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Narrative Review: Extraction and Characterization of Collagen from Various Fish Species

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ABSTRACT

Collagen is a significant biomaterial in medical applications because of its unique properties, such as biodegradability and low antigenicity. As a result, collagen has been used in drug delivery systems and tissue engineering. Most commercial collagen is derived from cow skin, pig skin, or chicken waste. These terrestrial animal sources are inappropriate for many religious and ethnic groups, present regulatory and quality control challenges, and might contain contaminants and biological toxins, including BSE (mad cow disease). This review summarizes an overview of recent research conducted from 2014 to 2024 on collagen extraction from fish. Data were retrieved from online publications on the Pubmed database and covered fish collagen, extracts, and characterization. Fish by-products have been shown to be useful for producing collagen that can substitute porcine and bovine sources. Collagen extracted from fish body parts is classified as type 1. Some typical marine collagen characterization methods thats are often used are FTIR and SDSPAGE. Fish by-products have been proven to be valuable to produce collagen which can replace porcine and bovine sources. Fish by-products may be an alternative to the more prevalent collagen extraction methods.

INTRODUCTION

Collagen is the most abundant protein found in the animal kingdom, and different types of collagen have been identified regarding their role in our body mainly due to their triple helix characteristics. Each type of collagen has its own identity depending on its structural characteristics (Silvipriya et al., 2015). Collagen is the foremost plenteous protein within the human body capable of structure, soundness, and quality, particularly within the dermal layer (Kwatra, 2020). The most common types of collagen incorporate type I collagen (found in skin, tendons, and bone tissue), type II (found in cartilage), and type III (found in skin and blood vessels). Collagen incorporates a wide range of applications. Collagen is broadly utilized, in pharmaceutical, cosmetic, drug, and nourishment businesses due to its high biocompatibility, and safety, and can be easily decomposed by the environment (Sionkowksa et al., 2020).

Collagen is a vital biomaterial in therapeutic applications because of its extraordinary characteristics, as portrayed as easily degraded by the environment and powerless antigenicity, collagen is one of the latest types of biomaterial and has been utilized in sedate conveyance frameworks and tissue building. Most commercial collagen is inferred from bovine stowaway, pig cover-up, or chicken excrement. These earthbound creature sources are not reasonable for numerous devout and ethnic bunches, confront administrative and QC (Quality Control) troubles, also may contain natural contaminants and toxins, including BSE (Bovine Spongiform Encephalopathy), TSE

(Transmissible Spongiform Encephalopathy), and FMD (Foot and Mouth Infection) (Aberoumand, 2012).

Therefore, it's interesting to look at unused origins of collagen determined coming out of fish and other seafood is curious, one of them comes from fish skin. Fish collagens from skin, bone, cartilage, and scales, are more bioavailable compared to bovine or porcine collagen and have a higher absorption capability (up to 1.5 times), also more rapid bloodstream circulation due to their low molecular weight and small particle size (Caruso, 2016). Fish collagen also has complex auxiliary proteins that offer assistance in maintaining the quality and adaptability of the skin, tendons, joints, hair, nails, bones, muscles, gums, eyes, blood vessels, and ligaments. Apart from that, the application of fish skin as an origin of collagen production also provides benefits in solving the problem of fisheries waste and assembly of household collagen requests as a halal ingredient (Hartati and Kurniasari, 2010).

METHODS

The data presented in this review were retrieved from the Pubmed online database. A date filter was applied to cover the last ten years of literature (2014–2024). The search terms used were as follows: fish collagen, extract, and characterization. The three keywords were combined using the Boolean operator: "AND". Of the 136 records identified and screened, 11 articles were eligible and included in this review.

RESULT AND DISCUSSION

Collagen is a fundamental protein in various connective tissues, including skin and bones. Even though its tertiary structure is very complex and shapes a coiled-coil structure, the amino acid solution of collagen is as it was shaped by irregular reiterations of glycine, proline, and hydroxyproline-containing tripeptides. The main types of collagen incorporate collagen type I (located in skin, ligaments, and bone tissue) with α1 peptide chains and α2 peptide chains, collagen type II (located in cartilage) with α1 peptide chains and collagen type III (located in skin and blood vessels) with α1 peptide chains (Jafari, 2020). Each type of collagen can be formed from three identical chains, or two or three different chains, based on the collagen type (Amirrah et al., 2022). Based on the articles that have been collected in this review article, there are several methods for extracting collagen, including Acid-Soluble Collagen (ASC) and Pepsin-Soluble Collagen (PSC). Also, there are several characterization methods, such as X-ray Diffraction (XRD), SDS-PAGE, and FTIR. **Table 1** appears as a rundown of later ponders on the extraction and characterization of a few collagens determined from a fish.

Collagen Extraction Methods Pretreatment

Collagen generation comprises pretreatment, extraction, and characterization. The initial phase, known as preparation, differs according to the raw material used. During extraction, sample contamination must be reduced through pretreatment operations such as cleaning, washing, and size reduction (Chandrasekaran et al., 2024). In the articles that we used, all of them used pretreatment before doing extraction. Acidic and alkali pretreatment are moreover extensively applied to fish to eliminate non-collagen components including proteins, lipids, and colors, resulting in higher purity while maximizing collagen output and quality (Matinong et al., 2022).

Pretreatment was carried out at a temperature of 10 oC by dousing the fish skin in 0.1 M NaOH solution from 1: 10 (w/v) for 12 hours with the changed solvent every 2 hours. The solution resulting from soaking the skin with NaOH was tested for dissolved protein. Testing for dissolved proteins in the soaking time. The fish skin was at that point washed with destilled water until the skin pH got to be unbiased (Tabarestani et al., 2012).

Acid-Soluble Collagen (ASC) Extraction Procedure

ASC (Acid-Soluble Collagen) may be a type of collagen extricated utilizing as it were acid. The acid-collagen response disrupts the connections within the collagen helix, improving the quality of the extracted collagen. As a result, the utilization of distinctive acids to optimize productivity, immaculateness, and surrender of collagen extraction has long been a subject of investigation (Gaikwad & Kim, 2024). Based on the Pirarucu collagen extraction, a working temperature in 20°C was kept up with steady mixing in an ultra thermostatic shower with circulating water associated with a mechanical stirrer until the collagen was acquired. The

extraction preparation was created in two primary stages (Carpio et al., 2023).

The first step used a 0.05 M NaOH solution for 12 hours, exchanging solution every 6 hours, in a ratio of 1:20 (w/v). Then, the samples were rinsed with ice-cold distilled water until neutral pH. The samples were put in a 10% butyl alcohol solution with a proportion 1: 20 (v/v) for 24 hours, with solution substitution every 12 hours, and afterward rinsed with distilled water at 4°C, to prepare the solution for collagen extraction. In the second stage, a solution of acetic acid was used at a concentration of 0.5 M and proportion of 1:40 (w/v) for 72 hours, with changes every 24 hours. Once the viscous solution was obtained, buffering was performed with a 0.9 M carbonate-bicarbonate buffer solution and 0.9 M sodium chloride until a pH of 7. Thereafter, centrifugation was done at 4 °C at 11,000 g for 40 minutes. The material collected from the centrifugation was submitted to three dialysis processes. The first in a solution of 0.1 M acetic acid and the last two dialyses in ice-cold distilled water. Each dialysis was performed for 12 hours. The dialyzed material was frozen to be lyophilized to obtain the dried ASC (Carpio et al., 2023).

Pepsin Soluble Collagen (PSC) Extraction Procedure

In common, another commonly utilized collagen extraction strategy is PSC (Pepsin-soluble collagen) extraction, in which pepsin is included in the extraction handle. Concurring to Pal et al. (2016), the swim bladders were cut into small pieces (0.5 x 0.5 cm2) and soaked in 0.1 M NaOH at a strong to fluid proportion of 1: 10 (w/v) with nonstop gentle stirring in two days. The alkali solution was changed at 12-hour intervals. The treated swim bladder build up was washed with cold distilled water to realize the neutral pH. The defatted swim bladder was defeated utilizing 100 mL butyl alcohol at a strong to fluid proportion of 1: 10 (w/v) in 24 hours. The defatted swim bladder buildup was altogether cleaned with a break even with a volume of cold distilled water (4 °C) and subjected to PSC extraction (Pal et al., 2016).

PSC was extricated utilizing 0.5 M acetic acid with 2 g (w/v) pepsin at a strong to fluid proportion of 1: 10 (w/v) at 4 °C for 48 hours with persistent gentle stirring. The mixture was sifted through a double-folded cheesecloth. Along these lines, the filtrate was spun in a centrifuged (41097 x g in 1 hour at 4 °C) utilizing an ultracentrifuge. The supernatant was collected and treated with NaCl to a last concentration of 2.6 M within the nearness of 0.05 M Tris (hydroxymethyl) aminomethane with pH 7.0 (neutral). The accelerated collagen was gathered by centrifugation (41097 x g in 1 hours at 4 °C) and broken up in the least amount of 0.5 M acetic acid. The coming about solution was subjected to dialysis (atomic weight cutoff ~ 12-14 kDa) towards 0.1 M acetic acid for 48 hours with solution substitution every 12 hours, followed by distilled water with water substitution until neutral pH was obtained. The dialyzed solution was freeze-dried. All strategies were performed at 4 °C. The gotten collagen was alluded to as PSC and put away at -20 °C until utilized (Pal et al., 2016).

Collagen Characterization Methods FTIR (Fourier transform infrared spectroscopy)

FTIR (Fourier transform infrared spectroscopy) is a common technique to analyze the secondary structure of proteins. FTIR is relevant because it detects every absorption wavenumber between 500 and 4000 cm-1. Most collagen from marine species is found to be type I collagen, and FTIR analysis has been proven in various studies to be an effective method for structural characterization of collagen (Oslan et al., 2022).

Type I collagen shows the highest absorption peak in the wavelength region of 220 to 240 nm. This peak is related to -C = O, -COOH, and -CONH2 in the polypeptide chain of the collagen molecule (Bhuimbar et al., 2019). According to Ampitiya (2022), the UV absorption spectrum was recorded between wavelengths of 190 nm and 400 nm, and the maximum absorption wavelength of the three selected fish species occurred at 217 nm. According to Nurubhasha et al. (2019), the maximum absorbance for the extracted protein was measured at 235 nm for collagen samples dissolved in acid and pepsin. Relying on Jaziri (2023), The spectrum was arranged among 4000 nm^{-1} and 800 cm^{-1}, He et al. (2019) adjusted the spectrum in the range of 650–4000 cm^{-1}. Carpio et al. (2023) used a spectrum in the range of 4000–400 cm^{-1}.

SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis)

SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) could be a

strategy that has been utilized to isolate proteins and protein parts based on estimates. SDS-PAGE can be utilized to decide the atomic weight of collagen. If the collagen groups are compared, collagen type can be decided by comparing it to collagen from different sources. Collagen consists of three α chains that can be indistinguishable or divergent, based on collagen type (Oslan et al., 2022).

Collagen can comprise three indistinguishable alpha chains or diverse alpha chains. For illustration, type I collagen usually consists of two α1 chains and one α2 chain, type II collagen consists three α1 chains and type IX collagen consists α1, α2, and α3 chains (Bielajew et al., 2020). According to Mau et al. (2023), α1 and α2 chains were shown in ASC and PSC tests at a proportion of ~2: 1, and dimeric (β chain) and trimeric (γ chain) shapes were moreover clearly obvious. Agreeing with Jaziri (2023), the α1 chain appeared a two-fold increment in band concentration compared to α2, demonstrating that parrotfish scale collagen is type I. Based on He et al. (2019), according to the SDS-PAGE design of GCC, CCC, and GSC. It can be seen that all have α1, α2, and β chain groups. Carpio et al. (2023) expressed that the test displayed two α chains within the proximal position (α 1 and α 2), with an atomic mass among 100 kDa and 120 kDa, also β chain and γ chain with an atomic mass over 200 kDa.

The brighter band in all collagen extractions comes from the α1 chain, suggesting that the collagen extraction contains collagen types I and II (Seixas et al., 2020). Pal et al. (2016) has the result, that the isolated PCS's electrophoretic pattern and mobility showed that PSCs comprised two α chains (α1 chain and α2 chain) also one β chain as the primary components. Wang et al. (2017) give results that the molecular masses for the α1 chain and α2 chain were 127 kDa and 115 kDa, separately. The escalation of the α1 chain was two times that of the α2 chain, demonstrating that the collagen comprised two indistinguishable α1 subunits and one α2 subunit. According to Kozlowska et al. (2015), collagen from Esox lucius scales incorporates two diverse α chains (α1 chain and α2 chain). Collagen in PSC and ASC features an atomic mass of approximately 118 kDa for α1 and 108 kDa for α2. High molecular weight parts, counting dimmer (β) and trimmer (γ) parts, were also watched in PSC and ASC. Yu et al. (2018) explained that PSC from Nibea japonica skin has one α2 chain and two α1 chains. This examination also recognized β and γ chains, as well as interrelated components. PSC from Nibea japonica skin might have an (α1)2α2 structure, which was designed as type 1 collagen. Based on the articles we used, all of them gave results that the collagen tested was type I collagen, except for Seixas et al. (2020), which gave results for collagen type I and II.

CONCLUSIONS

According to current research, considerable collagen extracted from fish body parts is classified as type 1. Some typical marine collagen characterization methods that are often used are FTIR and SDS-PAGE. Fish by-products have been proven to be valuable to produce collagen which can replace porcine and bovine collagen sources. Marine fish collagen can fulfill natural collagen needs from non-porcine and non-bovine sources. Fish by-products may be an alternative to the more prevalent collagen extraction methods.

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AUTHORS' CONTRIBUTIONS

Aidatul Mufidah conceptualized the study, designed the methodology, and was responsible for the literature search and primary content analysis. Wiranti Sri Rahayu and Pri Iswati Utami contributed to edited and revised the article. All authors reviewed and edited the final manuscript.

CONFLICT OF INTERESTS

The authors have no conflict of interests related to this publication.

ETHICAL CONSIDERATION

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, redundant, and double publication) have been completely observed by the authors.

Table 1. Summary of fish collagen characterization using different extraction techniques and collagen analysis from various species of fish

Fish Species	Collagen Source	Extraction Method	Characterization Method	Characterization Results	Types of Collagen	Reference
Elasmobranch	Cartilage	PSC (Pepsin-soluble collagen) and ASC (Acid-Soluble Collagen)	SDS-PAGE	The lighter bands in every single collagen extraction originated from α1 chains, suggesting that the collagen extractions contained both collagen type I and type II.	Type I Collagen Type II Collagen	(Seixas wt al., 2020)
Pirarucu Fish (*Arapaima gigas*)	Skin	ASC (Acid-soluble collagen)	SDS-PAGE and FTIR	The sample presented two α chains at the proximal position, along molecular masses among 100 kDa and 120 kDa, as well as β and γ subunits along molecular masses over 200 kDa. The equilibration of collagen triple helix structure was assessed by measuring the absorption ambit among amide III and 1454 cm^{-1}, which was near 1, indicating that ASC's molecular structure generated of pirarucu skin tissue was steady.	Type 1 collagen	(Carpio et al., 2023)
Sucker catfish (*Pterygoplichthys pardalis*)	Skin	PSC (Pepsin-soluble collagen) and ASC (Acid-Soluble Collagen)	UV-Vis and FTIR	The maximum absorbance for the extracted proteins was measured at 235 nm for collagen samples dissolved in acid and pepsin. PSC and ASC samples showed the appearance of a definite amide I peak between 1615 and 1680 cm^{-1} and a significantly less amount of amide II between 1530 and 1600 cm^{-1}, which could be in consequence of the existence of hydrogen bonds maintaining the three collagen helices.	Type I Collagen	(Nurubhasha et al., 2019)
Megalonibe fusca	Swim bladder	PSC (Pepsin-soluble collagen) and ASC (Acid-Soluble Collagen)	SDS-PAGE	The α1 and α2 chains are exist in both of PSC sample and ASC sample at a ratio of ~2:1, and trimeric (γ chain) and dimeric (β chain) forms are visible. The value of molecular weight for the bands in the PSC sample (α1 chain: 134 kDa & α2 chain: 120 kDa) are just below the molecular weight values in the ASC sample (α1 chain: 137 kDa & α2 chain: 124 kDa), which matches the pepsin related removal of the telopeptide area.	Type I Collagen	(Mo et al., 2023)
Grass Carp (*Ctenopharyngodon idella*) and Crucian Carp (*Carassius*	Skin and Scales	Acid-enzyme combination method	FTIR and SDS-PAGE	Five bands of type I collagen were found, and a ratio of one indicated a triple helix shape. According on the SDS-PAGE patterns of CCC, GCC, and GSC, it can be observed that all of them have β, α1, and α2 chain bands, indicating that the extracts are standard collagen type I.	Type I Collagen.	(He et al., 2019)

Fish Species	Collagen Source	Extraction Method	Characterization Method	Characterization Results	Types of Collagen	Reference
carassius)						
Parrotfish (*Scarus sordidus*)	Scales	Acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC).	FTIR and SDS-PAGE	PSC-PFS and ASC-PFS has same delta-v value (Δv = 95.05 cm^{-1}), suggesting that the parrotfish scale collagen's triple helical structure was retained. The α1 chain showed a twofold increase in band intensity compared to α2, indicating that the parrotfish scale collagen is type I.	Type I Collagen	(Jaziriet al., 2023)
Asian sea bass (*Lates calcarifer*, Seer fish (*Scomberomorus commerson*) and Yellowfin tuna (*Thunnus albacares*),	Skin	Acid-soluble collagen (ASC)	FTIR	The UV absorption spectra were measured from 190 nm and 400 nm for the three chosen fish species, and the highest absorption wavelength of the three chosen fish species occurred at 217 nm.	Type I Collagen	(Ampitiyaet al., 2022)
Rohu (*Labeo rohita*)	Swim bladder	Pepsin-soluble collagen (PSC).	SDS-PAGE	The isolated PCS's electrophoretic pattern and mobility showed that it consisted of two chains (α1 and α2) also β chain as the main components. The extracted collagen subunits had molecular weights of 90 kDa, 97 kDa, and 200 kDa for α1, α2, and β.	Type I Collagen	(Pal et al., 2016)
Loaches (*M. anguillicaudatus*)	Skin	PSC (Pepsin-soluble collagen) and ASC (Acid-Soluble Collagen)	SDS-PAGE	The molecular masses for α1 and α2 are 127 kDa and 115 kDa. The strength of the α1 chain is double of α2 chain, indicating that collagen is composed of two identical α1 subunits and one α2 subunit.	Type I Collagen	(Wang et al., 2017)
Northern pike (*Esox lucius*)	Scales	PSC (Pepsin-soluble collagen) and ASC (Acid-Soluble Collagen)	SDS-PAGE	Collagen from *Esox lucius* scales includes two distinct α chains (α1 and α2). Collagen in ASC and PSC has molecular masses of approximately 118 kDa for the α1 chain and 108 kDa for the α2 chain. High molecular weight parts, such as β (dimmer) and γ (trimmer) components, are observed in PSC and ASC.	Type I Collagen	(Kozlovska et al., 2015)
Giant Croaker (*Nibea japonica*)	Skin	Pepsin-soluble collagen (PSC).	SDS-PAGE	PSC from Nibea japonica skin has one α2 chain and two α1 chains. This study also identified β and γ chains and their interconnected elements. PSC derived from *Nibea japonica* skin might have an (α1)2α2 structure, called Type 1 collagen.	Type I Collagen.	(Yu et al., 2018)

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