of 200 °C, detector temperature of 230 °C, initial column temperature of 190 °C for 15 min, and final temperature of 230 °C for 20 min with a column flow rate of 10 °C/min. Hydrogen (H_2) was used as the carrier gas, with flow rates of 30 mL/min for H_2, 20 mL/min for N_2, and 200–250 mL/min for air (Panagan *et al.* 2012).

Heavy metal contamination analysis

Heavy metal content analysis (As, Hg, Cd, and Pb) was conducted on four types of samples, namely purple-spotted bigeye skin, lattice monocle bream skin, Bleeker's grouper skin, and a mixed sample consisting of a combination of the three fish skin types. Approximately 0.5–1 g of fish skin sample was placed into a vessel, followed by the addition of 10 mL of concentrated HNO_3. The vessel was tightly sealed and subjected to microwave digestion under the following operating conditions: ramping to 150 °C for 10 min and holding at 150 °C for 15 min. The digested sample was transferred into a 50 mL volumetric flask. A measured volume of 0.50

mL of 100 mg/L yttrium internal standard was added, and the solution was diluted to the mark with double-distilled water (ddH_2O) and homogenized. The solution was then filtered, and the sample was analyzed using an ICP-OES system. The concentration of heavy metals/ minerals in the sample was calculated using a standard calibration curve with the linear equation $Y = bx + a$, according to the following formula:

$$Metal\ Content(mg/kg) = \frac{\frac{Aspl - a}{b} \times V \times fp}{Wspl\ or\ Vspl}$$

Description:
$Aspl$ = Sample intensity
a = Intercept of the standard calibration curve
b = Slope of the standard calibration curve
fp = Sample dilution factor
V = Final volume of the sample volumetric flask (mL)
$WSpl$ = Sample weight (g)
$VSpl$ = Volume of pipetted sample (mL)

Data analysis

Data analysis conducted during the characterization of purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skins included proximate analysis, histological observation, amino acid analysis, fatty acid analysis, and heavy metal contamination analysis. The results were analyzed descriptively and presented in the form of tables and figures.

RESULTS AND DISCUSSION

Chemical composition

Physicochemical changes, including the chemical composition of fish, may be influenced by species, diet, season, activity level, stress during capture, and muscle type (Ebadi *et al.* 2019). The properties of fish skin, including amino acid composition, collagen content, microstructure, and mineral composition, as well as differences in body weight, may affect its chemical composition values (Rosmawati *et al.* 2018). The results of the chemical composition analysis, including moisture, ash, fat, protein, and carbohydrate contents, are presented in Table 1.

Proximate analysis was conducted on four types of samples, namely purple-spotted bigeye skin, lattice monocle bream skin, Bleeker's grouper skin, and a mixed sample consisting of a combination of the three fish skin types. The results demonstrated that purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skins possessed relatively similar characteristics and were suitable as raw materials for collagen sources. The moisture content of all samples was relatively high (61.05–66.99% wb), reflecting the collagen-rich and hydrophilic nature of fish skin tissue. The protein content of fish skin ranged from 17.88–20.87% (wb), indicating that fish skin is predominantly composed of structural proteins, particularly collagen, thereby exhibiting high potential for collagen production. These values are comparable to the protein content of marine fish skin reported by Karim and Bhat (2009), who stated that fish skin is a highly promising source of collagen. The low-fat content (<3% wb) observed in all samples is consistent with the general characteristics of fish skin as a gelatin raw material, which benefits from minimal lipid contamination.

The highest ash content was observed in purple-spotted bigeye skin at 14.11% (wb). The relatively higher ash content in this skin is associated with the presence of scales or more mineralized dermal structures, indicating the contribution of inorganic minerals such as calcium and phosphorus commonly bound to scaled tissues. Chuaychan *et al.* (2016) reported that fish scales contain hydroxyapatite compounds and calcium carbonate ($CaCO_3$), while the outer layer of fish scales consists of bone-like structures composed of collagen fibrils in mineralized form. Differences in mineral (ash) content among fish tissue fractions may result from variations in skin/scale physical structure and habitat characteristics.

The low carbohydrate content (<6.30% wb) indicates that the major components of fish skin are protein and water, thereby reflecting a proximate composition suitable for high-quality gelatin and collagen raw materials. However, on a dry basis, the carbohydrate content in several samples showed relatively higher values, reaching 17.86% (db) in lattice monocle bream skin and 14.13% (db) in Bleeker's grouper skin. These values were presumably derived from non-protein components such as glycoproteins, mucopolysaccharides, or residual tissues still attached to the skin during the preparation process. In addition, carbohydrate determination by the difference method may also contribute to the accumulation of values from other indirectly measured components. Overall, the proximate composition of purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skins was consistent with the characteristics of marine fish skin and supports their potential utilization as alternative sources

of collagen and gelatin that are safe, halal, and value-added.

Fish skin tissue

Fish skin generally consists of two main layers, namely the outer epidermis and the inner dermis. The epidermis appears as the outermost, relatively thin layer that functions as a protective barrier, whereas the dermis is thicker and dominates the overall skin structure. The dermis is composed of densely packed, parallel, and layered collagen fiber bundles, characterized by strong eosinophilic staining in histological preparations. The thickness of the epidermis varies depending on the species age, body size, and environmental conditions.

Masson's trichrome staining is commonly used to visualize connective tissue structures, where cell nuclei appear purple to black, collagen fibers appear blue to green, and muscles, cytoplasm, and keratin appear red (Wahyuni *et al.* 2015). The histological observations of scaled skin tissues from purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper, presented as whole-slide visual preparations with a 50 µm scale bar at 40× magnification, are shown in Figure 1.

Histological observations demonstrated that grouper skin exhibited a distinctly different dermal thickness compared to the other fish species. Grouper skin (C) showed the thickest and most compact dermal layer, characterized by densely packed and homogeneous collagen fiber arrangements. This condition indicates a higher structural collagen content compared to purple-spotted bigeye and lattice monocle bream skins. Lattice monocle bream skin (B) exhibited a moderately thick dermal layer with relatively well-organized collagen fibers, whereas purple-spotted bigeye skin (A) showed a thinner dermal layer with comparatively lower collagen fiber density. Differences in collagen layer characteristics are likely influenced by the number of collagen-forming components, particularly structural proteins of the extracellular matrix present in connective tissue. In addition, the protein content of fish skin is also correlated with the amount of collagen contained within the skin tissue (Haryati *et al.* 2019).

Table 1. Proximate composition of purple-spotted bigeye (*Priacanthus tayenus*), lattice monocle bream (*Scolopsis taenioptera*), and Bleeker's grouper (*Epinephelus bleekeri*) and mixed fish skins on a wet basis (wb) and dry basis (db).

Parameters	Purple-spotted Bigeye		Lattice Monocle Bream		Bleeker's Grouper		Mix	
(%)	(wb)	(db)	(wb)	(db)	(wb)	(db)	(wb)	(db)
Moisture content	61.05 ± 0.06	-	66.99 ± 0.21	-	62.39 ± 0.18	-	63.76 ± 0.08	-
Ash content	14.11 ± 0.31	36.22	6.59 ± 0.64	19.95	11.25 ± 0.44	29.91	12.16 ± 0.30	33.54
Fat content	1.52 ± 0.11	3.90	2.66 ± 0.28	8.04	1.29 ± 0.05	3.42	2.16 ± 0.19	5.95
Protein content	20.87 ± 0.16	53.57	17.88 ± 0.53	54.15	19.76 ± 0.58	52.54	19.48 ± 0.18	53.73
Carbohydrate content (by difference)	2.46 ± 0.02	6.30	5.89 ± 0.37	17.86	5.31 ± 0.37	14.13	2.46 ± 0.21	6.79

Note: (wb) wet basis; (db) dry basis. Data are presented as mean ± standard deviation without statistical analysis of significant differences among treatments.

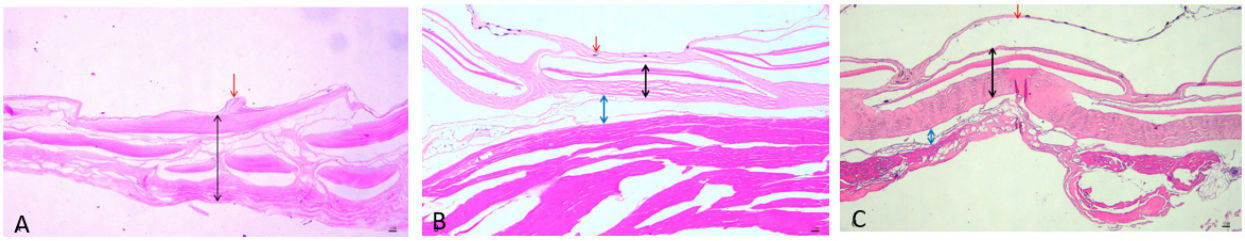

Figure 1. Histology of scaled skin from purple-spotted bigeyes (A), lattice monocle bream (B), and Bleeker's grouper (C) stained using Masson's trichrome at 40× magnification. Epidermal layer (red arrow), dermal layer (black arrow), and hypodermal layer (blue arrow). Scale bar: 50 µm.

Amino acid composition

Protein is an essential macromolecular component required by living organisms to carry out metabolic processes within the body (Rieuwpassa *et al.* 2020). Fish skin contains relatively high protein levels, as described previously. This characteristic has led to the extensive utilization of fish skin as a raw material for collagen and gelatin production. Collagen is a structural protein composed of amino acids, particularly glycine, proline, and hydroxyproline, which are essential for the formation and stability of its triple-helix structure. Therefore, the amino acid composition is an important parameter for evaluating the potential of fish skin as a collagen source. The results of the amino acid analysis of fish skin are presented in Table 2.

The results of the amino acid analysis presented in Table 2 indicate that fish skin contains various amino acids. The predominant amino acids identified in the mixed fish skin sample (a combination of purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skins) were glycine (3.99%), L-alanine (3.15%), and L-glutamic acid (2.60%). Meanwhile, L-arginine was not detected in the mixed fish skin sample. Based on these results, glycine and alanine were identified as the most abundant amino acids, which are recognized as the primary characteristic amino acids of collagen structure (Ward and Courts 1977). Amino acids can generally be classified into essential and non-essential amino acids. Essential amino acids are those that cannot be synthesized by the body, including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. In contrast, non-essential amino acids can be synthesized by the body, including alanine, asparagine, aspartate, glutamate, and serine (McGuire and Beerman 2013).

Overall, all amino acids that constitute proteins play crucial roles in maintaining body functions. Deficiency of amino acids may lead to health disorders, particularly affecting the development and repair of body tissues and the brain (Rieuwpassa *et al.* 2018).

Hydroxyproline content

Collagen is the primary group of structural proteins composing the extracellular matrix, arranged in a myofibrillar structure and consisting of more than 20 types of amino acids (Ferreira *et al.* 2012). Structurally, collagen is composed of polypeptide chains with a repeating triple-helix pattern of Gly–X–Y, in which glycine predominates together with two other amino acids, commonly proline and hydroxyproline (Gomez-Guillén *et al.* 2002).

Hydroxyproline is frequently used as an indicator for determining collagen content in tissues due to its specific presence in collagen protein (Kittiphattanabawon *et al.* 2005). According to Atma (2017), hydroxyproline is one of the important amino acids in collagen, in addition to glycine, proline, alanine, and glutamic acid. Therefore, the hydroxyproline content of a material can be used as a basis for evaluating its potential as a collagen source.

Table 2. Amino acid composition of mixed fish skin (a combination of purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skins).

Parameter	Result (% w/w)
L-alanine	3.15
L-Arginine	0.00
L-Aspartic acid	1.58
Glycine	3.99
L-Glutamic acid	2.60
L-Histidine	0.14
L-Isoleucine	0.35
L-Leucine	0.78
L-Lysine	1.03
L-Valine	0.64
L-Phenylalanine	0.55
L-Serine	0.83
L-Threonine	0.77
L-Tyrosine	0.24

In determining hydroxyproline levels, a standard solution with a concentration range of 0–1 µg was used to construct a calibration curve. The relationship between absorbance and hydroxyproline concentration showed a linear pattern with the regression equation $y = 1.3523x + 0.0310$ and a coefficient of determination (R^2) of 0.9758 or 97%, indicating good linearity (Figure 2).

Hydroxyproline analysis was performed on three types of samples, namely fish skin (K), swollen skin after swelling treatment (KS), and extracted gelatin (G). Quantification was conducted in duplicate, and the results were presented as mean values. The results demonstrated that hydroxyproline content increased from fish skin (0.0739 µg/µL) to swollen skin (0.1640 µg/µL), reaching the highest value in gelatin (0.4071 µg/µL). This increase indicates that the pretreatment and extraction processes contributed to improving collagen availability as detected by hydroxyproline analysis. These findings are also consistent with the amino acid profile, which showed the predominance of glycine and alanine as major components, characteristic of collagen protein. Therefore, the hydroxyproline data further confirm that purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skins are promising collagen sources with strong potential for further development. The analysis results are presented in Table 3.

Fatty acid composition

Fatty acid composition analysis was conducted to determine the lipid fraction characteristics of mixed fish skin samples consisting of purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skin. This information is important for evaluating the suitability of fish skin as a raw material for collagen and gelatin production, as the content and type of fatty acids may influence raw material stability and extraction process efficiency. The results of the fatty acid analysis are presented in Table 4.

Based on fatty acid classification, the saturated fatty acid (SFA) fraction was the dominant component, accounting for 34.53% (w/w), primarily consisting of palmitic acid (C16:0) and stearic acid (C18:0). The relatively high SFA content is commonly found in marine fish skin tissues and contributes to maintaining the structural stability of tissue membranes. Monounsaturated fatty acids (MUFA) accounted for 11.60% (w/w), with oleic acid (C18:1n9c) as the major component, whereas polyunsaturated fatty acids (PUFA) reached 9.30% (w/w). The presence of MUFA and PUFA reflects the typical lipid characteristics of demersal marine fish, which generally possess complex lipid profiles despite their relatively low total fat content.

The PUFA fraction in mixed fish skin contained essential omega-3 (ω-3) and omega-6 (ω-6) fatty acids at levels of 7.06% and 2.56% (w/w), respectively. The presence of ω-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) indicates that, although fish skin is considered a by-product, this tissue still contains bioactive compounds with functional value. However, the lower proportion of PUFA compared to SFA and MUFA suggests that mixed fish skin is more suitable for development as a collagen and gelatin source rather than as a fish oil source.

Overall, the fatty acid profile of mixed fish skin, characterized by low total fat content, dominance of SFA, and the presence of essential PUFA, supports the previous proximate and histological analyses, indicating that fish skin is predominantly composed of collagenous tissue. These characteristics further strengthen the potential of mixed fish skin as an efficient, stable, and value-added alternative raw material for collagen and gelatin production, while also supporting the sustainable utilization of the fishery industry by-products.

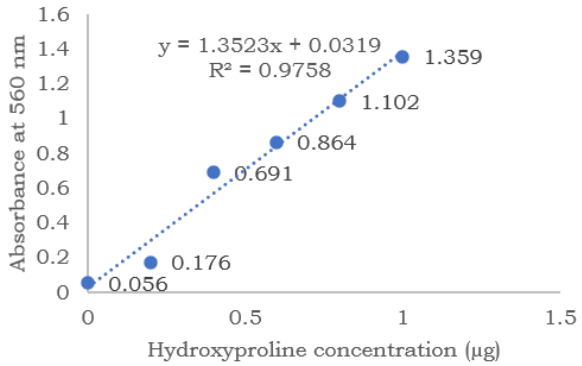

Figure 2. Standard calibration curve of hydroxyproline within the concentration range of 0–1 µg, showing the relationship between concentration and absorbance.

Table 3. Hydroxyproline content of mixed fish skin (K), swollen skin after swelling treatment (KS), and extracted gelatin (G).

Samples	Hydroxyproline (µg/µL)
Mixed fish skin (mix) (K)	0.0739 ± 0.0083
Skin after swelling treatment (KS)	0.1640 ± 0.0397
Extracted gelatin (G)	0.4071 ± 0.0341

Table 4. Fatty acid composition of mixed fish skin (purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper).

Parameters	Results (% w/w)
Butyric acid (C4:0)	0.10
Lauric acid (C12:0)	0.24
Myristic acid (C14:0)	3.57
Pentadecanoic acid (C15:0)	0.82
Palmitic acid (C16:0)	19.26
Palmitoleic acid (C16:1)	4.58
Heptadecanoic acid (C17:0)	1.23
Cis-10-Heptadecenoic acid (C17:1)	0.11
Stearic acid (C18:0)	7.67
Elaidic acid (C18:1n9t)	0.17
Oleic acid (C18:1n9c)	6.59
Linoleic acid (C18:2n6c)	0.61
γ-Linolenic acid (C18:3n6)	0.29
Linolenic acid (C18:3n3)	0.31
Arachidic acid (C20:0)	0.43
Cis-11-Eicosenoic acid (C20:1)	0.15
Heneicosanoic acid (C21:0)	0.19
Cis-11,14-Eicosadienoic acid (C20:2)	0.26
Behenic acid (C22:0)	0.59
Arachidonic acid (C20:4n6)	1.37
Cis-5,8,11,14,17-Eicosapentaenoic acid (EPA, C20:5n3)	3.69
Lignoceric acid (C24:0)	0.14
Cis-4,7,10,13,16,19-Docosahexaenoic acid (DHA, C22:6n3)	3.06
Total fatty acids	**55.43**

Heavy metal content

Heavy metal analysis was conducted to evaluate the safety of purple-spotted bigeye, lattice monocle bream, Bleeker's grouper, and mixed fish skin samples as potential raw materials for collagen and gelatin production. The presence of heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd), and lead (Pb) is an important concern because these contaminants may pose adverse health effects and determine the suitability of raw materials for food, pharmaceutical, and cosmetic applications. The results of the heavy metal analysis are presented in Table 5.

The results showed that the heavy metal contents in purple-spotted bigeye, lattice monocle bream, Bleeker's grouper, and mixed fish skin samples were present at very low levels. Arsenic (As) was detected in small amounts ranging from 0.01–0.02 mg/kg, whereas mercury (Hg), cadmium (Cd), and lead (Pb) were below the detection limits of the instrument. These findings indicate that the fish skins used in this study were relatively safe and did not exhibit significant heavy metal accumulation.

Table 5. Heavy metal contents (As, Hg, Cd, and Pb) in purple-spotted bigeye, lattice monocle bream, Bleeker's grouper, and mixed fish skins.

Heavy Metals	Results			
	Purple-spotted Bigeye	Lattice Monocle Bream	Bleeker's Grouper	Mix
Arsenic (As)	0.01	0.02	0.01	0.01
Mercury (Hg)	< 0.006	< 0.006	< 0.006	< 0.006
Cadmium (Cd)	< 0.002	< 0.002	< 0.002	< 0.002
Lead (Pb)	< 0.0002	< 0.0002	< 0.0002	< 0.0002

The low levels of heavy metals detected in all samples suggest that the fish skin raw materials originated from relatively uncontaminated environmental conditions and are suitable for utilization as value-added products. Furthermore, these results strengthen the potential use of fish skin as a safe source of collagen and gelatin, particularly for food, pharmaceutical, and cosmetic applications that require strict limits on heavy metal contamination.

CONCLUSION

Purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skins, as by-products of the fisheries industry, demonstrated strong potential as collagen sources. Histological observations revealed the predominance of the dermal layer with clearly arranged collagen fibers, particularly in grouper skin, which exhibited greater collagen layer thickness. Proximate analysis showed relatively high protein content and low-fat content, supported by the fatty acid profile of the mixed sample, which indicated low total fat levels dominated by saturated fatty acids with limited amounts of essential fatty acids. The predominant amino acids identified were glycine and alanine. Hydroxyproline analysis further confirmed the presence of collagen through the increasing hydroxyproline content from fish skin to gelatin. In addition, heavy metal contents in all samples were found to be very low or below the detection limits, indicating compliance with raw material safety requirements. Overall, purple-spotted bigeye, lattice monocle bream, and Bleeker's grouper skins have considerable potential to be developed as alternative raw materials for collagen and gelatin production that are safe, halal, and value-added.

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