IJBPAS, August, 2023, 12(8): 3837-3845

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ISOLATION AND CHARACTERIZATION OF ACID AND PEPSIN - SOLUBILISED COLLAGEN FROM THE SKIN OF BLACKTAIL SNAPPER (*L. FULVUS*)

B. SIVARANJANI AND K. MOHAN

Research Scholar, Pondicherry University

Research Scholar, Annamalai University

*Corresponding Author: B. Sivaranjani: E Mail: researchbio23@gmail.com

Received 15th Sept. 2022; Revised 15th Oct. 2022; Accepted 4th Dec. 2022; Available online 1st Aug. 2023

https://doi.org/10.31032/IJBPAS/2023/12.8.7380

ABSTRACT

Blacktail Snapper (*L. fulvus*) muscle obtained from by-catch resources was used as an alternative source for mammalian collagen. The aim of the study was to isolate the Acid soluble collagen (ASC) and Pepsin soluble collagen (PSC) from body muscles of Blacktail Snapper (*L. fulvus*) . Body muscles of Blacktail Snapper (*L. fulvus*) the exoskeleton was removed, the muscles were cut into small pieces (0.3-0.5 cm) and stored at -4°C until used. Blacktail Snapper (*L. fulvus*) was extracted with 0.1M NaOH to remove non-collagenous protein for 3 days. Then, the deproteinised muscles was washed with distilled water and lyophilized for further analysis. The net yield of ASC and PSC was provisionally estimated as 28% and 47% respectively in dry weight basis. The molecular masses of the ASC and PSC subunits (α1, α2) were about 67 kDa and 63 kDa. These finding shows the great potential of Blacktail Snapper (*L. fulvus*) muscle collagen as a new source biomedical materials, food and nutraceutical industries.

Keywords: Blacktail Snapper (*L. fulvus*) Collagen; SDS-PAGE; FT-IR spectroscopy. Acid solublised collagen (ASC), Pepsin solubilised collagen (PSC)

INTRODUCTION:

One of the lengthy, fibrous structural proteins, collagen serves a purpose very dissimilar from that of globular proteins, such as enzymes. In contrast to other proteins, collagen has a unique triple helical structure made up of three polypeptide chains. The majority of the protein in animal tissues is collagen, which accounts for 30% of the protein in the human body **[1]**. It has numerous uses in the food, pharmaceutical, cosmetics, and biomedical materials industries, as well as the leather and film industries **[2]**. Natural collagen is suitable for use in edible casings, vitreous implants, and wound dressings **[3]**. Marine natural products have recently garnered industrial uses due to both their positive impacts on human health as well as their potential as a source for pharmaceutical products. The number of marine compounds and natural products derived from marine species that find commercial applications in industries each year is steadily rising. Land-based animals, such as bovine or porcine skin and bone, have historically been the only suppliers of collagen for industrial usage. However, the recent foot-and-mouth disease (FMD) and bovine spongiform encephalopathy (BSE) crises have sparked consumer anxiety regarding collagen and items produced from land animals **[4]**. The reef-based fisheries of the Indo-Pacific region's subtropical and tropical coasts depend heavily on the black-tail snapper *Lutjanus fulvus* (Allen 1985). The island of **[5]** discovered that juvenile *L. fulvus* was primarily found in mangrove estuaries but occasionally appeared elsewhere while subadults and adults took place over reef flats. coral reefs (>120 mm TL) based on a quantitative visual census in several settings (mangrove seagrass bed, coral rubble, estuary, sand area, Reef flats with branching coral and tabular coral section of the reef slope). Throughout the day, *L. fulvus* Within each habitat, they vigorously swim, and at night they are found among the base of mangrove trees, pebbles on riverbanks and coral reefs **[6] [7]**.

The topic of this work is the separation and characterisation of collagen from Blacktail Snapper (*L. fulvus*) muscles that has been solubilized by acid and pepsin.

MATERIALS AND METHODS

Preparation of collagen sample

The muscle of *L. fulvus*. for three days, nepawas were extracted with 0.1M NaOH to eliminate non-collagenous protein. The deproteinized muscles were then lyophilized and cleaned with distilled water for additional research.

Isolation of Acid Soluble & pepsin soluble Collagen

0.5M acetic acid (1:3 w/v) was applied to the lyophilized muscle for three days, and the extracts were centrifuged at 5000 rpm for 30 minutes at 4°C. The residue was again extracted with the same solution for two days and centrifuged under the same circumstances after the supernatant was collected **[8]**. At a final concentration of 2.3 M at a neutral pH of 7.5, NaCl was added to each solution to combine and salt it out. The precipitates that resulted were collected and then redissolved in 0.5 M acetic acid. The finished product was lyophilized after being dialyzed with acetic acid and distilled water for three days. Acid soluble collagen was the name given to the dried substance (ASC). The acid-extracted insoluble residue was used to extract the collagen that was soluble with pepsin (Sigma, India). Residue was properly cleaned with distilled water before being solubilized for three days in 0.5 M acetic acid with 0.1% (w/v) pepsin. perpetual stirring At 4°C, the mixture was centrifuged for 60 minutes at 5000 rpm. NaCl was added to the extract's supernatant to salt it out, resulting in a final concentration of 2.3 M at a neutral pH 7.5. Centrifugation was used to separate the precipitate after spinning for 30 minutes at 5000 rpm and 4°C. The precipitate was then dissolved in 0.5 M acetic acid, and the resultant solution was dialyzed over a 3-day period against 0.1 M acetic acid and distilled water. The resultant dialysates were freeze-dried and regarded as collagen that is pepsin soluble (PSC) **[9]**.

Physical properties of collagen

The collagen sample was mixed with 1.5 M TrisHCl buffer (pH 6.8) containing 10% SDS and 11.14% 2-mercaptoethanol, 40% glycerol, and 0.02% bromo-phenol blue, heated at 100°C for 2 min, and electrophoresed at 50 v in vertical slab gels. SDS-PAGE was performed on gradient separating gel of 10% polyacrylamide using a 4% stacking gel as previously described by **[10]**. Each well received samples of ASC, PSC, and standards. Gels were stained in methanol, acetic acid, and water (5:2:5 v/v/v) with 0.1% Coomassie Brilliant Blue R-250, and then de-stained in 75% methanol, 25% acetic acid. Collagen sample's amide band patterning was examined using FTIR (Bio-Rad, FTIR 40 model, USA) spectroscopy. Under drying conditions, 100mg of potassium bromide (KBr) and about 0.5mg of lyophilized collagen sample were ground together. With 32 scans per sample, ranging from 4000 to 400 cm, the spectrum was acquired. Software called ORIGIN 8.0 was used to analyse the

generated spectrum data (Thermo-Nicolet, USA) **[11]**.

RESULTS AND DISCUSSION

Percentage yield of ASC and PSC collagen

Collagen that has been pepsinized and acid-solubilized was extracted from *L. fulvus* the yields of *L. fulvus* at 42% and 23% (dry weight basis), respectively. In the *L. fulvus* the 0.5 M acetic acid extraction did not completely solubilize the nepa muscle. PSC, on the other hand, was entirely solubilized when the residue was treated with pepsin in 0.5 M acetic acid. **Figure 1** demonstrates the ASC and PSC samples that were collected as fibrils with a whitish and greyish colour, possibly from the monochrome pigment that was still present in the collagen sample. On a dry weight basis, the yield of ASC was likewise greater than that of PSC. The Blacktail Snapper (*L. fulvus*) was caught off the coast of Parangipettai, frozen after capture, and the collagen was extracted from those tissues. According to preliminary estimates based on the wet weight of the skin, the extraction yields of pepsin-soluble (PSC) and acid-soluble collagens (ASC) were 5.1% and 7.7%, respectively. The reduced yield of ASC indicates that the 0.5 M acetic acid did not completely solubilize the skin. This outcome was quite similar to that described by Jongjareonrak, Benjakul, Visessanguan, and Tanaka (2005) who noted that the skin of bigeye snapper had only partially been dissolved. acetic acid, 0.5 M. The current finding was justified by the collagen of L. The condensation of aldehyde groups in the telopeptide region of fulvus molecules most likely resulted in covalent bonds that cross-linked the molecules **[12] [13]**.

Electrophoresis (SDS- PAGE)

The SDS-PAGE gel profile and the pixel positions of the bands obtained for the standard collagen, protein marker and ASC and PSC of *L. fulvus* was depicted in **Figure 2**. The gel obtained through SDS - PAGE showed two bands in lane 1 representing the protein marker recorded six bands with the molecular weight of 130, 100, 80, 60, 40 and 20 kDa and standard collagen (Human placenta, Sigma, USA) in lane 4 representing witha molecular weight of 92, 71 and 27 kDa, respectively and in that order; whereas the ASC in lane 2 depicted three bands with a molecular weight of 86, 67 and 26 kDa, and the PSC in lane 3 showed two bands with a molecular weight of 86 and 63 kDa respectively. The blacktail snapper fish collagen (ASC and PSC) possessed similar protein pattern comparing of α1, α2 and β as their major components. Pepsin cleaves cross-link containing telopeptide and β-chain was concomitantly cleaved into two- α chains (Sato

et al., 2000). In addition, the band intensity of the α1-chain was 2-fold higher than that of the α2 chain for both ASC and PSC suggesting that intra and inter molecular cross link of collagen as higher in ASC and PSC. Higher molecular cross-linked molecules such as β-component increased as per their animal maturity **[14]** and straving fish collagen has more cross linked than that well fed **[15] [16]**. However, the cross-linked rate of fish skin collagen was extremely slow and also highly cross-linking rate **[17]**.

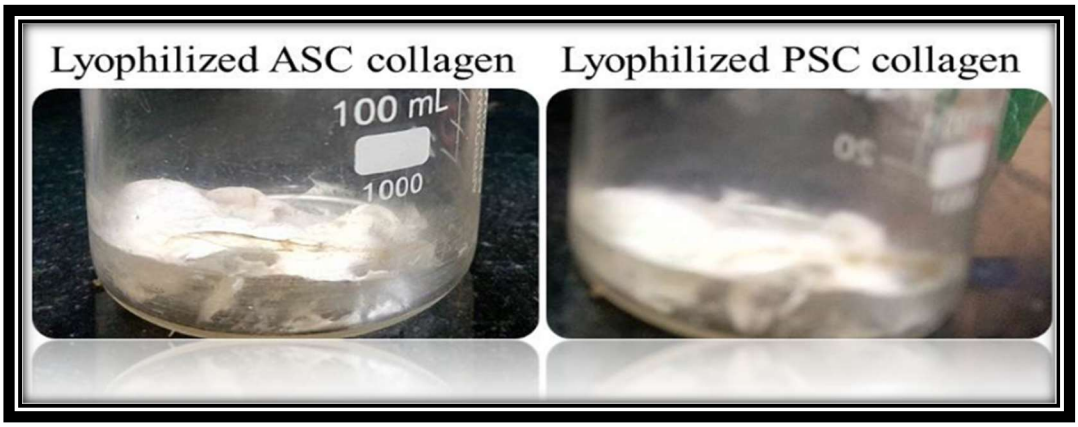

Figure 1*:* shows the isolated fish collagen (ASC and PSC)

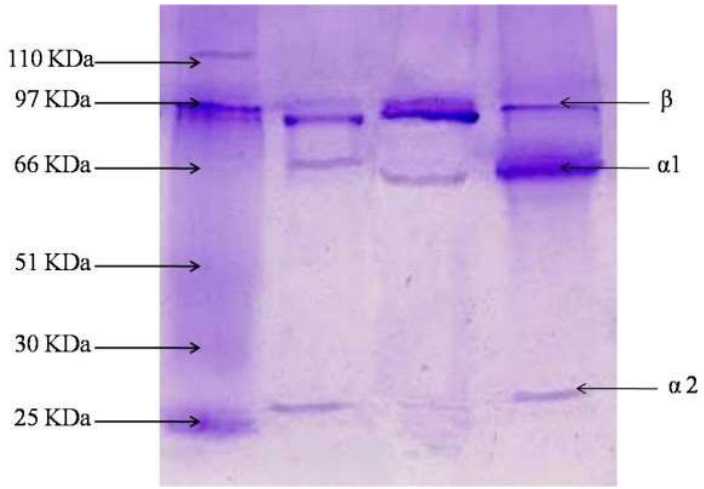

Figure 2: SDS- Polyacrylaminde Electrophorsis of Blacktail snapper (*L. fulvus*) Lane 1: protein marker, Lane 2: ASC sample, Lane 3: PSC sample, Lane 4: standard collagen

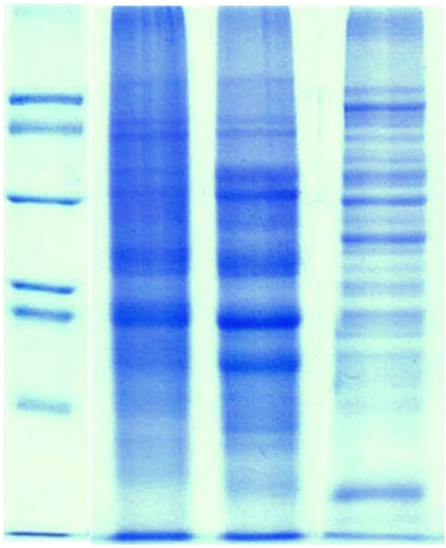

Figure 3: Peptide maps of ASC and PSC from Blacktail snapper (*L. fulvus*) digested by V8 protease. Lane 1: Marker, Lane 2: ASC, Lane 3: PSC and Lane 4: I type 1 calf skin collagen

The peptide maps of ASC and PSC of *L. fulvus* were revealed with V8 type-1 calf skin collagen as a control. The fragmented peptides within molecular weight were found to be 178, 162, 138, 123, 87, 76, 66, 65 and 54 kDa. After digestion of ASC and PSC with V8 protease, b- and c-components were almost completely hydrolysed which consists of high specific preference for glutamic acid and aspartic acid residues of proteins may due to lesser amount of glutamic acid and aspartic acid (75 and 45 residues/ 1000 residues) in calf skin collagen ASC (80 and 46 residues/1000 residues) and PSC (77 and 44 residues/1000 residues) might be more susceptible to hydrolysis by V8 protease. After hydrolysis with V8 protease, the following peptides with MW of 95.4, 66 and 25.7 kDa were obtained for ASC. In the case of PSC, peptides with MW of 71.1, 61.6 and 26.3 kDa were observed. However, type I calf skin collagen was resistant to the hydrolysis by V8 protease. When comparing the peptide maps between ASC and PSC hydrolysed by the V8 protease, PSC was more resistant to hydrolysis than ASC as indicated by a greater band intensity of the a-1 chain. As a result, chain lengths as well as amino acids at both the C- and N-termini were different. This might determine the accessibility of collagen molecules to proteases, leading to varying degrees of hydrolysis between ASC and PSC. The collagen peptide patterns were differing amongst the sources and species. Thus, ASC and PSC from the skin of *L. fulvus* might be different in terms of domain or cross-links and totally different from type I calf skin collagen in terms of sequence and composition of amino acids **(Figure 3)**.

Amino acid composition of acid soluble collagen and pepsin soluble collagen from the *Lutjanus fulvus*.

Table 1: Amino acid composition of ASC and PSC of *L. fulvus*

S. No.	Amino Acids	Code	ASC	PSC
1	Alanine	Ala	181	162
2	Arginine	Arg	94	106
3	Asparagine	Asn	42	45
4	Asparatic acid	Asp	36	27
5	Cysteine	Cys	-nil-	-nil-
6	Glutamic acid	Glt	63	68
7	Glycine	Gly	233	246
8	Histidine	His	8	5
9	Isoleucine	Ile	11	11
10	Leucine	Leu	18	14
11	Lycine	Lys	28	32
12	Hydroxylysine	Hyl	8	4
13	Methionine	Met	18	8
14	Phenylalaine	Phe	3	3
15	Hydroxylproline	Hyp	58	65
16	Proline	Pro	124	129
17	Serine	Ser	24	24
18	Threonine	Thr	13	12
19	Tyrosine	Try	7	4
20	Tryptophan	Try	-nil-	-nil-
21	Valine	Val	31	35
22	Imino acid		169	214

Amino acid composition of ASC and PSC were expressed as residues per 1000 total amino acids residues **(Table 1)**. The isolated ASC and PSC collagen had different amino acid profiles. At the outset, ASC and PSC was rich in Glycine (233% and 246%), alanine (181% and 162%), Proline (124% and 129%), hydroxyproline (58 %and 65%) tyrosine(7% and 4%), phenylalanine (3% and 3%), Hydroxylysine (8% and 4%), histidine (8% and 5%) and completely absence in tryptophan and cysteine respectively.

REFERENCES:

[1] Muyonga JH, ColeCCB, and Duoda KG: Characterization of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chem 2004; 85: 81-89.

[2] Kittiphattanabawon P, Benjakul S, Viessanguan W, NagaiT, and TanakaM: Characterization of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*).Food Chem2005; 89: 363-372.

[3] Senaratne LS, ParkPK, and Kim SK: Isolation and characterization of collagen from brown backed toadfish (*Lagocephalus gloveri*) skin. BioresourceTechnol 2006; 97: 191 - 197.

[4] JongjorecenarkA, EnjakulSB,Visessangaan W,Nagai T, and Tanaka M: Isolation and

characterization of acid and pepsin solubilised collagen from the skin Brownstripe red snapper (*Lutjanus vitta*). Food Chem 2005; 93: 475-484.

[5] Okazaki, E., & Osako, K. (2014). Isolation and characterization of acid-soluble collagen from the scales of marine fishes from Japan and Vietnam. *Food Chemistry*, *149*, 264-270.

[6] Nalinanon, S., Benjakul, S., Visessanguan, W., & Kishimura, H. (2007). Use of pepsin for collagen extraction from the skin of bigeye snapper (*Priacanthus tayenus*). *Food Chemistry*, *104*(2), 593-601.

[7] Singh, P., Benjakul, S., Maqsood, S., & Kishimura, H. (2011). Isolation and characterisation of collagen extracted from the skin of striped catfish (*Pangasianodon hypophthalmus*). *Food chemistry*, *124*(1), 97-105.

[8] Zhang, M., Liu, W., & Li, G. (2009). Isolation and characterisation of collagens from the skin of largefin longbarbel catfish (*Mystus macropterus*). *Food chemistry*, *115*(3), 826-831.

[9] Huang, Y. R., Shiau, C. Y., Chen, H. H., & Huang, B. C. (2011). Isolation and characterization of acid and pepsin-solubilized collagens from the skin of balloon fish (*Diodon holocanthus*). Food Hydrocolloids, 25(6), 1507-1513.

[10] Ahmad, M., Benjakul, S., & Nalinanon, S. (2010). Compositional and physicochemical characteristics of acid solubilized collagen extracted from the skin of unicorn leatherjacket (*Aluterus monoceros*). Food Hydrocolloids, 24(6-7), 588-594.

[11] Liu, D., Liang, L., Regenstein, J. M., & Zhou, P. (2012). Extraction and characterisation of pepsin-solubilised collagen from fins, scales, skins, bones and swim bladders of bighead carp (*Hypophthalmichthys nobilis*). Food Chemistry, 133(4), 1441-1448.

[12] Sinthusamran, S., Benjakul, S., & Kishimura, H. (2013). Comparative study on molecular characteristics of acid soluble collagens from skin and swim bladder of seabass (*Lates calcarifer*). Food Chemistry, 138(4), 2435-2441.

[13] Zhang, Y., Liu, W., Li, G., Shi, B., Miao, Y., & Wu, X. (2007). Isolation and partial characterization of pepsin-soluble collagen from the skin of grass carp (*Ctenopharyngodon idella*). Food chemistry, 103(3), 906-912.

[14] Ahmad, M., & Benjakul, S. (2010). Extraction and characterisation of pepsin-solubilised collagen from the skin of unicorn leatherjacket (*Aluterus monocerous*). Food Chemistry, 120(3), 817-824.

[15] Ogawa, M., Moody, M. W., Portier, R. J., Bell, J., Schexnayder, M. A., & Losso, J. N. (2003). Biochemical properties of black drum and sheepshead seabream skin collagen. Journal of Agricultural and food Chemistry, 51(27), 8088-8092.

[16] 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.

[17] Muyonga, J. H., Cole, C. G. B., & Duodu, K. G. (2004). Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food chemistry, 85(1), 81-89.