International Journal on Advanced Science Engineering Information Technology

Vol.15 (2025) No. 1
ISSN: 2088-5334

Evaluating the Physicochemical and Structural Properties of Collagen from Lizardfish (*Saurida tumbil* Bloch, 1795) Skin Prepared with the Optimal Enzymatic Process: in Comparison with Recent Studies

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***Abstract*—Marine fish byproducts are quiet potential raw material for protein-based commodities like collagen. Due to their abundant resources, safer from many zoonosis, and great biocompatibility, marine collagens are regarded as an ideal candidate biopolymer for such research and medical applications. Although lizardfish (*Saurida tumbil* Bloch, 1795) skin collagen had been extracted optimally by our previous research, the characterization of its physicochemical and microstructural properties has been reported yet. Thus, the aim of this article was to evaluate the characteristic of the pepsin soluble collagen (PSC) from lizardfish skin prepared using the optimal extraction process. The obtained PSC was categorized as type I collagen, as evidenced by the existence of two alpha chains following electrophoresis in acrylamide gel, and its imino acid was 187.54 residues/1000 residues. Analysis using ultraviolet-visible (UV-vis) techniques verified that the extracted collagen displayed an absorption peak at 232 nm, consistent with various fish collagens. The triple helical structure of PSC was preserved after being confirmed through X-ray diffraction (XRD) and Fourier transforms infrared spectroscopy (FTIR) tests. In terms of thermostability, the type I PSC exhibited high maximum transition temperature (T_{max} = 36.74°C). At low NaCl and acidic conditions, the relative solubility of optimized collagen was high (more than 80%). The PSC showed many multilayered forms with dense sheet-like film. Overall, the type I collagen processed optimally from the skin of lizardfish could be used as a promising source of biomaterials for industrial viewpoint.**

***Keywords*— Tropical marine species; optimized collagen; physicochemical properties; morphological structure evaluation.**

Manuscript received 20 Jul. 2024; revised 17 Dec. 2024; accepted 18 Jan. 2025. Date of publication 28 Feb. 2025.

I. INTRODUCTION

Collagen is considered a great source for biomaterial-based product development. It is extensively applied in a variety of medical and healthcare fields. In recent reports, for instance, some nanomaterials derived from different collagen sources have been developed as wound dressings, bandages, and tissue engineering scaffolds [1]. In addition, collagen is applicable in developing drug delivery systems, treating obesity, hypertension, and diabetes [2]. As a prospecting biomaterial, collagen is a fibrous protein that contributes to approximately one-third of total protein content, and it serves as the major structural component of skin, bones, ligaments, and tendons in vertebrates [3]. Collagen is characterized by its distinct triple helical configuration that twists to the right. This structure is made up of three helices resembling left-handed polyproline strands. Within each of these helices, there is a repeating Glycine-X-Y pattern, where the amino acids X and Y are frequently identified as proline and hydroxyproline [4]. So far, around twenty-nine types of collagens have been examined and those types were differed according to the structure of polypeptides, amino acids

sequences and molecular natures [5]. From these collagens, type I has attracted considerable intention among scientists due to its abundance and having certain traits, *i.e.*, low antigenicity, strong biocompatibility, and high biodegradability [2]. Type I collagen is typically sourced from the skin and bones of terrestrial animals, notably cattle and pigs. Nevertheless, the employment of cattle as a source has raised concerns among consumers due to documented diseases that can spread, such as foot-and-mouth disease (FMD) and bovine spongiform encephalopathy (BSE). In addition to these concerns, there are considerations regarding religious customs that prohibit the use of products derived from porcine for individuals practicing Islam and Judaism, whilst bovine-based commodities cannot be consumed by Hindus [6]-[7]. To tackle these limitations, an effective effort in finding alternative sources is needful.

Marine fish by-products, such as head, skin, frames, fins, scales, and swim bladder, are mostly used for collagen production because of their abundant resources and affordable materials, and the obtained collagen tend to be safer from many zoonosis, great biodegradability, and biocompatibility [8]. About 60% of fish by-products produced from seafood processing plants, representing 110.4 million tons from the global fish production [9]. By this number, underutilized fish by-products would be dumped in landfill or aquatic sites, leading to serious environmental pollution. The fish by-products generated from industrial processing plants are abundant in organic acids and fall into the category of specialized waste, necessitating treatment before they can be disposed of. This procedure can lead to increased expenses for the seafood industry [10]-[11]. On the other hand, most utilized fish by-products, such as silages, fertilizers, fish meals and biofuels were still considered as low value-added products [12]. Therefore, converting fish byproducts into collagen and collagen-based products has attracted increasing attention from researchers and processors. Numerous studies have extracted collagens from various marine fish species. For examples, threadfin bream (*Nemipterus japonicus*) [13], purple-spotted bigeye (*Priacanthus tayenus*) [14], barracuda (*Sphyraena* sp.) [15], puffer fish (*Lagocephalus inermis*) [16], unicorn fish (*Naso reticulatus*) [8],[17],[18], sin croaker (*Johniecop sina*) [19], barramundi (*Lates calcarifer*) [20], parrotfish (*Scarus sordidus*) [21], golden pompano (*Trachinotus ovatus*) [22], sardinella (*Sardinella fimbriata*) [23], and sharpnose stingray (*Dasyatis zugei*) [24]. Pepsin can be applied either independently or in conjunction with different concentrations of acetic acid. Literally, pepsin can effectively cleave the telopeptide regions within the triple helical structure of collagen molecules, thereby aiding in the release of collagen peptides into the solution and consequently enhancing extraction yields [20]. Collagen obtained through pepsin-assisted extraction is referred to as pepsin solubilised collagen (PSC).

Lizardfish (*Saurida tumbil*) is an important tropical marine fish species in Malaysia. It is main raw material for seafood processing industries, particularly surimi production, owing to its great gel strength and whiter flesh [12]. According to the Department of Fisheries Malaysia [25], around 85,000 tons of lizardfish were landed from 2018 to 2021. With a large quantity of by-products after processing, transforming these fish wastes into collagen is a right choice. Our previous findings successfully extracted and did the optimization process of the lizardfish's collagen with a high extraction yield was 21.80%. However, its physicochemical and structural properties have yet to be evaluated. In this study, therefore, we focused on the characteristics of lizardfish skin collagen prepared using the optimal conditions of PSC extraction (*i.e.*, pepsin concentration (1.87%), liquid-solid ratio (24.90 mL/g), and hydrolysis time (38.09 h).

II. Materials and Method

A. Chemicals

Chemicals used in this study were of analytical grade. For instances, pepsin (1:10,000) from bovine origin was obtained from HiMedia (Maharashtra, India). Lowry reagent was supplied from Sigma Aldrich, USA. Acetic acid (AcOH), Coomassie Blue R-250, sodium dodecyl sulphate, acrylamide powder, Folin-Ciocalteu reagent and N,N,N',N'-tetramethyl ethylene diamine were supplied from Merck, Germany.

B. Extraction of Pepsin Soluble Collagen (PSC)

The extraction procedure was adopted by our established method from previous studies [6],[7],[26],[27]. All steps of collagen extraction were conducted in a walk-in chiller, and the whole process is displayed in Fig.1.

C. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoresis process of the obtained PSC from lizardfish (*S. tumbil*) skin was carried out according to the established approach [28]. About two milligrams of lyophilized sample were immersed in 5% sodium dodecyl sulfate solution and the mixture was vortexed well. Then, the mixed sample was allowed to high temperature (85°C) for 1 h. The heated PSC was spun at 8,500× g for 10 min to eliminate undissolved matters. The supernatant (dissolved sample) was further pipetted out about 15 µL into a microcentrifuge tube and then added with 15 µL of prepared sample buffer. Afterwards, the mixture was heated at the same temperature for about 3 min. Following this, the prepared SDS-polyacrylamide gel, which comprised a 4% stacking gel and a 7.5% resolving gel, was loaded with the PSC sample. The gel was then subjected to electrophoresis under a consistent voltage of 120 volts for approximately 90 minutes. Upon completion of the electrophoresis process, the gel was immersed in a staining solution for approximately 5 minutes, followed by a rinse in a destaining solution. The electrophoretic band patterns of the PSC sample were then compared to those of the protein marker.

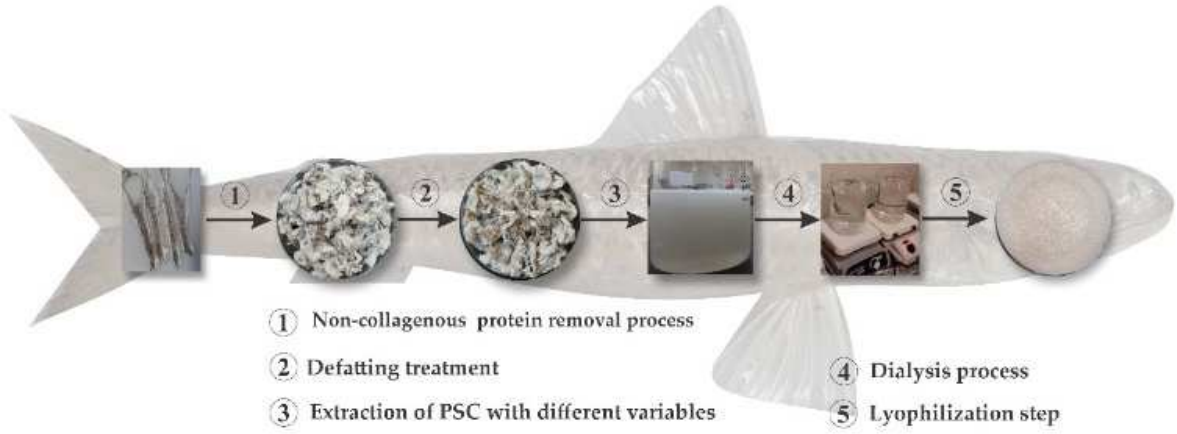

Fig. 1 The PSC extraction process

D. Amino Acids Analysis

The amino acid composition of PSC produced under the optimal conditions was assessed through a high-performance liquid chromatography (HPLC). Around 100 mg PSC samples were hydrolyzed with 5 mL of 6 M hydrochloric acid at 110°C in a sealed tube. The hydrolysates were then injected into an HPLC instrument. The amino acid composition of PSC was quantified as the number of residues per 1000 total residues [29].

E. Ultraviolet−visible (UV-Vis) evaluation

The UV-visible spectrum of the optimized collagen was adapted from our previous study [7]. About 10 mg of lyophilized collagen was solubilized in an acetic acid solution. The solubilized sample was thoroughly vortexed for around 3 min at room temperature. Afterward, the mixture was subjected to centrifugation at 8,500× g for 20 min. Following centrifugation, the solubilized PSC sample was transferred into a quartz cell and subjected to scanning within the wavelength range of 350 nm to 190 nm, utilizing a baseline standard of an acetic acid solution.

F. Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy (ATR–FTIR)

The obtained PSC sample was analyzed using an FTIR spectrometer. Approximately 20 mg of lyophilized PSC was positioned on the crystal cell bench of the IR spectrometer. The spectrum was configured within a wavenumber range of 4000–1000 cm^{-1}, employing a resolution of 2 cm^{-1} for 32 scans. The absorption peaks of interest were determined using the Agilent Microlab system [30].

G. X-ray Diffraction (XRD) Analysis

To analysis the diffraction angle of lizardfish skin collagen optimized under the optimal conditions, a XRD machine was used in a present study. The freeze-dried sample was subjected to scanning using copper Kα as the x-ray source. The range of scanning was configured from 10° to 40° (2θ), operating at a speed of 0.06° per second. The analysis was conducted with an electric current of 50 mA and a tube voltage of 40 kV, both of which were appropriately adjusted. The sample spectrum was collected and then assessed by comparing to other previous findings [31].

H. Differential Scanning Colorimetry (DSC) Assessment

The thermal stability of PSC was assessed under a DSC instrument, SDC7, Perkin−Elmer, USA. The dried collagen was hydrated by adding 40 volumes of distilled water and kept for 48 h in a refrigerator. After that, the hydrated sample was prepared by weighing around 8.0 mg into a Perkin-Elmer pan and subsequently sealed. Prior to scanning, the instrument was initially calibrated with an indium, and the sealed pan with the hydrated sample was scanned across a temperature range of 25°C to 45°C. An empty sealed pan was positioned as a reference in the right cell holder of DSC instrument. The endothermic peak of thermogram represents a maximum transition temperature (T_{max}), while the thermogram area denotes a denaturation enthalpy (ΔH) value [32].

I. Field Emission Scanning Electron Microscopy (FE-SEM)

A field emission scanning electron microscopy (FESEM), JEOL JSM-7900F, Japan, was applied to picture the microstructure of PSC sample. Prior to being captured, the freeze-dried PSC sample was readied for analysis by applying a thin layer of gold through sputter coating for a duration of 5 min using a coater. The gold-coated sample was then imaged with various magnifications (250, 1000, 15000×) [27].

J. Solubility Analysis

The solubility of the optimized sample was treated at various levels of sodium chloride (NaCl) and pH. Under NaCl concentrations, five milliliters of collagen solution were transferred into five milliliters of different salt concentrations, ranging from 0 to 60 g/L. The mixtures were subsequently stirred for approximately 60 min. Afterwards, the stirred PSC solutions were subjected to spinning at 8,500× g for 20 min. The resulting supernatants from each treatment were collected in new centrifuge tubes for further protein analysis. Regarding the pH treatment, the freeze-dried PSC was dissolved with an acid solution, and the mixtures were adjusted to different pH levels. Following pH adjustment, the collagen solutions were continuously stirred for 1 h and then centrifuged at 8,500× g for 20 min. The solubilized collagen in the various pH treatments was prepared for protein measurement using an established procedure [32]. To determine relative solubility

(%), all solubilized PSC samples were analyzed their protein content [33].

III. Results and Discussion

In this article, we highlighted the characteristics of biochemical and structural properties for pepsin soluble collagen derived from lizardfish (*S. tumbil*) skin by-product prepared using the optimal process. As previously reported, the PSC yielded 21.83% with optimal conditions composing of pepsin (1.87%), liquid-solid ratio (24.90 mL/g) and time (38.09 h) [34], and it was considered as a higher compared to some works in this fields [22],[35].

A. Protein Profile

The electrophoretic image of the PSC derived from the lizardfish skin is shown in Fig. 2. The molecular weight of α1 and α2 chains was predicted to be around 134.6 kDa and 115.9 kDa, respectively. In addition, our collagen sample was found the β− and γ−chain with MWs were approximately 246.4 kDa and 303.1 kDa, respectively, indicating dimer and trimer bands. The protein profile from PSC was also comparable to the commercial calf-skin collagen (CSC), as standard.

The obtained collagen was categorized as a type I due to having two alpha chains, as referred to in the study of Benjakul et al. [36]. Typically, the intensity of alpha-1 chain was two-fold higher compared to that of α2 chain, and it was comparable to other previous pepsin-solubilized fish skin collagens. For examples, purple-spotted bigeye snapper (*P. tayenus*) (α1 = 120 kDa and α2 = 110 kDa) [35], red stingray (*D. akajei*) (α1 = 129 kDa and α2 = 119 kDa) [31], and barramundi (*L. cacarifer*) (α1 = 118 kDa and α2 = 105 kDa) [29]. In terms of band intensity, the PSC exhibited greater than that of standard collagen (derived from calf skin). This suggests that the PSC extracted from lizardfish skin potentially contained a higher proportion of intra- and inter-crosslinks, indicating a more interconnected structure [36].

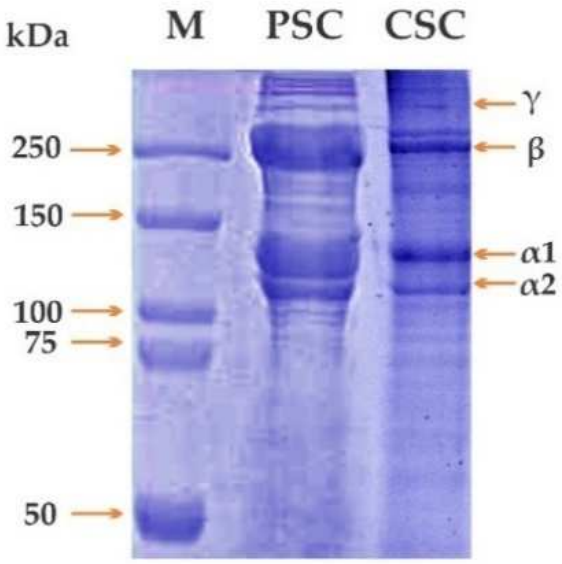

Fig. 2 Electrophoretic patterns of the selected collagen from the lizardfish (*S. tumbil*) skin and the standard. M, representing a protein marker; PSC and CSC are pepsin-solubilized collagen (this study) and calf skin collagen (commercial product), respectively.

B. Amino Acids Composition

The amino acid compositions of PSC extracted from lizardfish skin were evaluated and expressed as residues per 1000 total residues. Among these, glycine (203.7 residues/1000 residues) emerged as the predominant amino acid in the optimized lizardfish skin collagen. This was followed by alanine (118.4 residues/1000 residues), proline (104.2 residues/1000 residues), arginine (99.2 residues/1000 residues), glutamic acid (94.2 residues/1000 residues), and hydroxyproline (83.4 residues/1000 residues). These amino acid profiles were found to be comparable to those of PSC samples obtained from other collagen sources, as enumerated in Table 1. Regarding the analysis of imino acids (specifically proline and hydroxyproline), our data indicated that the content of imino acids in the PSC sample extracted from tropical lizardfish was 187.54 residues/1000 residues. Besides that, the levels of cysteine, histidine, tryptophan and tyrosine recorded in this work were lower than other amino acids, indicating that our PSC sample is associated with the type I collagen class.

For amino acids, generally, a left-handed triple helical collagen (type I class) is governed by the repetitive amino acid sequence, where Glycine-X-Y is the most abundant order (X and Y are repeatedly proline or hydroxyproline). However, this pattern is not consistent within the initial fourteen amino acid residues from the N-terminus and the first ten residues from the C-terminus of collagen molecules [37]. In our findings, the content of imino acids was recorded at 187.54 residues per 1000 residues. Although this value was lower than that of certain tropical fish species such as golden pompano (*T. blochii*) (198.2 residues/1000 residues) [22] and tilapia (*O. niloticus*) (191.51 residues/1000 residues) [38], it was higher than the imino acid contents observed in temperate fish species like Pacific cod (*G. macrocephalus*) (159.0 residues/1000 residues) [39] and Spanish mackerel (*S. niphonius*) [40], their imino acid contents (159.0 residues/1000 residues and 180.8 residues/1000 residues, respectively) were relatively lower than those of tropical fish collagen. However, despite the relatively higher imino acid content observed in all tropical fish sources, as presented in Table 1, their imino acid levels still fell below those seen in calf skin collagen (mammalian collagen). The stability of the collagen's triple helical structure is enhanced by a greater concentration of imino acids due to the constraints imposed by the pyrrolidine rings in imino acids, which limit changes in the secondary structure of the polypeptide chain. Additionally, this stability is partially maintained by the hydrogen bond capacity facilitated through the hydroxy group of hydroxyproline. Thus, the PSC optimized with optimal extraction condition is probably less stable than the other two collagens from tropical fish and calf skin collagen, but more stable from the mentioned cold-water fish species. In addition, a lower content of histidine, tryptophan, and tyrosine in the PSC from lizardfish skin was in accordance with the PSC derived from different fish sources. In other words, these PSC products contained a limited amount of disulfide bonds and low aromatic amino acids [7],[27].

TABLE I
THE AMINO ACIDS COMPOSITION OF PSC FROM VARIOUS FISH SKINS AND CALF SKIN AND THEIR VALUE IS EXPRESSED AS RESIDUES PER 1000 TOTAL RESIDUES

Amino acids	*S. tumbil*	*T. blochii* [1]	*O. niloticus* [2]	*G. macrocephalus* [3]	*S. niphonius* [4]	Calf skin [5]
Alanine	118.42	137.2	180.12	107	127.8	78.82
Arginine	99.22	53.1	40.24	51	48.7	73.28
Aspartic acid	51.48	45.2	50.21	52	47	68.73
Cysteine	3.01	0.5	0.94	2	0	1.08
Glutamic acid	94.19	71.1	90.73	80	66	109.35
Glycine	203.71	332	230.38	355	346.7	241.65
Histidine	17.41	5.3	10.37	10	6.8	11
Hydroxyproline	83.37	73.7	70.99	66	69.3	99
Isoleucine	11.65	8.8	10.36	12	13.7	17
Leucine	23.51	21	20.4	22	23.6	33
Lysine	41.29	26.7	30.78	27	28.5	4
Methionine	28.16	9.3	7	6	11.6	0
Phenylalanine	24.21	12.5	10.84	12	13.9	24
Proline	104.17	124.5	120.52	92	111.5	117
Serine	31.88	31.7	20.49	60	31.9	35
Threonine	34.11	24.6	20.15	22	19.3	20
Tyrosine	4.17	2.1	3.5	3	2.9	6
Valine	26.05	20.1	20.37	19	23.2	28
Imino acids	187.54	198.2	191.51	159	180.8	216

[1] [22]
[2] [38]
[3] [39]
[4] [40]
[5] [41]

C. IR Spectra Analysis

FTIR spectrum of the PSC produced using the optimal extraction conditions was studied. There were five prominent peaks characterizing the collagen helices, as noted in Fig. 3A. Amide A is often known as the N-H stretching vibrations with H-bonds, and commonly occupied at wavenumber of 3200–3440 cm^{-1} [42] as detected in the figure. In terms of Amide B, it reflects the CH_2 asymmetric stretching [39], which was found at the wavenumber area of 2933.49 cm^{-1}. Next, the Amide I region obtained from the PSC sample was recorded at approximately 1628.89 cm^{-1}, aligning with the stretching vibration of backbone carbonyl (C = O) groups in collagen's polypeptide chain. This frequency is often linked with the secondary structure of proteins, as observed in previous studies [43]. The robust presence of the Amide I band in lizardfish skin collagen was consistent with findings from other investigations [20],[21],[44]. Furthermore, the regions of Amides II and III are widely employed for evaluating the triple helical configuration of collagen [45]. Specifically, Amide II is recognizable within the wavenumber range of 1500−1600 cm^{-1}, while Amide III appears between 1200−1350 cm^{-1} [46]. These Amide regions correspond to N–H bending vibrations coupled with C–N and C–H stretching vibrations, respectively.

Those peaks, particularly Amides I−III, served as indicators for assessing the stability of the triple helical structures present in the PSC obtained from lizardfish skin. Some researchers have indicated that the stability of the triple helix is maintained when the difference in wavenumbers of Amide I and Amide II ($\Delta v = vI - vII$) remains below 100 cm^{-1} [7]. Our findings demonstrate that the triple helical structure within the optimized PSC was preserved, evidenced by a Δv value of 87.6 cm^{-1}. Another method involves assessing the ratio of the Amide III peak to the 1450 cm^{-1} band (AIII/A1450), as outlined by Doyle [47]. Our analysis confirms that the triple helical structure remained stable throughout the optimization process, as indicated by the absorption ratio value of approximately 1.0. This suggests that the use of optimized extraction conditions facilitates collagen extraction without causing any alteration in its underlying structure.

D. UV-vis Absorption Spectra

The UV-visible absorption spectrum is commonly used to examine the distinctive attributes of the polypeptide chains within fish collagen. Specifically, for type I collagen, a strong connection exists with functional groups such as amides ($CONH_2$), carboxyl (-COOH), and carbonyl (C=O), as previously elucidated [48], and also confirmed that the prominent peak of collagen product is generally located at around 210−240 nm [7]. The PSC observed in our study had a significant UV-vis absorption peak at about 232 nm (Fig. 3B), while at the wavelengths of 300−250 nm, the absorption peak of PSC decreased sharply.

It could be noted that the low peak of PSC from the lizardfish (*S. tumbil*) skin composed of aromatic amino acids, and this observation aligns with the expected composition of aromatic amino acids (Fig.3). Previous reports relating to the UV−vis absorption spectra in the PSC derived from different fish species also agreed with our findings, such as tilapia (*O. niloticus*) (232−234 nm) [30], red stingray (*D. akajei*) (228 nm) (Chen et al., 2019), loach (*M. anguillicaudatus*) (218 nm) [49] and puffer fish (*L. inermis*) (230) [16].

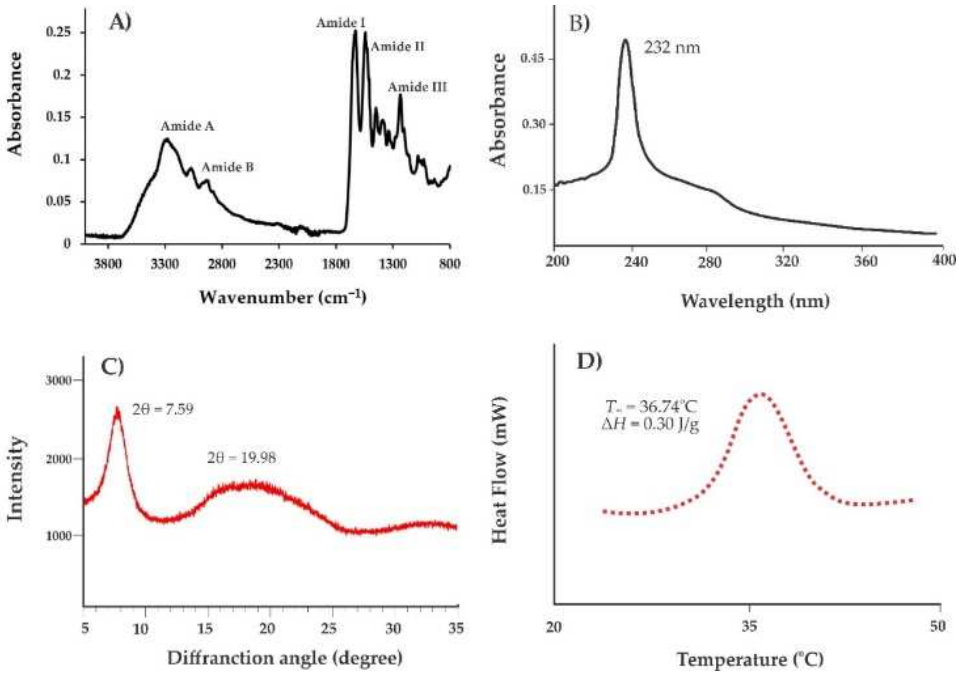

Fig. 3 Various analyses of optimized PSC from the lizardfish (*S. tumbil*) skins. A) FTIR, B) UV-vis absorption spectra, C) XRD image and D) Thermogram picture.

E. XRD Evaluation

Fig. 3C. illustrates the XRD result of lizardfish (*S. tumbil*) skin collagen prepared with the optimal extraction conditions. In general characteristic of collagen helices, there are two significant peaks detected *i.e.*, sharp and broad peaks, respectively [29]. As portrayed in the graph, the prominent peak of PSC sample was pointed at the diffraction angle (2θ) of 7.59° and 19.98°. Those peaks were in accordance with the PSC isolated from the skins of Pacific cod (*G. macrocephalus*) [39], barramundi (*L. calcalifer*)[29], golden pompano (*T. blochii*) [22] and tilapia (*O. niloticus*) [50]. According to [51], the peak one (around 7°) describes the spacing of each molecular chains in collagen helices, and its distance was around 1.2 nm after being measured using the Bragg formula. Another distinct peak, labelled as peak two (at approximately 20°), corresponds to the spacing between the frameworks of the collagen's triple helix. The findings exhibited a resemblance to the dimensions of a single left-handed and triple helical arrangement within collagen molecules. This alignment underscores the retention of the triple helix in the optimized PSC derived from lizardfish skin.

F. Thermostability Profile

The DSC curve of the optimized PSC derived from the lizardfish (*S. tumbil*) skin is depicted in Fig. 3D. The maximum transition temperature (T_{max}) of PSC obtained in this study was 36.74°C. Other literatures working with pepsin soluble collagen from various fish sources documented the T_{max} values were around 33−37°C. For instances, purple-spotted bigeye snapper *(P. tayenus*) (T_{max} = 33.21°C) [35], loach (*Misgurnus anguillicaudatus*) (T_{max} = 33.61°C) [49], rohu (*Labeo rohita*) (T_{max} = 35.48°C) [52], grass carp (*C. idellus*) (T_{max} = 35.8°C) [53], cobia (*Rachycentron canadum*) (T_{max} = 36.03°C) [54] and golden pompano (*T. blochii*) (T_{max} = 37.04°C) [22].

Our finding relating to the thermostability test in the PSC sample showed that a high thermal stability was recorded and comparable to other tropical fish sources, as aforementioned. However, when compared to tempered fish species, such as Pacific cod (*G. macrocephalus*) (14.5°C) [39] and Spanish mackerel (*S. niphonius*) (16.8°C) [40], our data and various tropical fish species had greater thermostability (T_{max} value). The reason might be due to the levels of imino acids (proline and hydroxyproline), which display a direct and positive correlation with protein thermal stability through hydrogen bonding [55]. Moreover, it is established that proline and hydroxyproline's pyrrolidine rings enforce conformational constraints on the polypeptide chain, enhancing the resilience of the triple helical structure within fish collagen [36]. Consequently, variations in T_{max} values of fish collagen can be influenced by several factors, including amino acid composition, diverse fish species, distinct extraction methods, the fish's habitat, and water temperature conditions [7],[26].

G. Morphological Structure Property

The morphological study was carried out by subjecting the PSC sample to field emission scanning electron microscopy (FESEM), with the detailed structure displayed in Fig. 4. The findings revealed the presence of numerous multi-layered structures characterized by irregular dense sheet-like films interlinked by randomly coiled filaments in the lizardfish skin collagen. Additionally, some fibrillar formations were evident within the PSC sample. Furthermore, the structure of the optimized collagen displayed a wrinkled, porous, and loose appearance, which could be attributed to dehydration during the freeze-drying process, as suggested in previous research [56].

In the context of morphological structure comparison, the extracted collagen had similar structure to previous fish collagens, including the skin of marine eel (*Evenchelys macrura*) [57], the skin of black ruff (*Centrolophus niger*) [58], and the scale of miiuy croaker (*M. miiuy*) [59]. Due to

having fiber structures, the fish collagens might be a promising source of biomaterials in generating new valuable products, especially for biomedical and pharmaceutical applications; for examples, used for new tissue formation, wound treatment, cell seeding component, cell growth and migration materials, as well as coating application [2].

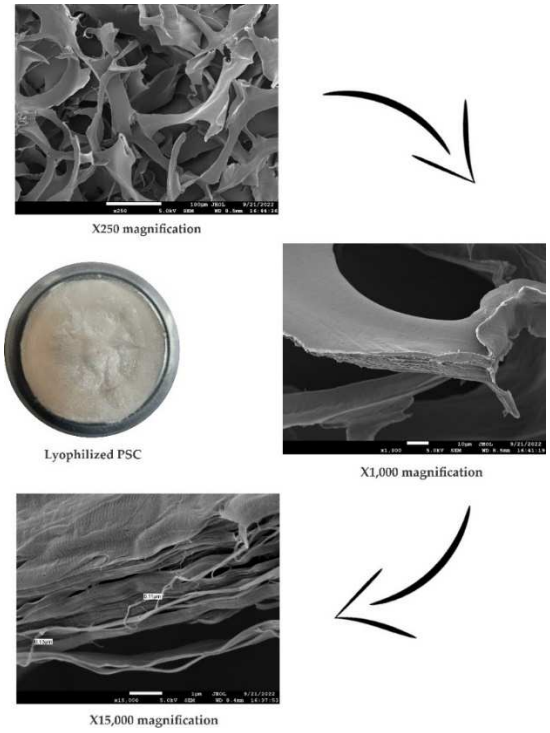

Fig. 4 FESEM image of optimized PSC from the lizardfish (*S. tumbil*) skins.

H. Solubility Profile

Fig. 5. presents the solubility profile of the PSC from lizardfish (*S. tumbil*) skin prepared with the optimal extraction conditions. At different NaCl concentrations, the lower amounts of salt concentration (0−30 g/L) added to the prepared collagen solution, the relative solubility increased more than eighty percent (>80%), whilst at the NaCl concentrations of 40−50 g/L, the solubility in the collagen sample declined less than 60% (Fig. 5A). At various pH levels, the solubility of PSC sample increased in acidic conditions (pH 1.0−5.0), with the greatest solubilization was observed at pH 2.0, as shown in Fig. 5B. However, the relative solubility decreased gradually (below 60%) after being treated with the pH 6.0−9.0.

For solubility analysis, less than 60% of relative solubility value was collected in the higher sodium chloride concentration (40-60 g/L), and it could be assumed that elevated NaCl concentrations enhance hydrophobic-hydrophobic interactions within collagen's polypeptide chains. Additionally, the augmentation of interactions between water molecules and salt ions contributes to heightened protein precipitation. Our experimental findings align with those involving pepsin-solubilized collagens sourced from tilapia (*O. niloticus*) [60], golden pompano (*T. blochii*) [22] and red drum (*Sciaenops ocellatus*) [46]. Furthermore, solubility remained lower (<60%) within the pH range of 6.0 to 9.0. This observation suggests that an increase in hydrophobic-hydrophobic interactions among protein molecules might correspond to the isoelectric point, which is generally observed under slightly acidic and neutral conditions [54]. Moreover, variations in collagen solubility across different pH treatments can be attributed to discrepancies in molecular characteristics and conformations of collagen molecules [20]. Our PSC sample was equivalent to other marine collagens produced from the skins of Spanish mackerel (*S. niphonius*) [40], golden pompano (*T. blochii*) [22] and bigeye tuna (*T. obesus*) [61].

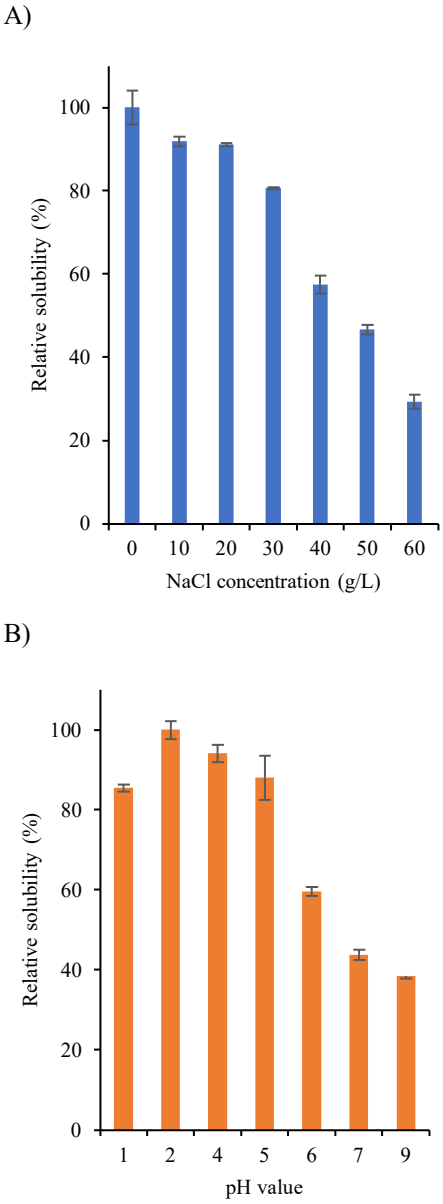

Fig. 5 Solubility values of optimized PSC from the lizardfish (*S. tumbil*) skins treated with (A) different NaCl concentrations and (B) different pH values.

V. Conclusion

The biochemical properties and morphological structure of pepsin soluble collagen prepared with the optimal enzymatic process have been assessed. The extracted PSC was classified as type I after being assessed by SDS-PAGE, and its triple helical structure was maintained under FTIR and XRD tests. Higher contents of glycine amino acid and imino acid (proline and hydroxyproline) were recorded in the PSC, as comparable

to other marine fish collagens. The type I PSC had a high T_{max} value, indicating high thermostability property, and might be due to a greater hydroxyproline. This collagen showed a good solubilization in the acidic condition and low sodium chloride concentrations. Based on those properties, our PSC may be applied for many industrial product developments.

ACKNOWLEDGMENT

The authors would like to thank to the Ministry of Higher Education Malaysia for the funds provided through the Fundamental Research Grant Scheme (FRGS) with a grant number FRGS/1/2019/STG03/UMS/02/5, and to the Universiti Malaysia Sabah (UMS) for the support to accomplish this research paper. We are also would like to thank Universitas Brawijaya for Adjunct Professor program batch 2, 2024, contract number 01643/UN10. A0101/B/KS/2024.

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