International Journal on Advanced Science Engineering Information Technology

Vol.14 (2024) No. 4
ISSN: 2088-5334

Comparison of Physicochemical Properties of Barracuda *(Sphyraena barracuda* Edwards, 1771) Skin Collagen Hydrolyzed Using Two Different Pepsin Sources

Leshanthiy A/P K Santhi Rasegaran [a], Abdul Aziz Jaziri [a,b], Ahmad Hazim Abdul Aziz [a], Rossita Shapawi [c], Ruzaidi Azli Mohd Mokhtar [d], Wan Norhana Md. Noordin [e], Rahmi Nurdiani [b], Nurul Huda [f,*]

[a] *Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, Kota Kinabalu, 88400, Sabah, Malaysia*
[b] *Department of Fish Product Technology, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Malang 65415, East Java, Indonesia*
[c] *Higher Institution Centre of Excellence (HICoE), Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS 88400, Kota Kinabalu, Sabah, Malaysia*
[d] *Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, Kota Kinabalu 88400, Sabah, Malaysia*
[e] *Fisheries Research Institute, Batu Maung, 11960, Penang, Malaysia*
[f] *Faculty of Sustainable Agriculture, Universiti Malaysia Sabah. Sandakan 90509, Sabah, Malaysia*
Corresponding author: [*]drnurulhuda@ums.edu.my

***Abstract*—This research focused on the comparison of extractability and physicochemical properties of barracuda (*Sphyraena barracuda* Edwards, 1771) skin collagens prepared using pepsins from bovine (PSC-B) and porcine (PSC-P). The PSC-P sample had a significantly higher ($p<0.05$) collagen extractability (31.16%) compared to the BCPB (19.48%). Based on the Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profiles, all hydrolyzed collagens were identified as a type I collagen with two different alpha chains (α1 and α2). The Infrared spectra showed that the collagen's triple-helical structure was maintained in the PSC-B and PSC-P samples, as mostly reported from other literatures. In terms of the thermal stability, the T_{max} value of BCP-B (43.63°C) was greater than that of BCP-P (T_{max} = 37.49°C), and their values were comparable to other literatures related on marine fish skin collagens. Overall, the by-product skin of barracuda (*S. barracuda* Edwards, 1771) can be utilized for alternative collagen products.**

***Keywords*— *S. barracuda;* fish skin by-product; pepsin-assisted extraction; physicochemical characteristics.**

Manuscript received 26 Oct. 2023; revised 9 Jan. 2024; accepted 21 Jun. 2024. Date of publication 31 Aug. 2024.

I. INTRODUCTION

Collagen serves as the primary structural protein within connective tissues, making up over 30% of all proteins in vertebrates. It forms a supportive framework that imparts strength and shape to the body, playing a vital role in binding cells together and contributing elasticity to the skin. Typically, collagen's molecular structure features a distinctive repeating pattern of glycine (Gly), proline (Pro), and hydroxyproline (Hyp) in its polypeptide chain [1]. There are currently over 29 identified collagen types [2], and type I collagen accounts for over 90% of the body's collagen and is primarily sourced from the connective tissue of mammals. Also, it is utilized extensive applications in the food, cosmetics, pharmaceutical, and biomedical industries [3]. Concerns can arise with land animal-derived collagen due to potential infectious diseases like mad cow disease and bird flu (H_5N_1). Moreover, dietary restrictions among certain religious groups, such as Jews and Muslims abstaining from pork-based commodities and Hindus avoiding beef and its derivatives, can further complicate its use [4]. Consequently, collagen from fish source has attracted much attention over a decade ago. It is abundant resources, and it is considered more safer source, free from infectious disease concerns, as well as exhibits favorable biochemical properties after modification [5]. Numerous studies have documented the extraction processes of fish collagen from various fish species. For examples, lizardfish (*Saurida tumbil*) [6-7], pink ear emperor (*Lethrinus lentjan*) and [8], needlefish (*Tylosurus acus*

melanotus) [9], purple-spotted bigeye (*Priacanthus tayenus*) [10], parrotfish (*Scarus sordidus*) [11], and unicorn (*Naso reticulatus*) [12-13]. Their attributes have also undergone thorough assessment. These investigations suggest that collagen extracted from fish skin presents a potentially advantageous alternative, offering a greater yield compared to collagen sourced from bones, cartilage, or bone fragments [14]. Furthermore, fish skin collagen holds promise for the development of novel collagen products, especially from an industrial standpoint.

The collagen extraction process plays a crucial role in obtaining collagen from fish skin. Presently, various techniques are extensively employed, including acid, base, salt, and enzyme treatments [15]. Among these, extraction involving the use of acids and enzymes is favored due to its ability to yield a higher quantity of collagen. Enzymes offer greater specificity in their reactions and have fewer adverse effects on collagen proteins. Several enzymes, such as pepsin, trypsin, papain, Novozymes, Alcalase, and Flavourzyme, can be employed in collagen extraction. However, pepsin-based extraction is known to yield significantly more collagen, as pepsin can cleave telopeptide bonds from both sides of the collagen's triple helical structure [16-17]. Moreover, enzymatic methods generate less waste and reduce processing time. Enzymatic hydrolysis stands out as a top choice for breaking down fish skin while preserving its nutritional value. This technique is particularly preferred in the food and pharmaceutical industries, as it leaves no residual organic solvents or harmful chemicals in the final product [18].

Barracuda (*Sphyraena barracuda* Edwards, 1771) is a member of the Sphyraenidae family, and it is considered as a significant economic importance in Malaysia as a tropical marine fish species. The total production of barracuda from 2016 to 2019 amounted to approximately 7,895 metric tons [19]. These fish are typically found near the water's surface and are characterized by their silvery bodies with multiple dark lines on the upper part. Due to its substantial production, barracuda is widely utilized in various fish processing methods, including salting, filleting, and the production of surimi. However, a significant portion of fish by-products, including skin, bones, entrails, heads, scales, and fins, is generated during the fish processing stage, estimated to be around 50 percent [20-21]. This excess waste has detrimental effects on both the environment and the financial aspects of the industry. From an environmental standpoint, the underutilization of fish by-products leads to wastage in bodies of water or landfills, resulting in damage to the ecosystem. Moreover, these by-products contain abundant organic compounds and fall under regulated waste, demanding appropriate handling before disposal. This procedure can result in elevated operational expenses for the fish processing sector [22]. Hence, converting barracuda skin by-product into fish collagen is a great approach, and this research has been much less documented yet, particularly employing two different pepsin sources. Thus, our study aimed to obtain a comparison of yield extractability and physicochemical properties observed in *S. barracuda* skin collagen prepared using different pepsins derived from bovine and porcine.

II. Materials and Method

A. Sample Preparation and Extraction of Pepsin-solubilized Collagen (PSC)

Barracuda (*S. barracuda*) were purchased from a local wet market in Kota Kinabalu, Malaysia. Upon arrival, the fish was cleansed using tap water and underwent separation utilizing an SFD-8 deboner. After separation, the skin portions were gathered and extensively rinsed. The cleaned fish skins were then preserved in a freezer until further experiments were conducted. The extraction of pepsin-solubilized collagen (PSC) was performed following the methodology established by Jaziri et al. [23] with minor adjustments, as depicted in Fig. 1. Approximately 50 g of fish skin were pre-treated with 0.1 M NaOH for 6 h. After neutralization, the fish skins were subjected to immersion in 10% butyl alcohol for 24 h to remove fat from the samples. Next, the samples were solubilized in acidic condition containing pepsins from bovine and porcine. The solubilization process was carried out for 48 h with continuous stirring. The mixture was filtered and then salted out in a sodium chloride and Tris-HCl solution. The solution was then rotated at 15,000× g for 15 min using a centrifuge. After this, the resulting pellets were resuspended in acid solution, and the solution was further dialyzed for 72 h. The dialysate was freeze-dried and then stored in a freezer for future experimental use.

Fig. 1 Extraction process of PSC from barracuda (*S. barracuda*) skin.

B. Swelling and Yield Determination

The swelling percentage was calculated based on the approach outlined by Huda et al. [24]. After treating the fish skin to remove non-collagenous proteins, it was filtered and allowed to sit undisturbed for 15 min. Subsequently, the skin was weighed using a digital scale. For yield determination, we used the procedure outlined in the method detailed by Jongjareonrak et al. [25].

C. Color Analysis

The color characteristics of PSC isolated from barracuda (*S. barracuda*) skins were evaluated following the established procedure [4], utilizing a colorimeter to evaluate the color attribute values. The attributes considered in this study included L^*, a^*, and b^*.

D. Ultraviolet-visible (UV-vis) Absorption Analysis

The spectra of PSC samples were employed using a UV-visible spectrophotometer. To prepare the samples, around 5 mg of lyophilized collagens were immersed in an acid solution and thoroughly mixed. Subsequently, the mixtures were spinned out at 8,500× g for 10 min to obtain solubilized components. The soluble collagens were further transferred into a quartz cell. The spectral range examined in this study encompassed wavelengths from 400 nm to 200 nm [4].

E. X-ray Diffraction (XRD) Profile

The dried PSCs were analyzed using an XRD instrument, employing copper Kα as the x-ray source. The scanning range for all extracted collagens was configured to span from 10° to 40° (2θ), with a scanning speed of 0.06° per second. Data acquired during the analysis were collected and evaluated. The XRD methodology employed in this experiment was referred from the study of Chen et al. [26].

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

The SDS-PAGE analysis was performed using a Mini-PROTEAN electrophoresis apparatus, following a method adopted by Laemmli [24] with minor adjustments. Initially, lyophilized collagen (2.5 mg) was dissolved in a 5% SDS solution and gently mixed. The mixture was then heated at 85°C for 1 h and then spinned out at 8,500× g for 5 min to remove any undissolved components. After this step, around 20 µL of the resulting supernatants were pipetted out into a mini centrifuge tube, and subsequently added sample buffer. The mixture was heat-treated for around 5 min and then loaded onto a prepared gel, consisting of a 4% stacking gel and a 7.5% resolving gel. Electrophoresis was conducted for approximately 80 min at a constant voltage of 120 volts. Next, the electrophorized gel was stained using a staining solution for 10 min. The stained gel was then destained using a destaining solution. The resulting electrophoretic bands were compared to a protein marker.

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

The infrared (IR) spectra of PSC samples were evaluated using an ATR-FTIR spectrometer. The method employed in this study was adapted from the methodology outlined in the research by Jaziri et al. [6]. To conduct the analysis, about 10 mg of dried PSC were put onto the crystal cell of a spectrometer. All Each spectrum was captured at a 2 cm^{-1} resolution across a wavenumber range from 4000 to 500 cm^{-1}, with an average of 32 scans. Following this, the spectral data were analyzed using an Agilent Microlab software tool.

H. Thermostability Analysis

We used a differential scanning calorimetry (DSC) instrument to determine the thermostability of collagens. To prepare the PSC samples, they were dissolved in distilled water and kept in a chiller for 48 h. Following incubation, approximately 8 mg of the sample was accurately weighed and placed in an aluminum pan, and subsequently sealed. Prior to conducting the analysis, the instrument was subjected to calibration. After that, the prepared sample was introduced into the DSC's cell holder and subjected to heating, starting from 25°C and gradually increasing to 50°C at a rate of 1°C per minute, all under a nitrogen gas flow. An empty pan was used as a reference. The parameters measured included the maximum heat flow (T_{max}) and the enthalpy denaturation (ΔH), which were determined based on the area under the endothermic peak observed in the DSC thermogram [27].

I. Statistical Analysis

This study was conducted with three replicates, and the data collected were presented as the mean values along with their respective standard deviations. A one-way ANOVA and Duncan's multiple range test was used in our analyses using SPSS Statistics version 28.0 (IBM Corp., Armonk, New York).

III. Results and Discussion

A. Yield of PSC Samples

Yields of both PSC samples derived from the skin of barracuda (*S. barracuda*) are tabulated in Table 1. The obtained results show the yield of PSC-P (31.18%) was significantly higher ($P<0.05$) than that of PSC-B (19.48%). This reason might be due to the pre-treatment process, particularly during dissolving with NaOH solution. As presented in Table 1, the swelling percentage of collagen hydrolyzed with pepsin from porcine yielded significantly greater ($P<0.05$) compared to that of PSC-B (pepsin from bovine). Swelling plays an essential role in the collagen extraction process, as it can disrupt the inner molecular structures of proteins and promote collagen formation by breaking non-covalent bonds, as described by Cheng et al. [28]. When a controlled-temperature solution like NaOH permeates the skin structure, it causes the skin to swell to two or three times its initial volume, leading to the cleavage of non-covalent bonds both within and between molecules. NaOH is particularly effective as a pre-treatment for skin because it induces significant swelling, enhancing collagen extraction by accelerating mass transfer within the tissue matrix, as highlighted by Schmidt et al. [29]. All collagen treatments exhibited a substantial swelling percentage (>250%), nearly five times the weight of the 50g fish skin. In terms of collagen yield, it was comparable to other fish skin collagen prepared using pepsin enzyme, such as golden pompano (*Trachinotus blochii*) (21.81%) [30], tilapia (*Oreochromis niloticus*) (19.61%) [31], bigeye tuna (*Thunnus obesus*) (16.7%) [15], and purple-spotted bigeye snapper (*P. tayenus*) (12.44%) [32].

TABLE I
YIELD, COLOR ATTRIBUTES, AND UV-VIS SPECTRA OF PSC DERIVED FROM BARRACUDA (*S. BARRACUDA*) SKIN.

Parameters	***Pepsin-solubilized collagen***		
	PSC-B	***PSC-P***	***CFCH***
Yield (%)	19.48 ± 3.08^{b}	31.18 ± 2.64^{a}	-
Swelling (%)	252.60 ± 17.25^{b}	395.00 ± 11.65^{a}	-
*L**	53.29 ± 0.40^{a}	59.41 ± 0.75^{b}	88.36 ± 0.01^{c}
*a**	3.10 ± 0.09^{b}	1.90 ± 0.33^{c}	-1.90 ± 0.02^{a}
*b**	6.20 ± 0.03^{b}	4.21 ± 0.70^{a}	4.77 ± 0.03^{a}
UV-vis spectra	232.0 nm	231.0 nm	230.0 nm

PSC-B: Barracuda skin hydrolyzed with pepsin from bovine.
PSC-P: Barracuda skin hydrolyzed with pepsin from porcine.
CFCH: Commercial fish protein hydrolysate

B. Color Attributes

Table 1. presents the color attributes of PSC samples from barracuda (*S. barracuda*) skin, compared to those of commercial fish collagen hydrolysate (CFCH). The data indicated significant differences ($p < 0.05$) in the values of L*, *a**, and *b** for all samples. Notably, the CFCH exhibited the lightest color compared to both PSC samples. Moreover, our current study also recorded a lower *L** value compared to the study on lizardfish (*S. tumbil*) skin collagen (72.76) [4] but it was a slightly higher than that of barramundi (*Lates calcalifer*) skin collagen (*L** = 44.76) [33]. In contrast, collagen extracted from snakehead fish (*Channa argus*) skin using a H_2O_2 solution exhibited a brighter color (*L** = 89.49) [18] than our results, indicating that the use of H_2O_2 solution has the potential to yield a collagen product with enhanced brightness. As highlighted in the study by Sadowska et al. [34], a collagen product exhibiting a more vibrant color is highly desirable for innovative product applications, particularly within the realms of food, pharmaceuticals, and medical sectors, as it minimizes or eliminates interference with the product's original color. In terms of the *a** and *b** measurements, PSC-B exhibited the most elevated values. When a product has a higher *L** value, it tends to correspond to lower *a** and *b** values, and conversely, when the *L** value is low, the *a** and *b** values are typically higher.

C. UV-vis Absorption Spectra

The UV-vis spectra of the collagens isolated from different pepsin sources are presented in Table 1. In general, fish collagen exhibits a prominent UV absorption spectrum in the range of 210 nm to 240 nm [35]. The results obtained in this study indicate that all collagen samples fell within the recommended maximum spectral ranges established by previous research (Wu et al. 2015). The highest peak positions found in our data aligned with those reported from numerous marine collagens, including Siberian sturgeon (*Acipenser baerii*) [36], red drum (*Sciaenops ocellatus*) [37], and puffer fish (*Lagocephalus inermis*) [38]. The spectra recorded are linked to functional groups such as carboxyl, carbonyl, and amides, which are found in the collagen's polypeptide chains. This alignment with the findings from the study carried out by Jaziri et al.[4] reinforces these identifications.

D. Protein Pattern of PSC

Fig. 2. shows the electrophoretic patterns of the PSC samples obtained from barracuda (*S. barracuda*) skin. The gel had two alpha (α) chains, a β-chain, and a γ-chain, and each chain showed different molecular weights (MW). Notably, our findings classified these collagens as type I collagen due to the double alpha chains presence, a characteristic consistent with previous studies on fish skin collagens, including bigeye tuna (*T. obesus*) [15], purple-spotted bigeye (*P. tayenus*) [32], and lizardfish (*S. tumbil*) [6].

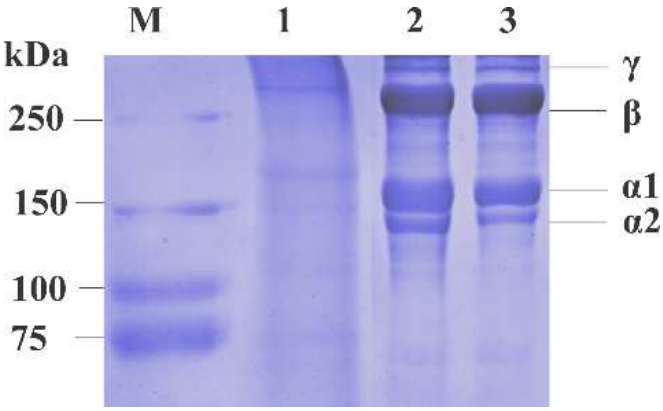

Fig. 2 Electrophoretic patterns of PSC from barracuda (*S. barracuda*) skin. M: protein marker; 1: commercial fish protein hydrolysate (CFCH); 2: barracuda skin hydrolyzed with pepsin from bovine (PSC-B); 3: Barracuda skin hydrolyzed with pepsin from porcine (PSC-P).

E. IR Spectra Analysis

We examined the IR spectra of all collected sampels, and the prominent peaks are illustrated in Fig. 3, with detailed descriptions provided in Table 2. As mentioned earlier, there were five significant peak areas identified in all samples. These spectral peaks were also consistent with those found in various investigations on marine collagens, of purple-spotted bigeye snapper (*P. tayenus*) [32], lizardfish (*S. tumbil*) [6], and sturgeon fish (*Huso huso*) [39]. More interestingly, Amide I-III can serve as indicators of the stability of triple helical structures. Researchers have proposed an equation involving $\Delta\nu(\nu_I\text{-}\nu_{II})$, where the difference in wavenumber (cm^{-1}) between Amide I and Amide II is less than 100 cm^{-1}, signifying the preservation of the triple helical structure of the extracted collagen [40]. Our findings reveal that the triple helical structures of PSCs were maintained, suggesting that the use of acidic solvents in conjunction with pepsins effectively solubilized collagens without altering their structures. Alternatively, by utilizing the ratio of Amide III to the 1450 cm^{-1} band (AIII/A1450) [41], we found that the triple helical structures of PSC samples remained stable, as indicated by the absorption ratio values (~1.0).

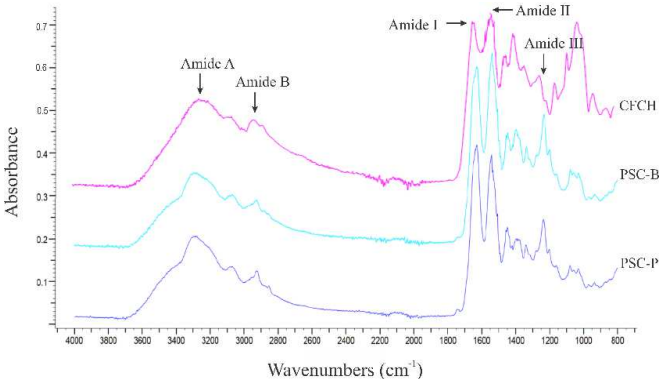

Fig. 3 IR spectra of PSC from barracuda (*S. barracuda*) skin.

PSC-B: Barracuda skin hydrolyzed with pepsin from bovine;
PSC-P: Barracuda skin hydrolyzed with pepsin from porcine;
CFCH: Commercial fish protein hydrolysate.

TABLE II
THE PEAK AREA AND DESCRIPTION OF PSC DERIVED FROM BARRACUDA (*S. BARRACUDA*) SKIN

Peak	Type of collagen			Peak assignment
	PSC-B	PSC-P	CFCH	
Amide A	3280.14 cm^{-1}	3285.73 cm^{-1}	3259.64 cm^{-1}	Stretching of N-H with hydrogen chain
Amide B	2926.03 cm^{-1}	2922.31 cm^{-1}	2931.62 cm^{-1}	Asymmetric stretching of CH_2
Amide I	1628.89 cm^{-1}	1628.89 cm^{-1}	1636.34 cm^{-1}	Stretching of C=O / hydrogen chain with COO-
Amide II	1541.29 cm^{-1}	1541.29 cm^{-1}	1522.66 cm^{-1}	N-H bond with stretching of C-N
Amide III	1235.64 cm^{-1}	1235.64 cm^{-1}	1241.23 cm^{-1}	N-H bond with C-H dan C-O stretching

PSC-B: Barracuda skin hydrolyzed with pepsin from bovine.
PSC-P: Barracuda skin hydrolyzed with pepsin from porcine.
CFCH: Commercial fish protein hydrolysate.

F. Thermostability Evaluation

All PSC samples derived from barracuda (*S. barracuda*) skin exhibited higher thermostability (T_{max}) values, as depicted in Fig. 4. A higher T_{max} value in collagen indicates a greater hydroxyproline content [40]. This could be attributed to the composition of imino acids, particularly hydroxyproline and proline, which play a role in the formation of pyrrolidine rings partially stabilized by hydrogen bonding through the hydroxyl group of hydroxyproline (Hyp), as suggested by Benjakul et al. [40]. Furthermore, Hyp has the capacity to enhance the triple helical stability structure through hydrogen bonding within the coiled-coil alpha chains, as elucidated by Bae et al. [42]. These findings align with the observations of several researchers working with various marine fish collagens, including loach (*Misgurnus anguillicaudatus*) skin (T_{max} = 36.03°C) [17], seabass (*Lates calcarifer*) skin (T_{max} = 39.32°C) [43], and lizardfish (*S. tumbil*) skin (T_{max} = 40.24°C) [4].

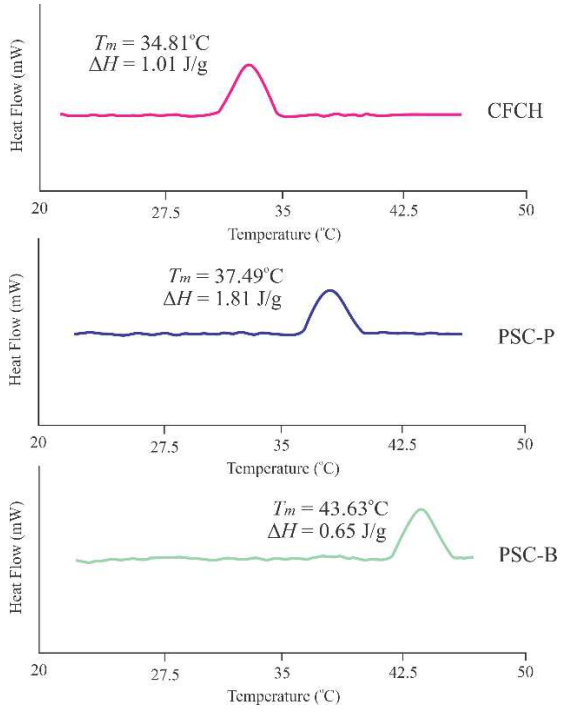

Fig. 4 Thermogram images of PSC from barracuda (*S. barracuda*) skin. PSC-B: Barracuda skin hydrolyzed with pepsin from bovine; PSC-P: Barracuda skin hydrolyzed with pepsin from porcine; CFCH: Commercial fish protein hydrolysate.

IV. CONCLUSION

Barracuda (*S. barracuda* Edwards, 1771) skin collagens have been isolated using two different pepsin sources (i.e., bovine and porcine). A greater yield ($p<0.05$) was observed in collagen isolated with pepsin from porcine (PSC-P) (31.16%), compared to that of collagen isolated with pepsin from bovine (PSC-B) (19.48%). All PSC samples showed high thermostability (T_{max}) values (>37°C), especially found in PSC-B, and those could be due to the composition of hydroxyproline, as stated many researchers that hydroxyproline can stabilize the triple helical structure of collagen. In terms of IR parameter, the triple helical structures of barracuda (*S. barracuda*) skin collagens obtained in our study were still maintained, indicating all samples were stable during solubilizing with acids and pepsins solution, and interestingly, these PSC samples were considered as type I with existing two alpha chains (α1 and α2). Overall, PSC-B sample is more suitable for further study due to higher thermostability.

ACKNOWLEDGMENT

This research received external funding by the Ministry of Education Malaysia through the Fundamental Research Grant Scheme (FRGS) with a grant number FRGS/1/2019/STG03/UMS/02/5. Authors also would like to thank Universiti Malaysia Sabah (UMS) for the support to accomplish this research.

REFERENCES

[1] A. Sorushanova, L. M. Delgado, Z. Wu, N. Shologu, A. Kshirsagar, R. Raghunath, A. M. Mullen, Y. Bayon, and A. Pandit "The Collagen Suprafamily: from Biosynthesis to Advanced Biomaterial Development," *Advanced Materials*, vol. 31, no. 1, p. 1801651, Jan. 2019, doi: 10.1002/adma.201801651.

[2] Y. S. Lim, Y. J. Ok, S. Y. Hwang, J. Y. Kwak, and S. Yoon, "Marine collagen as a promising biomaterial for biomedical applications," *Marine Drugs*, vol. 17, no. 8, p. 467, Aug. 2019, doi:10.3390/md17080467.

[3] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Biochemical analysis of collagens from the bone of lizardfish (*Saurida tumbil* Bloch, 1795) extracted with different acids," *PeerJ*, vol. 10, e13103, Mar. 2022, doi: 10.7717/peerj.13103.

[4] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Microstructural and Physicochemical Analysis of Collagens from the Skin of Lizardfish (*Saurida tumbil* Bloch, 1795) Extracted with Different Organic Acids," *Molecules*, vol. 27, no. 8, Apr. 2022, doi: 10.3390/molecules27082452.

[5] X. Zhang, S. Xu, L. Shen, and G. Li, "Factors affecting thermal stability of collagen from the aspects of extraction, processing and modification," *Journal of Leather Science and Engineering*, vol. 2, no. 1, 2020, doi: 10.1186/s42825-020-00033-0.

[6] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Physicochemical and Microstructural Analyses of Pepsin-Soluble Collagens Derived from Lizardfish (*Saurida tumbil* Bloch, 1795) Skin, Bone and Scales," *Gels*, vol. 8, no. 8, Aug. 2022, doi:10.3390/gels8080471.

[7] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, S. B. Zulfigar, and N. Huda, "Optimization Process of the Pepsin-Solubilized Collagen from Lizardfish (*Saurida tumbil* Bloch, 1795) Skins by-Product," *Indonesian Food Science and Technology Journal,* vol. 7, no. 1, Dec. 2023, doi: 10.22437/ifstj.v7i1.30797.

[8] A. A. Prihanto, A. A. Jaziri, M. D. Pratomo, S. E. Putri, C. Fajriati, R. Nurdiani, and M. Firdaus, "Characteristics of Collagen from Parrotfish (*Chlorurus sordidus*), Tiger Grouper (*Epinephelus fuscoguttatus*) and Pink Ear Emperor (*Lethrinus lentjan*): Effect of Acetic Acid Concentration and Extraction Time," *Online Journal of Biological Science*, vol. 22, no. 1, pp. 26–35, Jan. 2022, doi:10.3844/ojbsci.2022.26.35.

[9] S. Z. Ramle, S. N. H. Oslan, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Biochemical Characteristics of Acid-Soluble Collagen from Food Processing By-products of Needlefish Skin (*Tylosurus acus* melanotus)," *Applied Sciences*, vol. 12, no. 24, Dec. 2022, doi: 10.3390/app122412695.

[10] H. Hasanuddin, A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Effect of different acids during collagen extraction the bone and fins from purple-spotted bigeye (*Priacanthus tayenus* Richardson, 1846) and their physicochemical properties," *Food Research*, vol. 8, no. 1, pp. 326–335, Feb. 2024, doi:10.26656/fr.2017.8(1).224.

[11] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. Md. Noordin, and N. Huda, "Extraction and Characterization of Type I Collagen from Parrotfish (*Scarus sordidus* Forsskål, 1775) Scale solubilized with the Aid of Acetic Acid and Pepsin," *International Journal of Biomaterials*, vol. 2023, pp. 1–10, Apr. 2023, doi:10.1155/2023/7312447.

[12] N. S. Fatiroi, A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Biochemical and Microstructural Characteristics of Collagen Biopolymer from Unicornfish (*Naso reticulatus* Randall, 2001) Bone Prepared with Various Acid Types," *Polymers*, vol. 15, no. 4, Feb. 2023, doi: 10.3390/polym15041054.

[13] D. F. Harianto, A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, S. M. Rambli, and N. Huda, "Assessing the Organic Acids-solubilized Collagen Derived from the Skin of Unicornfish (*Naso reticulatus* Randall, 2011) on Physicochemical and Structural Characteristics," *ASEAN Journal on Science and Technology for Development*, vol. 40, no. 2, p. 1-12, Feb. 2024, doi: 10.61931/2224-9028.1535.

[14] G. K. S. Arumugam, D. Sharma, R. M. Balakrishnan, and J. B. P. Ettiyappan, "Extraction, optimization and characterization of collagen from sole fish skin," *Sustainable Chemistry and Pharmacy*, vol. 9, pp. 19–26, Sep. 2018, doi: 10.1016/j.scp.2018.04.003.

[15] R. Ahmed, M. Haq, and B. S. Chun, "Characterization of marine derived collagen extracted from the by-products of bigeye tuna (*Thunnus obesus*)," *International Journal of Biological Macromolecules*, vol. 135, pp. 668–676, 2019, doi:10.1016/j.ijbiomac.2019.05.213.

[16] F. Yu, C. Zong, S. Jin, J. Zheng, N. Chen, J. Huang, Y. Chen, F. Huang, Z. Yang, Y. Tang, and G. Ding, "Optimization of extraction conditions and characterization of pepsin-solubilised collagen from skin of giant croaker (*Nibea japonica*)," *Marine Drugs*, vol. 16, no. 1, Jan. 2018, doi: 10.3390/md16010029.

[17] J. Wang, X. Pei, H. Liu, and D. Zhou, "Extraction and characterization of acid-soluble and pepsin-soluble collagen from skin of loach (*Misgurnus anguillicaudatus*)," *International Journal of Biological Macromolecules*, vol. 106, pp. 544–550, Jan. 2018, doi:10.1016/j.ijbiomac.2017.08.046.

[18] W. Liu, Y. Zhang, N. Cui, and T. Wang, "Extraction and characterization of pepsin-solubilized collagen from snakehead (*Channa argus*) skin: Effects of hydrogen peroxide pretreatments and pepsin hydrolysis strategies," *Process Biochemistry*, vol. 76, pp. 194–202, Jan. 2019, doi: 10.1016/j.procbio.2018.10.017.

[19] Department of Fisheries Malaysia, "Fisheries Statistics I 2023," accessed on 22 May 2024, https://www.dof.gov.my/en/resources/fisheries-statistics-i/

[20] A. A. Jaziri, R. Shapawi, R. A. Mohd Mokhtar, W. N. Wan, and N. Huda, "Tropical Marine Fish Surimi By-products: Utilisation and Potential as Functional Food Application," *Food Review International*, vol. 39, no. 6, p. 3455-3480, Dec. 2021, doi:10.1080/87559129.2021.2012794.

[21] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, S. M. Rambli, and N. Huda, "Byproducts of Tropical Marine Processing as a Candidate for Industrial Food Ingredients an Appraisal on Macro and Micronutrients," *ASEAN Journal on Science and Technology for Development*, vol. 40, no. 2, Feb. 2024, doi: 10.61931/2224-9028.1535.

[22] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Chemical composition of lizardfish surimi by-product: Focus on macro and micro-minerals contents," *Current Research in Nutrition and Food Science*, vol. 9, no. 1, pp. 52–61, Apr. 2021, doi:10.12944/CRNFSJ.9.1.06.

[23] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Physicochemical and Microstructural Analyses of Pepsin-Soluble Collagens Derived from Lizardfish (*Saurida tumbil* Bloch, 1795) Skin, Bone and Scales," *Gels*, vol. 8, no. 8, pp. 1–18, 2022, doi:10.3390/gels8080471.

[24] N. Huda, E. K. Seow, N. M. Nor, N. A. N. Muhammad, A. Fazilah, and A. M. Easa, "Effect of duck feet collagen addition on physicochemical properties of surimi," *International Food Research Journal*, vol. 20, no. 2, p. 537-544, Oct. 2013.

[25] A. Jongjareonrak, S. Benjakul, W. Visessanguan, and M. Tanaka, "Skin gelatin from bigeye snapper and brownstripe red snapper: Chemical compositions and effect of microbial transglutaminase on gel properties," *Food Hydrocolloids*, vol. 20, no. 8, pp. 1216–1222, Dec. 2006, doi: 10.1016/j.foodhyd.2006.01.006.

[26] J. Chen, J. Li, Z. li, R. Yi, S. Shi, K. Wu, Y. Li, and S. Wu, "Physicochemical and functional properties of type I collagens in red stingray (*Dasyatis akajei*) skin," *Marine Drugs*, vol. 17, no. 10, Sep. 2019, doi: 10.3390/md17100558.

[27] K. Matmaroh, S. Benjakul, T. Prodpran, A. B. Encarnacion, and H. Kishimura, "Characteristics of acid soluble collagen and pepsin soluble collagen from scale of spotted golden goatfish (*Parupeneus heptacanthus*)," *Food Chemistry*, vol. 129, no. 3, pp. 1179–1186, Dec. 2011, doi: 10.1016/j.foodchem.2011.05.099.

[28] F. Y. Cheng, F. W. Hsu, H. S. Chang, L. C. Lin, and R. Sakata, "Effect of different acids on the extraction of pepsin-solubilised collagen containing melanin from silky fowl feet," *Food Chemistry*, vol. 113, no. 2, pp. 563–567, Mar. 2009, doi: 10.1016/j.foodchem.2008.08.043.

[29] M. M. Schmidt, R. C. P. Dornelles, R. Mello, E. H. Kubota, M. Mazutti, A. P. Kempka, and I. M. Demiate, "Collagen extraction process," *International Food Research Journal*, vol. 23, no. 3, p. 913-922, Oct. 2013.

[30] J. Cao, Q. Duan, X. Liu, X. Shen, and C. Li, "Extraction and Physicochemical Characterization of Pepsin Soluble Collagens from Golden Pompano (*Trachinotus blochii*) Skin and Bone," *Journal of Aquatic Food Product Technology*, vol. 28, no. 8, pp. 837–847, Sep. 2019, doi: 10.1080/10498850.2019.1652216.

[31] W. K. Song, D. Liu, L. L. Sun, B. F. Li, and H. Hou, "Physicochemical and Biocompatibility Properties of Type I Collagen from the Skin of Nile Tilapia (*Oreochromis niloticus*) for Biomedical Applications," *Mar Drugs*, vol. 17, no. 3, Feb. 2019, doi: 10.3390/md17030137.

[32] S. N. H. Oslan, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Characterization of Acid- and Pepsin-Soluble Collagen Extracted from the Skin of Purple-Spotted Bigeye Snapper," *Gels*, vol. 8, no. 10, Oct. 2022, doi: 10.3390/gels8100665.

[33] J. Bakar, U. Hartina, M. Razali, D. Hashim, and A. Q. Sazili, "Properties of collagen from barramundi (*Lates calcarifer*) skin." *International Food Research Journal*, vol. 20, no. 2, p. 835-842, Nov. 2012.

[34] M. Sadowska, I. Kołodziejska, and C. Niecikowska, "Isolation of collagen from the skins of Baltic cod (*Gadus morhua*)." *Food Chemistry*, vol. 81, no. 2, pp. 257-262, May. 2003, doi:10.1016/S0308-8146(02)00420-X.

[35] Q. Q. Wu, T. Li, B. Wang, and G. F. Ding, "Preparation and characterization of acid and pepsin-soluble collagens from scales of croceine and redlip croakers," *Food Science and Biotechnology*, vol. 24, no. 6, pp. 2003–2010, Dec. 2015, doi: 10.1007/s10068-015-0264-z.

[36] Q. Bin Luo, C. F. Chi, F. Yang, Y. Q. Zhao, and B. Wang, "Physicochemical properties of acid- and pepsin-soluble collagens from the cartilage of Siberian sturgeon," *Environmental Science and Pollution Research*, vol. 25, no. 31, pp. 31427–31438, Nov. 2018, doi: 10.1007/s11356-018-3147-z.

[37] S. Chen, H. Chen, Q. Xie, B. Hong, J. Chen, F. Hua, K. Bai, J. He, R. Yi, and H. Wu, "Rapid isolation of high purity pepsin-soluble type I collagen from scales of red drum fish (*Sciaenops ocellatus*)," *Food Hydrocolloids*, vol. 52, pp. 468–477, Jan. 2016, doi:10.1016/j.foodhyd.2015.07.027.

[38] S. Iswariya, P. Velswamy, and T. S. Uma, "Isolation and Characterization of Biocompatible Collagen from the Skin of Puffer Fish (*Lagocephalus inermis*)," *J Polym Environ*, vol. 26, no. 5, pp. 2086–2095, May 2018, doi: 10.1007/s10924-017-1107-1.

[39] M. Atef, S. M. Ojagh, A. M. Latifi, M. Esmaeili, and C. C. Udenigwe, "Biochemical and structural characterization of sturgeon fish skin collagen (*Huso huso*)," *Journal of Food Biochemistry*, vol. 44, no. 8, Aug. 2020, doi: 10.1111/jfbc.13256.

[40] S. Benjakul, Y. Thiansilakul, W. Visessanguan, S. Roytrakul, H. Kishimura, T. Prodpran, and J. Meesane, "Extraction and characterisation of pepsin-solubilised collagens from the skin of bigeye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*)," *Journal of the Science of Food and Agriculture*, vol. 90, no. 1, pp. 132–138, Jan. 2010, doi: 10.1002/jsfa.3795.

[41] B. B. Doyle, E. G. Bendit, E. R. Blout, “Infrared Spectroscopy of Collagen and Collagen-Like Polypeptides,” *Biopolymers*, vol. 14, no. 5, pp. 937-57, May. 1975, doi: 10.1002/bip.1975.360140505.

[42] I. Bae, K. Osatomi, A. Yoshida, K. Osako, A. Yamaguchi, and K. Hara, “Biochemical properties of acid-soluble collagens extracted from the skins of underutilised fishes,” *Food Chemistry*, vol. 108, no. 1, pp. 49–54, May 2008, doi: 10.1016/j.foodchem.2007.10.039.

[43] S. Chuaychan, S. Benjakul, and H. Kishimura, “Characteristics of acid- and pepsin-soluble collagens from scale of seabass (*Lates calcarifer*),” *LWT – Food Science and Technology*, vol. 63, no. 1, pp. 71–76, Sep. 2015, doi: 10.1016/j.lwt.2015.03.002.