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PHYSICOCHEMICAL CHARACTERIZATION AND PEPTIDE PROFILING OF GELATIN FROM THE INVASIVE SPECIES *Amphilophus labiatus*

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Abstract

Protein- and peptide-based bioactive compounds are a key focus in pharmaceutical and cosmetic development because of their superior biocompatibility, high target specificity, and effectiveness at low dosages. Fish collagen and its derivatives, such as gelatin, are promising candidates as alternative sources of functional biomolecules. Therefore, this study aimed to investigate the potential of red devil fish (*Amphilophus labiatus*) skin, an abundant invasive species in Indonesia, as a novel source of high-value gelatin. Gelatin was extracted at varying acetic acid concentrations (0, 1, 2, and 3%) and subsequently characterized for its physicochemical properties (gel strength and viscosity) and peptide profile. The results showed that optimal treatment was achieved using 3% acetic acid. A 12.95% gelatin was produced, characterized by a high protein content (87.2%), gel strength of 257.17±17.1 bloom, and viscosity of 6.84±0.87 cP. Advanced characterization using high-resolution tandem mass spectrometry has identified 20 distinct collagen types. The most abundant protein was type I collagen, along with proteins such as collagen IV NC1 and fibrillar collagen NC1. These findings underscore the dual significance of valorizing the skin of the invasive red devil fish, not only as an ecological management strategy but also as a sustainable and innovative source of bioactive proteins for pharmaceutical and cosmetic applications..

Keywords: collagen, mass spectrometry, peptides, red devil

Karakteristik Fisikokimia dan Komposisi Peptida Gelatin Ikan Invasif *Amphilophus labiatus*

Abstrak

Bahan aktif berbasis protein dan peptida telah menjadi fokus utama dalam pengembangan farmasi dan kosmetik modern karena keunggulannya dalam biokompatibilitas, spesifisitas target, serta efektivitas pada dosis rendah. Kolagen ikan dan produk degradasinya, yaitu gelatin, merupakan kandidat menjanjikan untuk sumber biomolekul fungsional tersebut. Penelitian ini mengeksplorasi potensi kulit ikan setan merah (*Amphilophus labiatus*), spesies invasif yang melimpah di Indonesia sebagai sumber alternatif gelatin bernilai tambah. Gelatin diekstraksi dengan metode asam menggunakan 3 variasi konsentrasi asam asetat, yaitu 1, 2, dan 3% (0% sebagai kontrol) dan dikarakterisasi berdasarkan sifat fisikokimia (kekuatan gel dan viskositas) serta profil peptidanya. Perlakuan terbaik diperoleh pada perlakuan menggunakan asam asetat 3% yang menghasilkan rendemen 12,95% dengan kadar protein tinggi (87,2%), kekuatan gel 257,17±17,1 bloom,

dan viskositas 6,84±0,87 cP. Analisis lanjutan menggunakan spektrometri massa tandem resolusi tinggi mengidentifikasi 20 jenis kolagen berbeda, dengan kolagen tipe I sebagai yang dominan, serta protein lain dengan domain mirip kolagen seperti kolagen IV NC1 dan fibrillar collagen NC1. Temuan ini menegaskan potensi kulit ikan red devil tidak hanya sebagai strategi pemanfaatan spesies invasif, tetapi juga sebagai sumber biomaterial inovatif untuk aplikasi farmasi dan kosmetik berbasis protein..

Kata kunci: kolagen, *mass spectrometry*, peptida, setan merah

INTRODUCTION

Recent trends in pharmaceutical and cosmetic development include the use of protein- and peptide-based preparations, including di-peptides (Larder *et al*., 2022), collagen tripeptides (Lee *et al*., 2022), acetyl hexapeptode-8 (Kraeling *et al*., 2015; Raikou *et al*., 2021), and natural proteins or peptides (Apone *et al*., 2019; Oslan *et al*., 2022). Claims of greater target specificity, better biocompatibility, and higher potency at lower doses have driven the growth of studies and businesses in these fields (Fu *et al*., 2020; Tinoco *et al*., 2022). Peptides are chemical compounds that consist of short chains of amino acids. They can be chemically synthesized (Tatsumi *et al*., 2023) or derived from natural protein sources of animal or plant origin, commonly referred to as protein hydrolysates (Castro-Jácome *et al*., 2021; Henriques *et al*., 2021; Arslan *et al*., 2024). Collagen is one of the most widely produced natural proteins. It is often hydrolyzed into short peptides, such as collagen tripeptides, which are used in cosmetic preparations as anti-aging agents or as collagen peptides for pharmaceutical preparations to aid wound healing (Fujita *et al*., 2003; Ambrosini *et al*., 2022). The primary sources of collagen are bovine (Noorzai *et al*., 2020), porcine (Gorlov et al., 2018), and marine species (Shaik *et al*., 2024). Since these sources are often associated with outbreaks, such as bovine spongiform encephalopathy (BSE) or mad cow disease, are subject to religious prohibitions in Hinduism and Islam, and pose challenges in cultivation, exploring safer and easier alternatives is desirable.

The exploration and utilization of naturally occurring invasive species is highly desirable for developing new collagen-derived sources while protecting the environment. The red devil fish (*Amphilophus labiatus*) is an omnivorous and invasive species that can disrupt ecosystems and reduce their value and diversity. It also threatens biodiversity, damages and extirpates native species, and impairs the health of humans, animals, and plants. This species is abundant in the Sermo Reservoir in Yogyakarta, Indonesia (Habibie *et al*., 2015). Dominance is a significant issue because of their capacity to breed year-round, produce hundreds of eggs in a single spawn, prey on tiny fish, and compete with native breeds (Jakubčinová *et al*., 2017). Despite its abundance, red devil fish is not popular as a food choice because of its large bones and inferior meat quality. Therefore, alternative uses should be explored to address environmental issues and increase economic value. This study investigated the use of red devil fish skin as an alternative gelatin protein source. Fish skin contains a large amount of collagen, including structural proteins that provide stability and tensile strength. Collagen can be converted into gelatin through acid or alkaline treatment, facilitated by heating (Liu *et al*., 2015; Gaikwad & Kim, 2024). Gelatin is a natural polymer derived from hydrolytic degradation, and its amino acid structure is widely used in pharmaceuticals and cosmetics. The extraction of polymers from fishery by-products, such as bones and heads, is becoming increasingly popular; however, skin waste remains rarely used (Valcarcel *et al*., 2021).

Fish skin is known to produce a high amount of collagen. It also possesses a high amount of oil, carbohydrates, and possibly many other non-collagen proteins that need to be eliminated. Furthermore, the extraction process should aim to produce high-quality gelatin. The method of choice for preparation involves the use of weak acids, which is preferable because the collagen content in the skin can be converted faster compared to when using a base. A weak acid also provides an advantage, as it is easier to control the bonds

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of the polypeptide chain from damage, leading to high gel strength. Acetic acid is among the types used in the extraction process (Liu *et al*., 2015; Nugraheni *et al*., 2021). The quality of gelatin can be affected by the concentration of acetic acid. High concentrations may break hydrogen bonds and open collagen structures. This contributes to a lower protein content and decreased water-binding capacity, which affect the strength and viscosity of the gelatin (Zhang *et al*., 2020). Therefore, the acetic acid concentration used for extraction should be optimized. Early investigations have been conducted on the skin and bones of tuna, sharks, *kurisi*, carp, snapper, tilapia, catfish, grouper, and *gelik fish* (Atma & Ramdhani, 2018; Nurilmala *et al*., 2021; Prajaputra *et al*., 2024). Based on existing publications, the potential of red devil fish as a gelatin-collagen source has not been reported.

In addition to the amount and physical quality of the gelatin-extracted collagen, identifying the protein and peptide profiles is essential. A major challenge associated with natural protein products is the complex mixture of proteins derived from a single source. This becomes more complicated after hydrolysis, as the peptides are derived from various proteins (Castro-Jácome *et al*., 2021; Kumazawa *et al*., 2016). This heterogeneous mixture of peptides or proteins in the final pharmaceutical or cosmetic products may affect their effectiveness, efficiency, and safety. Therefore, thorough characterization is necessary to ensure that other undesirable substances are absent from the primary source of the oil. In this study, liquid chromatography coupled with high-resolution tandem mass spectrometry (LC-MS/MS) was used to characterize the proteins and their respective peptides in the extraction product. The results provide initial scientific knowledge for the development of red devil fish for food, pharmaceutical, and cosmetic applications.

MATERIALS AND METHODS

The materials used included red devil fish (*Amphilophus labiatus*), acetic acid, formic acid, sulfuric acid, hydrochloric acid, boric acid, trifluoroacetic acid, methyl red, methyl blue, and acetone (all from Sigma-Aldrich (St. Louis, USA). Ammonium bicarbonate, sodium hydroxide, LC-MS-grade acetonitrile, LC-MS-grade water, and Kjeldahl catalysts were purchased from Merck KGaA (Darmstadt, Germany). Pierce trypsin protease MS grade was purchased from Thermo Fisher (Massachusetts, USA).

Sample Preparation

The red devil fish was collected from fishermen in the Sermo Reservoir, Kulon Progo Regency, Yogyakarta, Indonesia. The fish were transferred to the laboratory in a cool ice box, skinned, and stored at -20°C. The samples were analyzed for proximate content and part proportion. Subsequently, the skin was separated using a sharp knife under flowing water, as described by Nurilmala *et al*. (2021) and Nurilmala *et al*. (2022). Non-collagenous proteins were removed by alkali treatment, which involved soaking in 0.05 M sodium hydroxide at a 1:10 (w/v) ratio for 3 h at 300 rpm at RT. The solvent was changed every hour, and after alkali treatment, the skin was washed under running water until the pH was neutral.

Gelatin Collagen Extraction

Gelatin collagen was extracted by treatment with acetic acid (AA). In this study, four different concentrations of acetic acid (0, 1, 2, and 3%) were optimized. The fish skin was soaked in each concentration of the solution for 12 h at a 1:4 ratio. The swollen skin was washed repeatedly until the pH reached 5-6. Gelatin collagen was extracted using distilled water at a ratio of 1:3 for 3 h at 80±3°C. The filtrate was then filtered using a cloth and dried in an oven at 40-50°C until dry. The resulting gelatin was powdered into fine granules and used for further analysis.

Gel Strength Analysis

Gel strength analysis was conducted according to Lassoued *et al*. (2014) using a universal texture analyzer. Gelatin (6.67 g) was dissolved in 100 mL of distilled water and heated at 60°C for 15 min. The solution was then incubated at 7 °C for 16–18 h or until a gel was formed. The gelatin in a container was placed below a universal texture machine

plunger with a probe speed of 1 mm.min^{-1}, depth pressure of 4 mm, diameter of 12.7 mm, and surface area of 126.728 mm^2.

Viscosity Analysis

Viscosity was analyzed according to the Gelatin Manufacturers Institute of America (GMIA) standard (2012). Gelatin 6.67 % (w/v) was dissolved at 60°C in a water bath shaker. The viscosity of the solution was measured using a Brookfield viscometer at 50°C using spindle 61 at 60 rpm. The measurement results were attributed to the conversion factor on the spindle based on the Brookfield Manual 2002 (centipoise (cP)).

Moisture Content Analysis

Moisture content was analyzed using an Ohaus moisture analyzer (MB120) according to the manufacturer's instructions.

Proximate analysis

The proximate composition, including crude protein, lipid, and ash content, was determined following the standard methods of the AOAC International (2019). Crude protein content was measured using the Kjeldahl method. The protein content was calculated using a nitrogen-to-protein conversion factor of 6.25, as follows: Lipid content was determined by Soxhlet extraction with petroleum ether as the solvent, while ash content was determined by incineration in a muffle furnace at 550 ± 25 °C for 6 h.

Gelatin pH Analysis

The pH of the gelatin solution was analyzed using a 1.5% (b/v) gelatin sample dissolved in a 100 mL beaker containing distilled water. The sample was heated at 60°C until completely dissolved. The solution was cooled to 25°C, and the acidity was measured using a calibrated pH meter (GMIA, 2019).

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Analysis

Approximately 100 mg of the sample was obtained from each treatment and used for tryptic digestion. The sample was prepared by dissolving the powder in double-distilled water, and acetone was added at a 1:1 ratio to precipitate the proteins. The supernatant was then removed, and the protein was redissolved in 50 mM ammonium bicarbonate and incubated for 10 min at 90°C. Tryptic digestion was performed overnight at 37 °C with a trypsin: protein ratio of 1:100. TFA (1%) was added, and the mixture was centrifuged for 10 min at 8,000 × *g*. Subsequently, the supernatant was filtered using a 0.2 µL PVDF filter.

The peptides were chromatographically separated using a two-buffer system. These included buffer A, 0.1% FA in MS-grade water, and buffer B, 0.1% FA in MS-grade acetonitrile, in a Thermo Scientific Vanquish Horizon UHPLC with a binary pump system (Germering, Germany). An Acclaim PepMap 100 C18 column (150 mm × 1 mm, 3 µm particle size) was used for separation using a 45-min gradient elution, with buffer B increasing from 5% to 50% over 30 min. Eluting peptides were ionised by a heated electrospray ionisation (H-ESI) positive mode with a spray voltage of 4000, transferred into the mass spectrometry (Orbitrap Exploris 240 HRMS, Bremen, Germany), and analyzed in data-dependent acquisition (DDA) mode. Full scan MS was performed in the range of 350–1,500 m/z with a maximum injection time of 100 ms, an intensity threshold of 8,000, and a mass tolerance of 5 ppm. The resolutions for the full-scan MS1 and MS2 were 120,000 and 15,000 FWHM, respectively.

Raw MS data were analyzed using Proteome Discoverer 2.5 software (Thermo Fisher Scientific). Protein identification was conducted using the *Amphilophus labiatus* FASTA file from the UniProt and NCBI databases. The data obtained from the software were further processed using an Excel spreadsheet.

Statistical Analysis

This study adopted a completely randomized design for the data analysis. The treatments in this study were four different concentrations of acetic acid: 0, 1, 2, and 3%. The protein hydrolysate data, including the degree of yield, pH, viscosity, gel strength, protein, moisture, and ash content from red

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devil fish skin, were subjected to ANOVA, followed by Tukey's post hoc test to identify pairwise differences when significant effects were detected with a 95% confidence level. All analyses were conducted using the IBM SPSS Statistics software (version 25).

RESULTS AND DISCUSSION

The red devil fish showed a favorable proximate composition in both its flesh and skin, with appreciable levels of protein and other essential nutrients (Table 1). The nutritional profile underscores the potential of this species not only as a promising raw material for human food but also as an alternative ingredient for animal feed. Given its abundance as an invasive species in Indonesian waters, valorizing red devil fish provides the dual benefits of mitigating ecological threats and supplying valuable biomaterials for the food and feed industries.

Proximate analyses of the meat and skin of red devil fish were conducted. The results showed that the protein content in fish skin was similar to that in fish meat. The skin of red devil fish had a similar moisture content as the yellowtail species (68.5%) and tilapia (65.72%). However, the protein content was lower than that of yellowtail skin (17.87%) and tilapia skin (47.01%). The ash content of red devil fish (1.8%) was comparable to that of tilapia (1.23%) (Nurjanah, 2017) and yellowtail (0.74 %) (Astiana *et al*., 2016).

Skin Yield

The skin used in this study was 8.90±1.0% of the entire fish, while the meat was 25.44±1.15%, the head was 32.27±1.03%, the bone gave 25.33±1.41%, and the viscera was 8.04±1.36%. Although the yield of the red devil appears to be modest. A previous study reported that the skin typically accounts for only 8-10% of the fish's body weight. This is also equal to the skin yield of brackish-water and freshwater fish, which is 7% and 8%, respectively.

Gelatin Yield

Gelatin is the main component of collagen hydrolysis. The yield of gelatin ranged from 6.82 to 12.95%, with the lowest yield obtained from the treatment without acetic acid. The highest proportion was obtained using 3% acetic acid. The complete characteristics are listed in Table 2.

The increase in yield from 1 to 3% was consistent with the findings of Al Hajj et al. (2024), who reported that the yield increased with higher acidity and acid concentration. This is because a higher acid concentration produces more H+ ions in the solvent, facilitating the extraction of more gelatin (Ridhay *et al*., 2016). Compared to catfish skin gelatin (18.11%), tilapia (19.64%), and tuna (16.95%), the yield of red devil fish gelatin is relatively low (Nurilmala *et al*., 2021). However, it was higher than that of starry triggerfish (7.93%), as reported by Nugraheni *et al.* (2021). The obtained protein levels showed that the addition of acetic acid to the gelatin had a significant impact. The higher the acidity or acid concentration, the more H+ ions denatured the collagen, and the higher the protein production (Nugraheni *et al*., 2021).

Table 2 shows that the total protein content of the red devil fish skin increased with increasing acetic acid concentration, from 59.87 to 87.2%. The obtained levels indicate the impact of adding acetic acid to gelatin on the protein content. The protein content of red devil fish skin was in accordance with GMIA

Table 1 Proximate analysis of the meat and skin of red devil fish

Parameters (%)	Fish parts	
	Meat	Skin
Protein	14.11±0.76	12.81±0.63
Fat	1.93±0.8	3.42±0.1
Ash	0.35±0.01	1.8±0.35
Moisture	66.14±4.41	69.38±1.54

Table 2 Proximate analysis of the meat and skin of red devil fish

Parameters	Acetic acid concentration (%)			
	0	1	2	3
Yield (%)	6.82±0.65[a]	8.97±1.76[ab]	11.31±1.92[bc]	12.95±0.91[c]
pH	6.63±0.32[b]	5.9±0.14[a]	5.83±0.09[a]	5.57±0.23[a]
Viscosity (cP)	4.68±0.58[a]	6.49±0.94[ab]	6.74±0.88[ab]	6.84±0.87[b]
Gel Strength (bloom)	55.16±19.15[a]	237.44±36.07[b]	239.85±24.34[b]	257.17±17.1[b]
Protein (%)	59.87±3.99[a]	81.37±3.81[b]	86.62±0.87[b]	87.2±1.8[b]
Moisture (%)	3.99±0.43[b]	2.41±0.24[a]	2.22±0.24[a]	1.79±0.28[a]
Ash (%)	0.81±0.09[a]	1.11±0.03[b]	1.13±0.09[b]	1.25±0.09[b]

Different superscript letters within a single row denote a significant difference (p<0.05).

2019, which was approximately 87.26%. This value was higher than that of starry triggerfish skin gelatin (82.57 %) (Nugraheni *et al*., 2021).

Gelatin pH

pH analysis was necessary because it affected other gelatin parameters, including viscosity and gel strength. At a neutral pH, gelatin is stable and can be used as an ingredient in food and non-food products (Nurilmala *et al*., 2021). The pH of red devil fish skin gelatin ranged from 5.57 to 6.63, with the highest pH value. The highest was observed in the treatment without acid, while the lowest was obtained at a 3% acetic acid concentration level. According to a previous study (Nurilmala *et al*., 2021), the pH values of catfish skin (5.56), tilapia (5.67), and tuna (5.65) were nearly identical to that of red devil fish with a 3% acetic acid concentration.

Moisture Content

Moisture content is crucial as it affects the appearance, texture, and flavor of food (Jaziri *et al*., 2019). According to the test results, the moisture content of red devil fish skin gelatin was significantly affected by varying acetic acid concentrations, ranging from 1.79 to 3.99%. The change in value across each treatment was typically influenced by the total amount of collagen generated in gelatin. A decrease was observed during oven-like drying because the hydrogen bonds evaporated with water (Maciej *et al*., 2015). The moisture content of red devil skin gelatin was lower than that of tuna (3.96%), tilapia (7.04%), and catfish (6.43%) (Nurilmala *et al*., 2021).

Viscosity and Gel Strength

The viscosity of the gelatin solution at a specific temperature and concentration was determined using a test. The viscosity of gelatin must be considered when it is used in food product preparations (Nurilmala *et al*., 2021). The results from the red devil fish skin showed that increasing the acetic acid concentration significantly increased the viscosity. The obtained values were 4.68-6.84 cP, as shown in Table 3. This complied with SNI No. 06-3735-1995 and GMIA (2019) standards (1.5 – 7.5 cP).

Another significant physical characteristic of gelatin is its gel strength, which is associated with its ability to form a reversible gel from a solution. The gel strength test results showed that the acetic acid concentration treatment had a significant effect. Acidic conditions tend to cause hydrolysis and breakdown of the polypeptide chain (Goudie *et al*., 2023). The red devil fish skin gelatin had a value of 55.16-257.17 bloom. This satisfied both GMIA (2019) and SNI No. 06-3735-1995 criteria, where the ideal gelatin gel strength was between 50-300 blooms. The value recorded in starry trigger fish skin gelatin was higher at 294.47 blooms (Nugraheni *et al*., 2021) than that in red devil fish. The gel strength of red devil fish skin was reported to be higher than that of catfish,

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tilapia, and tuna skin gelatin, at 204.01, 59.73, and 59.43 bloom, respectively (Nurilmala *et al*., 2021). Several factors affect viscosity, including extraction temperature, extraction time, acid concentration, and the number of molecules dissolved (Ahmad *et al*., 2017).

Viscosity and water content are closely related. The ability to bind water to form a gel increased as the moisture content decreased. Meanwhile, the gel strength decreased with increasing moisture content (Portanguen *et al*., 2023). The measured viscosity was affected by the solution thickness, which was determined by the amount of water bound to the gelatin. The viscosity of red devil fish skin was approximately the same that as of catfish (6.16 cP), tilapia (6.63 cP), and tuna (6.5 cP) (Nurilmala *et al*., 2021).

Protein and Peptide Profiling

Accurate and comprehensive characterization of proteins or peptides is a prerequisite for their application as primary or auxiliary components in pharmaceuticals and cosmetics, particularly when sourced from natural origins. In most cases, a homogeneous mixture of a single type of protein or peptide is highly desirable to ensure the effectiveness and safety of the product. To some extent, separating the main protein or peptide of interest from the rest of the mixture is essential. A crucial step before separation is the confident identification of all components in the mixture. For proteins extracted from natural sources such as plants, animals, and fish, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used as a confirmation method to detect target proteins based on the presence of bands in the gel. The molecular weight was approximately determined by comparing the sample band with the protein marker. In the case of gelatin collagen, when a band with a molecular weight of approximately ~120 Da (the theoretical mass of most collagen species) appeared, collagen was considered present. This method was practical but lacked confidence and accuracy in identification of the species. There was a high possibility that many other proteins were present and remained undetected because of the resolution and sensitivity limitations of the gel-based analytical method. Therefore, SDS-PAGE is beneficial for early identification but insufficient for comprehensive characterization.

This study identified collagen and over 700 proteins from red devil skin using liquid chromatography coupled with high-resolution tandem mass spectrometry (LC-MS/MS). Several different types of collagen were identified, as shown in Table 3. In the protein extract of red devil fish, 13 collagen types (20 molecular species) were identified out of the 28 collagen types reported (Ricard-Blum, 2011). Table 3 shows that for collagen type I, both alpha chains 1 and 2 were identified with the highest confidence and abundance, followed by collagen types II and IV. This result is consistent with that of a previous study, in which types I-IV accounted for more than 90% of the content in vertebrates. Type I is the most abundant in fish skin, accounting for 70% (Salvatore *et al*., 2020). Type II was identified at lower abundance, as expected. This form is primarily localized in the hyaline cartilage rather than the skin (Wu *et al*., 2021). Collagen type III was not detected in the sample, possibly due to the maturity of the fish. Previous studies on various freshwater species have reported that type III is typically present in juvenile fish (Santhanam *et al*., 2022). Based on observations, it is absent in marine species (Salvatore *et al*., 2020). In the present study, fish were collected from the wild, preventing control over their developmental stage and complicating direct conclusions regarding the relationship between undetected collagen type III and age-related factors.

In addition to the primary collagen types, several others were identified in this study, including types V and VI, which have also been reported in marine species such as the shortbill spearfish (Han *et al.*, 2024). Types IX-XII, as well as types XVIII, XIX, and XXII were detected in the red devil fish skin. According to previous studies, these types are not typically observed in the skin of other fish species. Collagen diversity reflects variations in amino acid sequences, alpha-chain variation, and post-translational modifications arising from alternative promoters and splicing (Ricard-Blum, 2011). Consistent with this,

Table 3 Type of collagen identified in the red devil fish skin gelatin

No.	Type of collagen identified*	Molecular weight (Da)	Identified in the % acetic acid concentration used for extraction
1	Collagen, type I, α2	125.2	0, 1, 2, 3
2	Collagen, type I, α1a	132.6	0, 1, 2, 3
3	Collagen, type I, α1b	118.7	0, 1, 2, 3
4	Collagen, type VI, α3	38.4	0, 2
5	Collagen, type VI, α1	101.4	0, 1
6	Collagen, type VI, α2	105	0, 2
7	Collagen, type XII, α1b	317.3	0, 2
8	Collagen type XXVIII α2a	109.4	0, 2, 3
9	Collagen, type XII, α1a	327.5	0
10	Collagen, type II, α1b	110.9	0, 1, 2, 3
11	Collagen, type XI, α1b	150.5	0, 1, 2, 3
12	Collagen, type XXVIII α2b	101.7	0, 2, 3
13	Collagen, type X, α1b	62.9	0, 2
14	Collagen, type II, α1a	139.7	0, 2
15	Collagen, type XIX, α1	39.1	0
16	Collagen type V, α3a	166.6	0, 2
17	Collagen, type IX, α1b	54.3	0, 3
18	Collagen type XXII α1	132.8	0, 3
19	Collagen type IV, α1	119.2	2, 3
20	Collagen type V, α2a	121.9	2, 3

*Listed from most to least confident identification level.

multiple alpha chains, such as a1 and a2, were identified, along with a unique a3-subunit in type VI collagen. Several proteins containing collagen-like domains were identified in this study. The proteins were not collagen but were components of larger molecules. The complete structure of collagen typically comprises a central triple helix domain flanked by non-collagenous N- and C-terminal globular domains (NC domains), some of which have specific motifs, such as the EGF region (Boyle, 2018). The presence of these proteins has rarely been reported in previous studies, largely due to the limitations of the commonly used gel-based characterization method. In contrast, mass spectrometry enables a more comprehensive and confident identification of collagen types, collagen-related proteins, and the presence of other proteins in protein samples extracted from natural sources.

Collagen forms a triple helix, stabilized by glycine (Gly) at every third residue. Therefore, it is often hydrolyzed and used as a collagen tripeptide rather than a higher-molecular-weight peptide. The repeating unit follows a Gly–X–Y sequence, where X and Y are commonly proline (Pro) and hydroxyproline (Hyp), accounting for 50–60% of the sequence (Lee *et al.,* 2024). In a study of red devil fish skin collagen, trypsin was used to digest the protein extract before tandem mass spectrometry, enabling the identification of higher-molecular-weight peptides. Numerous Gly–X–Y motifs were detected, with greater variation in the X and Y positions. In type I collagen, the sequence coverage reached 12–19%, including positions where X and Y were amino acids other than

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Pro or Hyp (Table 4). Although Gly–X–Y is the main structural motif, the repeat may be interrupted by other residues, as listed in Table 4. These interruptions contribute to molecular recognition and structural flexibility within an otherwise uniform helix (Bella *et al.,* 2006). Differences in the X and Y residues and overall sequences among collagen types lead to distinct properties and functions.

The diversity of collagen types supports broad applications in nutraceutical, pharmaceutical, and cosmetic industries. Type I collagen, which is abundant in the skin, is widely used in cosmetic formulations because of its structural role in the skin, tendons, bones, ligaments, and other connective tissues. Hydrolyzed type I collagen is commonly used for antiaging effects, moisture retention, and wrinkle reduction (Prajaputra *et al.,* 2024). In pharmaceutical

Table 4 List of peptides of collagen type I

Collagen type I α1 (12% sequence coverage)
GAR-GEP-GAA-GAR
GDA-GAQ-GAR-GPE-GPS-GAR
GDK-GET-GEA-GER
GET-GEA-GER
GFS-GLD-GAK-GDS-GPA-GPK-GEA-GTP-GEN-GTP-GAM-GPR
GFT-GMQ-GPP-GPP-GAS-GEA-GPA-GAA-GPA-GPR
GPA-GAA-GSA-GKD-GMS-GLP-GPT-GPP-GPR
GPS-GPQ-GAR
GRA-GAT-GAA-GPT-GPA-GPP-GFP-GGP-GPK-GDA-GAQ-GAR
GAT-GEP-GRT-GEP-GLP-GAK-GMT-GSP-GSP-GPD-GK
GEA-GPP-GPA-GFA-GPP-GAD-GQP-GAK-GEP-GDN-GAK
GFS-GLD-GAK-GDS-GPA-GPK-GEA-GTP-GEN-GTP-GAM-GPR
GFT-GMQ-GPP-GPP-GAS-GEA-GPA-GAA-GPA-GPR
GHR-GFT-GMQ-GPP-GPP-GAS-GEA-GPA-GAA-GPA-GPR
GTM-GPT-GPA-GAP-GKD-GDV-GAQ-GPG-**PA***-GER
Collagen type I α2 (19% sequence coverage)
GAP-GPA-GPR
GAT-GPT-GLR
GEA-GPS-GSA-GAV-GPA-GAR
GEL-GPG-GPA-GPA-GQS-GPA-GPS-GPA-GPT-GAR
GER-GPS-GAK-GEL-GPG-GPA-GPA-GQS-GPA-GPS-GPA-GPT-GAR
GIP-GEP-GPA-GAA-GGK-GER
GPA-GAP-GPD-GGK-GEP-GLA-GAA-GGP-GHQ-GAG-GMP-GER-GAA-GGP-GPK
GPA-GPH-GPA-GKD-GRP-GAH-GTM-GAP-GPR
GPS-GAK-GEL-GPG-GPA-GPA-GQS-GPA-GPS-GPA-GPT-GAR
GPT-GEL-GAT-GLA-GAR
GAD-GNV-GPS-GPA-GPL-GAA-GPP-GFP-GGP-GPK-GEN-GPA-GAT-GPS-GPQ-GAR
GPP-GPD-GQD-GKP-GLP-GPP-GPP-GPP-GLG-GNF-**AAQYD***-GVK-**APDP***-GPG-**PM***-GLM-GPR

*Interruptions of Gly-X-Y

applications, collagen functions as a wound-healing material (Revert-Ros *et al.,* 2024) and carrier in drug delivery systems (Chacko & Sudheesh, 2023). Most pharmaceutical and cosmetic products use low-molecular-weight collagen peptides, typically below 3 kDa in size. Hydrolysis conditions and enzyme selection influence peptide length and amino acid composition, which determine biological activity. Fish collagen hydrolysates containing peptides of 1–3 kDa have shown anti-inflammatory effects by reducing TNF-α gene expression (Sivaraman, 2021). In addition to molecular weight, the amino acid sequence of a peptide plays a key role in determining its activity. The tripeptide GPO (Gly–Pro–Hyp) inhibits the activity of a hormone involved in blood glucose regulation (Hatanaka *et al.,* 2014). ACE-inhibitory activity increases when a hydrophobic amino acid occupies the C-terminus (Chen *et al.,* 2020). A key challenge is the isolation of specific collagen types or targeted peptide sequences. In the present study, 20 collagen molecular species were identified. When specific Gly–X–Y peptides are required for pharmaceutical or cosmetic use, high purity, homogeneity, and strong activity are essential, making extensive purification necessary.

CONCLUSIONS

In conclusion, gelatin was successfully derived from red devil fish skin using acetic acid treatment. The optimum value was achieved after soaking in 3% acetic acid. The characteristics of the gelatin complied with standards, such as GMIA 2019 and SNI 06-3735-1995, in terms of viscosity, gel strength, pH, moisture, and ash content. Mass spectrometry identification revealed 13 collagen types (20 molecular species) out of the 28 discovered. Proteins such as collagen IV NC1 domain, fibrillar collagen NC1 domain, collagen and calcium-binding EGF domain, procollagen galactosyltransferase, and procollagen proline were also identified. These results demonstrate the high potential of red devil fish skin as an alternative collagen source for both pharmaceutical and cosmetic applications.

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REFERENCES

Ahmad, T., Ismail, A., Ahmad, S. A., Khalil, K. A., Kumar, Y., Adeyemi, K. D., & Sazili, A. Q. (2017). Recent advances on the role of process variables affecting gelatin yield and characteristics with special reference to enzymatic extraction: A review. *Food Hydrocolloids*, 63, 85–96. https://doi.org/https://doi.org/10.1016/j.foodhyd.2016.08.007

Al Hajj, W., Salla, M., Krayem, M., Khaled, S., Hassan, H. F., & El Khatib, S. (2024). Hydrolyzed collagen: exploring its applications in the food and beverage industries and assessing its impact on human health - A comprehensive review. *Heliyon*, 10(16), e36433. https://doi.org/10.1016/j.heliyon.2024.e36433

Ambrosini, S., Prinsi, B., Zamboni, A., Espen, L., Zanzoni, S., Santi, C., Varanini, Z., & Pandolfini, T. (2022). Chemical characterization of a collagen-derived protein hydrolysate and biostimulant activity assessment of its peptidic components. *Journal of Agricultural and Food Chemistry*, 70(36), 11201–11211. https://doi.org/10.1021/acs.jafc.2c04379

AOAC International. (2019). Official methods of analysis of AOAC International (21st ed.). AOAC International.

Apone, F., Barbulova, A., & Colucci, M. G. (2019). Plant and microalgae-derived peptides are advantageously employed as bioactive compounds in cosmetics. *Frontiers in Plant Science*, 10, 756. https://doi.org/10.3389/fpls.2019.00756

Arslan, B., Xiong, Y. L., & Soyer, A. (2024). Antioxidant properties of bovine liver protein hydrolysates and their practical application in biphasic systems. *Journal of the Science of Food and Agriculture*, 104(5), 2980–2989. https://doi.org/10.1002/jsfa.13190

Astiana, I., Nurjanah, N., & Nurhayati, T. (2016). Characterization of acid-soluble

collagen from redbelly yellowtail fusilier fish skin (*Caesio cuning*). *Jurnal Pengolahan Hasil Perikanan Indonesia*, 19(1), 79–93. https://doi.org/10.17844/jphpi.2016.19.1.79

Atma, Y., & Ramdhani, H. (2018). Gelatin extraction from the indigenous pangasius catfish bone using pineapple liquid waste. *Indonesian Journal of Biotechnology*, 22(2), 86. https://doi.org/10.22146/ijbiotech.32472

Bella, J., Liu, J., Kramer, R., Brodsky, B., & Berman, H. M. (2006). Conformational effects of gly–X–gly interruptions in the collagen triple helix. *Journal of Molecular Biology*, 362(2), 298–311. https://doi.org/https://doi.org/10.1016/j.jmb.2006.07.014

Boyle, A.L. (2018). Applications of de novo designed peptides. *Peptide Applications in Biomedicine, Biotechnology and Bioengineering*, 51–86. https://doi.org/10.1016/B978-0-08-100736-5.00003-X

Castro Jácome, T., Alcantara, L., Montalvo-González, E., Chacón, A., Kalixto-Sánchez, M., Rivera, M., López-García, U., & Tovar-Pérez, E. (2021). Skin-protective properties of peptide extracts produced from white sorghum grain kafirins. *Industrial Crops and Products*, 167, 113551. https://doi.org/10.1016/j.indcrop.2021.113551

Chacko, I. A., & Sudheesh, M. S. (2023). 7 - Collagen for drug delivery applications. In R. Jayakumar & V. P. B. T.-N. B. in D. D. and T. E. Murali (Eds.), Woodhead Publishing Series in Biomaterials (pp. 157–177). Woodhead Publishing. https://doi.org/https://doi.org/10.1016/B978-0-323-98827-8.00024-2

Chen J., Sun S., Li Y., Liu R. (2020). Proteolysis of tilapia skin collagen: identification and release behavior of ACE-inhibitory peptides. *LWT*, 139: 110502. https://doi.org/10.1016/j.lwt.2020.110502

Fu, T.-K., Kuo, P.-H., Lu, Y.-C., Lin, H.-N., Wang, L. H.-C., Lin, Y.-C., Kao, Y.-C., Lai, H.-M., & Chang, M. D.-T. (2020). Cell penetrating peptide as a high-safety anti-inflammation ingredient for cosmetic applications. *Biomolecules*, 10(1). https://doi.org/10.3390/biom10010101

Fujita, A., Kawakita, H., Saito, K., Sugita, K., Tamada, M., & Sugo, T. (2003). Production of tripeptide from gelatin using collagenase-immobilized porous hollow-fiber membrane. *Biotechnology Progress*, 19(4), 1365–1367. https://doi.org/10.1021/bp030004i

Gaikwad, S., & Kim, M. J. (2024). Fish by-product collagen extraction using different methods and their application. *Marine Drugs*, *22*(2), 60. https://doi.org/10.3390/md22020060

Gelatin Manufacturers Institute of America (GMIA). (2012). *Gelatin Handbook*. United States of America: Gelatin Manufacturers Institute of America.

Gorlov, I. F., Titov, E. I., Semenov, G. V., Slozhenkina, M. I., Sokolov, A. Y., Omarov, R. S., Goncharov, A. I., Zlobina, E. Y., Litvinova, E. V., & Karpenko, E. V. (2018). Collagen from porcine skin: a method of extraction and structural properties. *International Journal of Food Properties, 21*(1), 1031–1042. https://doi.org/10.1080/10942912.2018.1466324

Goudie, K. J., McCreath, S. J., Parkinson, J. A., Davidson, C. M., & Liggat, J. J. (2023). Investigation of the influence of pH on the properties and morphology of gelatin hydrogels. *Journal of Polymer Science*, 61(19), 2316–2332. https://doi.org/https://doi.org/10.1002/pol.20230141

Habibie, S. A., Djumanto, & Rustadi. (2015). Penggunaan otolit untuk penentuan umur dan waktu pemijahan ikan red devil, *Amphilophus labiatus* [Günther, 1864] di waduk sermo, Yogyakarta. *Jurnal Iktiologi Indonesia*, 15(2), 87–98. https://doi.org/https://doi.org/10.32491/jii.v15i2.63

Han Q., Koyama T., Watabe S., Ishizaki S. (2024). Functional and structural properties of type v collagen from the skin of the shortbill spearfish (*Tetrapturus angustirostris*). *Molecules*, 29(11):2518. https://doi.org/10.3390/molecules29112518

Hatanaka T, Kawakami K, Uraji M. (2014). Inhibitory effect of collagen-derived

tripeptides on dipeptidylpeptidase-IV activity. *J. Enzyme Inhib. Med, Chem,*; 29: 823-828. https://doi.org/10.3109/14756366.2013.858143

Henriques, A., Vázquez, J. A., Valcarcel, J., Mendes, R., Bandarra, N. M., & Pires, C. (2021). Characterization of protein hydrolysates from fish discards and by-products from the north-west spain fishing fleet as potential sources of bioactive peptides. *Marine Drugs*, 19(6). https://doi.org/10.3390/md19060338

Jakubčinová, K., Simonović, P., Števove, B., Čanak Atlagić, J., & Kováč, V. (2017). What can morphology tell us about ecology of four invasive goby species?. *Journal of Fish Biology*, 90(5), 1999–2019. https://doi.org/10.1111/jfb.13283

Jaziri, A. A., Muyasyaroh, H., & Firdaus, M. (2019). Karakteristik fisikokimia gelatin kulit ikan ayam-ayam (*Abaliste stellaris*) dengan pra-perlakuan konsentrasi asam sitrat. *Buana Sains*, 19(1), 1. https://doi.org/10.33366/bs.v19i1.1522

Kraeling, M. E. K., Zhou, W., Wang, P., & Ogunsola, O. A. (2015). In vitro skin penetration of acetyl hexapeptide-8 from a cosmetic formulation. *Cutaneous and Ocular Toxicology*, 34(1), 46–52. https://doi.org/10.3109/15569527.2014.894521

Kumazawa, Y., Taga, Y., Iwai, K., & Koyama, Y.-I. (2016). A Rapid and simple LC-MS method using collagen marker peptides for identification of the animal source of leather. *Journal of Agricultural and Food Chemistry*, 64(30), 6051–6057. https://doi.org/10.1021/acs.jafc.6b02132

Larder, C. E., Iskandar, M. M., Sabally, K., & Kubow, S. (2022). Complementary and efficient methods for di- and tri-peptide analysis and amino acid quantification from simulated gastrointestinal digestion of collagen hydrolysate. *LWT*, 155, 112880. https://doi.org/https://doi.org/10.1016/j.lwt.2021.112880

Lassoued, I., Jridi, M., Nasri, R., Dammak, A., Hajji, M., Nasri, M., & Barkia, A. (2014). Characteristics and functional properties of gelatin from thornback ray skin obtained by pepsin-aided process in comparison with commercial halal bovine gelatin. *Food Hydrocolloids*, 41, 309–318. https://doi.org/https://doi.org/10.1016/j.foodhyd.2014.04.029

Lee, J. S., Yoon, Y. C., Kim, J. M., Kim, Y.-H., Kang, Y.-H., & Shin, Y. C. (2024). Liquid collagen from freshwater fish skin ameliorates hydration, roughness and elasticity in photo-aged skin: a randomized, controlled, clinical study. *Nutrition Research and Practice*, 18(3), 357–371. https://doi.org/10.4162/nrp.2024.18.3.357

Lee, Y. I., Lee, S. G., Jung, I., Suk, J., Lee, M.-H., Kim, D.-U., & Lee, J. H. (2022). Effect of a topical collagen tripeptide on antiaging and inhibition of glycation of the skin: a pilot study. *International Journal of Molecular Sciences*, 23(3). https://doi.org/10.3390/ijms23031101

Liu, D., Wei, G., Li, T., Hu, J., Lu, N., Regenstein, J. M., & Zhou, P. (2015). Effects of alkaline pretreatments and acid extraction conditions on the acid-soluble collagen from grass carp (*Ctenopharyngodon idella*) skin. *Food Chemistry*, 172, 836–843. https://doi.org/10.1016/j.foodchem.2014.09.147

Noorzai, S., & Verbeek, C. J. R. (2020). Collagen: From waste to gold. In T. P. Basso, T. O. Basso, & L. C. Basso (Eds.), Biotechnological Applications of Biomass (Chapter 6). IntechOpen. https://doi.org/10.5772/intechopen.94266

Nugraheni, A. W., Anggo, A. D., & Dewi, E. N. (2021). Pengaruh jenis asam terhadap karakteristik gelatin kulit ikan ayam-ayam (*Abalistes stellaris*). *Jurnal Ilmu dan Teknologi Perikanan*, 3(2), 78–85. https://doi.org/https://doi.org/10.14710/jitpi.2021.13144

Nurilmala, M., Nasirullah, M. T., Nurhayati, T., & Darmawan, N. (2021). Karakteristik fisik-kimia gelatin dari kulit ikan patin, ikan nila, dan ikan tuna. *Jurnal Perikanan Universitas Gadjah Mada*, 23(1), 71. https://doi.org/10.22146/jfs.59960

Nurilmala, M., Suryamarevita, H., Husein Hizbullah, H., Jacoeb, A. M., & Ochiai, Y. (2022). Fish skin as a biomaterial for halal collagen and gelatin. *Saudi*

Journal of Biological Sciences, 29(2), 1100–1110. https://doi.org/10.1016/j.sjbs.2021.09.056

Nurjanah, A. (2017). Kajian karakteristik fisik-kimia dan sensori opak dari jagung manis dan kulit ikan nila. *Jurnal Rekayasa Pangan dan Pertanian* 6(1), 1–8.

Oslan, S. N. H., Li, C. X., Shapawi, R., Mokhtar, R. A. M., Noordin, W. N. M., & Huda, N. (2022). Extraction and characterization of bioactive fish by-product collagen as promising for potential wound healing agent in pharmaceutical applications: current trend and future perspective. *International Journal of Food Science*, 2022(1), 9437878. https://doi.org/https://doi.org/10.1155/2022/9437878

Portanguen, S., Dumoulin, C., Duconseille, A., Meurillon, M., Sicard, J., Théron, L., Chambon, C., Sayd, T., Mirade, P.-S., & Astruc, T. (2023). Impact of water content and bloom index on gelatin glycation. *Food Hydrocolloids*, 145, 109096. https://doi.org/https://doi.org/10.1016/j.foodhyd.2023.109096

Prajaputra, V., Isnaini, N., Maryam, S., Ernawati, E., Deliana, F., Haridhi, H. A., Fadli, N., Karina, S., Agustina, S., Nurfadillah, N., Arisa, I. I., Desiyana, L. S., & Bakri, T. K. (2024). Exploring marine collagen: Sustainable sourcing, extraction methods, and cosmetic applications. *South African Journal of Chemical Engineering*, 47, 197–211. https://doi.org/https://doi.org/10.1016/j.sajce.2023.11.006

Raikou, V., Kalogria, E., Varvaresou, A., Tsirivas, E., & Panderi, I. (2021). Quantitation of acetyl hexapeptide-8 in cosmetics by hydrophilic interaction liquid chromatography coupled to photo diode array detection. *In Separations* 8(8). https://doi.org/10.3390/separations8080125

Revert-Ros, F., Ventura, I., Prieto-Ruiz, J. A., Hernández-Andreu, J. M., & Revert, F. (2024). The versatility of collagen in pharmacology: targeting collagen, targeting with collagen. *International Journal of Molecular Sciences* 25 (12). https://doi.org/10.3390/ijms25126523

Ridhay, A., Musafira, Nurhaeni, & Khasanah, B. N. (2016). Pengaruh variasi jenis asam terhadap rendemen gelatin dari tulang ikan cakalang (*Katsuwonus pelamis*). *Kovalen*, 2(2), 44–53.

Salvatore, L., Gallo, N., Natali, M. L., Campa, L., Lunetti, P., Madaghiele, M., Blasi, F. S., Corallo, A., Capobianco, L., & Sannino, A. (2020). Marine collagen and its derivatives: Versatile and sustainable bio-resources for healthcare. *Materials Science and Engineering*: C, 113, 110963. https://doi.org/https://doi.org/10.1016/j.msec.2020.110963

Santhanam, R., Ramesh, S., Nivendhitha, S., Balasundari, S. (2022). Pharmaceuticals and nutraceuticals from fish and fish wastes (1st ed.). *Apple Academic Press*. https://doi.org/10.1201/9781003180548

Shaik, M. I., Rahman, S. H. A., Yusri, A. S., Ismail-Fitry, M. R., Kumar, N. S. S., & Sarbon, N. M. (2024). A review on the processing technique, physicochemical, and bioactive properties of marine collagen. *Journal of Food Science*, 89(9), 5205–5229. https://doi.org/10.1111/1750-3841.17273

Sivaraman, K., & Shanthi, C. (2021). Role of fish collagen hydrolysate in attenuating inflammation— An in vitro study. *Journal of Food Biochemistry*. 00, e13876. https://doi.org/10.1111/jfbc.13876

Tatsumi, T., Sasamoto, K., Matsumoto, T., Hirano, R., Oikawa, K., Nakano, M., Yoshida, M., Oisaki, K., & Kanai, M. (2023). Practical N-to-C peptide synthesis with minimal protecting groups. *Communications Chemistry*, 6(1), 231. https://doi.org/10.1038/s42004-023-01030-0

Tinoco, A., Martins, M., Cavaco-Paulo, A., & Ribeiro, A. (2022). Biotechnology of functional proteins and peptides for hair cosmetic formulations. *Trends in Biotechnology*, 40(5), 591–605. https://doi.org/10.1016/j.tibtech.2021.09.010

Valcarcel, J., Hermida-Merino, C., Piñeiro, M. M., Hermida-Merino, D., & Vázquez, J. A. (2021). Extraction and characterization

of gelatin from skin by-products of seabream, seabass and rainbow trout reared in aquaculture. *International Journal of Molecular Sciences* 22(22). https://doi.org/10.3390/ijms222212104

Wu Z., Korntner S.H., Mullen A.M., Zeugolis D.I. (2021). Collagen type II: From biosynthesis to advanced biomaterials for cartilage engineering. *Biomater Biosyst*, 4:100030. https://doi.org/10.1016/j.bbiosy.2021.100030

Zhang, X., Xu, S., Shen, L., & Li, G. (2020). Factors affecting thermal stability of collagen from the aspects of extraction, processing and modification. *Journal of Leather Science and Engineering*, 2(1), 19. https://doi.org/10.1186/s42825-020-00033-0