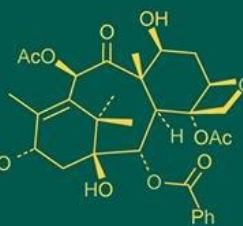
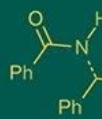


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(9): 502-507
www.biochemjournal.com
Received: 15-07-2025
Accepted: 18-08-2025

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A review on methods extraction of collagen fish and it's applications in various industries

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i9g.5681>

Abstract

Collagen, the most abundant structural protein in vertebrates, plays a critical role in tissue regeneration, wound healing, and structural integrity of skin, bones, tendons, and cartilage. With growing demand across biomedical, cosmetic, pharmaceutical, food, and packaging industries, fish-derived collagen has emerged as a sustainable and safer alternative to mammalian sources, avoiding zoonotic risks and cultural concerns. This review highlights methods of extracting collagen from fish by-products such as skin, scales, bones, and swim bladders, focusing on acid-soluble collagen (ASC), pepsin-soluble collagen (PSC), hydro-extraction, neutral salt extraction, enzymatic techniques, and gelatin production. Each method varies in yield, purity, cost, and application potential, with PSC and enzymatic extraction offering higher recovery and reduced immunogenicity, while gelatin dominates in food and pharmaceutical industries due to its scalability. Comparative studies demonstrate species- and tissue-specific variations in yield and structural stability, further influencing industrial applications. Beyond extraction, this review emphasizes collagen's versatility in food processing, nutraceuticals, cosmetics, wound healing, drug delivery, biomaterials, and smart packaging. Advances in green chemistry, enzyme-assisted processes, and nanotechnology are improving efficiency, sustainability, and bioactivity. Overall, fish collagen extraction represents a promising strategy for transforming low-value by-products into high-value biomaterials with significant biomedical and industrial potential.

Keywords: Collagen extraction, fish by-products, biomedical applications, food packaging, pepsin-soluble collagen

Introduction

Collagen is ^[1] the primary biomedical applications of collagen were in biomaterials, especially as drug and gene carriers, tissue engineering, absorbable surgical suture, osteogenic and bone filling materials, hemostatic agents, immobilization of therapeutic enzymes, and burn/wound cover dressings ^[2]. Collagen is a crucial component of the wound-healing process; it acts as a natural structural scaffold or substrate for new tissue growth and plays an essential role in all phases of wound healing, including hemostasis, inflammation, proliferation, and remodeling ^[3]. The collagen produced by organisms is defined as endogenous, which consists of three long helicoidally shaped chains of amino acids ^[4]. Chains of polypeptide constituted by the repeated sequence (Gly-X-Y)_n form collagens, where X and Y can be occupied by any amino acid, although these positions are commonly occupied by proline and 4-hydroxyproline ^[5, 6]. Collagen breaks down due to aging, exposure to ultraviolet light, and tobacco. The degradation of collagen results in wrinkles, sagging skin, and dry skin, and therefore, it is essential to identify new resources of collagen for regenerative tissue applications ^[7]. Figure 1. Shows the chemical structure of collagen type I. Figure 2. Shows the Triple-helix structure of collagen.

Collagen from vertebrate fish can be isolated through several initial stages. The initial stages involve preparing the fish parts intended for isolation, including separation and cleaning, size reduction, and the removal of non-collagen components. Parts of the fish such as the skin and bones, which serve as collagen sources, are separated from the meat or other impurities and then cleaned with water. Subsequently, the sample is reduced in size or ground to facilitate.

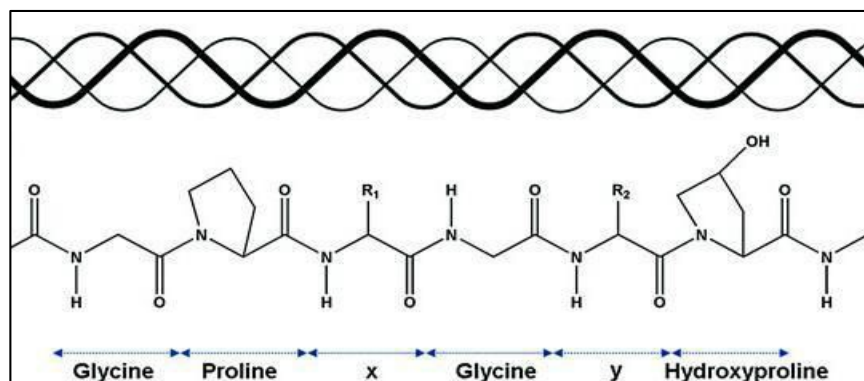


Fig 1: Chemical structure of collagen type I. Adapted from [4].

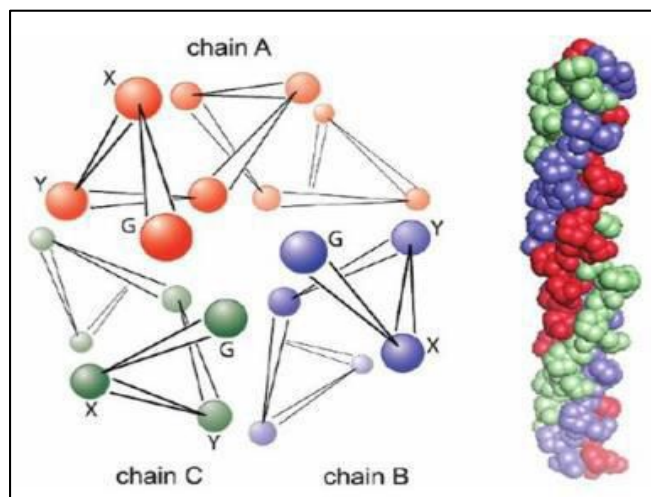


Fig 2: Triple-helix structure of collagen. Adapted from [8].

Vertebrates are animals characterized by having an internal backbone or spinal column. Vertebrate animals encompass more than 85,000 species [9] they are categorized into mammals, fish (pisces), reptiles, amphibians, and birds. Among vertebrate animals, fish constitute the largest group, with approximately 25,000 recorded species. Fish are cold-blooded creatures known for their distinctive features, including a backbone, gills, fins, an aquatic habitat, and the use of fins for maintaining balance in water [10]. Various types of fish, such as tilapia, catfish, cod, short-finned fish, and others, offer significant health benefits and can also serve as alternative sources of collagen. The purification processes. The removal of non-collagen components such as fat, minerals, pigments, and odors is carried out using NaOH. After the initial or pre-treatment stages, the extraction phase is conducted using an extraction method. This is followed by collagen precipitation and drying to obtain collagen yield [11].

Methods of collagen extraction

1. Acid-soluble collagen (ASC)

Acid-soluble collagen (ASC) extraction is one of the most traditional methods of recovering collagen from animal by-products. The procedure typically employs dilute organic acids such as acetic or citric acid, which disrupt hydrogen bonding and solubilize collagen molecules [12]. ASC is particularly effective in tissues with immature aldimine cross-links, as these are reversible under acidic conditions [4]. Fish skins such as tilapia, cod, and carp provide higher yields compared to mammalian tissues due to lower hydroxyproline content and weaker cross-linking [13].

Historically, rat tail tendon was the first tissue successfully used for ASC, paving the way for studies on collagen biochemistry [12]. The extracted collagen retains its triple-helical structure, making it a useful material in biomedical scaffolding and tissue engineering applications [14]. However, the retention of telopeptides has been linked with immunogenicity issues, which limits its direct use in clinical settings [4]. Yields are generally less than 5% in mature mammalian tissues, restricting its scalability [13]. Despite this, ASC is cost-effective as it avoids expensive enzyme treatments [12]. *in vitro*, ASC exhibits strong fibrillogenesis ability, offering insights into collagen self-assembly mechanisms [14]. Acid treatment can slightly modify amino acid residues, but optimized conditions minimize damage to the helical domains [13]. Process intensification methods, such as ultrasound-assisted ASC, have improved extraction efficiency in recent years. ASC is frequently used as a preparatory step before enzymatic digestion, boosting final yields [14]. Marine-derived ASC is particularly attractive in markets with concerns about zoonotic diseases [13]. Applications also extend to wound dressings and biomaterials, given its structural compatibility with tissues [4]. Although ASC offers lower yields, its simplicity and reproducibility ensure its continued relevance [12]. Overall, ASC extraction remains an accessible technique for both research and industrial purposes.

2. Pepsin-soluble collagen (PSC)

Pepsin-soluble collagen (PSC) improves upon ASC by incorporating enzymatic digestion. In this approach, pepsin selectively cleaves the telopeptide regions, solubilizing more collagen without disrupting the triple-helical core. As a result, PSC yields are considerably higher than ASC, sometimes exceeding 30% in chicken feet or fish skins. By removing telopeptides, PSC reduces immunogenicity, which enhances its suitability for biomedical applications. This feature is particularly valuable for mature mammalian tissues where extensive cross-linking prevents efficient acid solubilization [15]. Studies report that comminution of tissue prior to pepsin digestion further enhances recovery. However, the use of porcine pepsin poses ethical and cultural concerns in certain regions. Over-digestion is another challenge, as excessive exposure to pepsin can damage triple-helical domains [16]. Fish-derived PSC generally demonstrates lower thermal stability compared to mammalian PSC, reflecting the habitat temperatures of the source species. Despite these limitations, PSC collagen has been applied successfully in wound healing formulations and regenerative scaffolds [17]. Industrially, PSC is increasingly utilized for cosmetics and nutraceutical

supplements ^[15]. Compared to ASC, PSC is less immunogenic, making it a better fit for clinical-grade products. Purification steps, such as ultrafiltration, are essential to remove degraded non-collagenous peptides ^[16]. Although enzyme costs make PSC more expensive, the higher yields offset this disadvantage. PSC extraction is widely applied in marine industries, turning fishery by-products into value-added collagen ^[15]. PSC also serves as a starting material for collagen peptide production, extending its utility in nutraceuticals ^[17]. The method remains the most effective strategy for maximizing yields across a variety of tissues ^[16]. Ultimately, PSC is regarded as a gold standard for biomedical-grade collagen recovery.

3. Hydro-extraction (Water-based method)

Hydro-extraction, or water-based extraction, is among the mildest techniques for collagen recovery. In this method, tissues are immersed in distilled or deionized water, which leaches soluble proteins including collagen fragments ^[16]. It is particularly effective for tissues containing immature collagen or partially denatured fibrils ^[17]. Compared with acid or enzymatic extraction, hydro-extraction yields are significantly lower, but the collagen remains free from chemical residues ^[16]. This purity makes it especially attractive for food and pharmaceutical uses, where absence of chemicals is crucial. Extraction times are longer than other methods, sometimes requiring several days for measurable recovery ^[17]. Hydro-extraction aligns with green chemistry principles, as it avoids acids, alkalis, and enzymes. The process generates eco-friendly collagen suitable for nutraceuticals and natural cosmetics ^[17]. Structural analysis shows that water-extracted collagen retains its native helicity but demonstrates weaker fibrillogenesis than ASC ^[16]. Industrial scalability remains a challenge due to long processing times and poor efficiency. Nevertheless, it provides analytical-grade samples for biochemical research ^[17]. Hydro-extraction can also be used as a first step before applying enzymatic digestion for enhanced yields ^[16]. Advances in membrane-assisted hydro-extraction are under investigation to improve productivity ^[17]. While unlikely to replace ASC or PSC in industrial production, hydro-extraction provides unparalleled purity ^[16]. This method is thus best suited for niche applications prioritizing safety and sustainability.

4. Neutral salt extraction

Neutral salt extraction utilizes high concentrations of NaCl at neutral pH to extract collagen. It was developed following

the limitations observed with ASC in terms of yield and cross-link disruption. This method enables the recovery of intact collagen molecules that closely resemble native forms. Electron microscopy has confirmed that salt-extracted collagen reforms into fibrils with characteristic D-banding patterns. However, yields remain very low, often lower than ASC or PSC ^[16]. Neutral salt extraction is particularly effective in juvenile tissues with incomplete cross-linking ^[15]. Unlike acid treatment, neutral salts do not reverse immature aldimine bonds, further restricting yields. Despite limited applications, the collagen obtained is exceptionally pure. Biomedical applications are restricted due to scalability issues ^[17]. Instead, neutral salt extraction is valued for isolating precursor collagens for laboratory studies ^[15]. In some cases, it is combined with enzymatic digestion for higher yields ^[16]. It provides collagen in the most native-like state of all extraction techniques ^[14]. Thus, while unsuitable for mass production, it remains important in collagen biochemistry research.

5. Enzymatic extraction

Enzymatic extraction uses proteases to cleave non-helical regions while preserving the triple helix. Pepsin is the most common enzyme, but papain, trypsin, chymotrypsin, and microbial enzymes are also used. Bacterial collagenase is unique in that it digests intact helices into small peptides ^[16]. Yields from enzymatic extraction are generally higher than ASC or neutral salt methods. Different enzymes generate distinct peptide profiles, influencing collagen's functional properties ^[17]. For example, papain and alcalase produce smaller peptides than pepsin ^[16]. Enzymatic extraction is the preferred route for collagen hydrolysates. It also reduces immunogenicity by removing telopeptides. Enzyme costs, however, can be a limiting factor for large-scale production. Pretreatment steps, such as thermal denaturation, often improve enzyme accessibility ^[16]. Purification via ultrafiltration is needed to remove degraded non-collagenous proteins ^[17]. Biomedical-grade collagen is often sourced via enzymatic extraction due to its safety and structural integrity ^[15]. Enzymes also allow tailoring of peptide lengths for nutraceutical applications ^[17]. Recent innovations include thermostable enzymes for improved efficiency ^[16]. Overall, enzymatic extraction balances yield, purity, and functionality better than most alternatives. Table 1 provides information about collagen type I sources, extraction methods, and yields from various fish species across different regions.

Table 1: Collagen type I sources, extraction methods, and yields from various fish species across different regions.

Species	Habitat	Tissue	Collagen Type	Extraction Method	Yield (%)	Reference
<i>Oreochromis</i> sp.	Mexico	Skin, gills	I	Sequential Tris buffer, 0.5 M Acetic acid, 1% Pepsin	1.46, 3.02, 2.52 (dry weight)	[18]
Sole fish	India	Skin	I	0.54 M Acetic acid + 1.9 M NaCl (32 h)	19.27 mg/g protein	[20]
<i>Centrolophus niger</i>	India	Skin	I	0.5 M Lactic acid/Acetic acid (72 h, 4 °C)	45 (wet basis), 25	[21]
Small-spotted catshark (<i>Scyliorhinus canicula</i>)	NE Atlantic	Skin	I	1 M Acetic acid (34 h, 25 °C)	61.24	[22]
Rabbitfish	NE Atlantic	Skin	I	0.5 M Acetic acid	20	[22]
Tilapia & Grey mullet	Freshwater	Scales	I	0.5 M Acetic acid (3 days)	40 (ASC)	[23]

Applications of Collagen in Different Industries

1. Food Industry

Food Additives and Stabilizers

Collagen, particularly gelatin, is widely employed in food processing for its rheological properties that improve texture, viscosity, and elasticity of products like desserts, dairy items, and sausages [18, 25]. Additionally, collagen hydrolysates act as natural antioxidants, preventing lipid peroxidation in meat systems.

Functional Foods and Nutraceuticals

Bioactive peptides derived from collagen hydrolysis have demonstrated antioxidant, antihypertensive, and metabolic regulatory effects, making them ideal for functional foods and supplements [18, 27, 28]. Clinical trials have shown that regular collagen peptide supplementation improves skin elasticity, bone density, and joint health, supporting its dual role in nutrition and therapy.

Biodegradable Food Packaging

Gelatin-based films are increasingly explored as sustainable packaging materials due to their biodegradability and gas barrier properties. However, they often require reinforcement through crosslinking or blending to overcome poor mechanical stability [29, 30]. Functionalization with natural extracts such as rosemary or grape enhances antioxidant and antimicrobial performance, supporting their use in active packaging.

2. Cosmetic Industry

Moisturizing and Anti-Aging

Hydrolyzed collagen is extensively used in creams, gels, and masks because of its superior solubility and water-binding properties. It helps retain skin moisture, reduce wrinkles [18, 32, 33]. Collagen peptides also act as antioxidants, providing UV protection and anti-photoaging benefits.

Injectable Dermal Fillers

Collagen-based fillers are applied in dermatology to reduce scars, wrinkles, and aging-related skin depressions. Their biodegradability and ability to stimulate fibroblast proliferation make them a reliable alternative to synthetic fillers [34, 36]. Despite newer filler materials, collagen remains relevant in aesthetic medicine.

Nail and Hair Formulations

Collagen peptides are added to shampoos and conditioners to strengthen keratin fibers, reducing breakage and enhancing tensile strength. Oral supplementation has also been linked to improved nail growth and reduced brittleness [35, 28]. These applications extend collagen's role in personal care beyond skincare.

3. Pharmaceutical Industry

Drug Delivery Systems

Collagen hydrogels and nanoparticles are attractive carriers for proteins, genes, and anticancer drugs, thanks to their biodegradability and capacity for controlled release [18]. They allow targeted therapy, reducing side effects and improving bioavailability.

Wound Healing Materials

Collagen dressings accelerate tissue repair by stimulating fibroblast migration, angiogenesis, and extracellular matrix

formation [18, 40]. Enrichment with antimicrobial agents such as silver or tannic acid further enhances their healing potential.

Nutraceutical Supplements

Oral collagen supplementation has been widely applied in pharmaceutical nutraceuticals to treat osteoarthritis, osteoporosis, and metabolic disorders. It improves joint flexibility, reduces inflammation, and supports bone formation [41, 28]. Its high bioavailability ensures efficient absorption and therapeutic impact.

4. Packaging Industry

Edible Sausage Casings

One of the earliest applications of collagen is in the production of sausage casings. These provide strength, permeability, and elasticity, serving as hygienic and uniform alternatives to animal intestines [42, 43]. Their use remains widespread in the meat industry.

Active Packaging Films

Collagen-based films combined with natural extracts such as rosemary, oregano, or grape act as antioxidant and antimicrobial packaging solutions [44, 45]. These systems extend shelf life and enhance food safety without compromising sensory properties.

Smart Packaging

Innovative collagen blends incorporating natural pH-sensitive indicators are used to monitor food freshness. Anthocyanins and betalains embedded in collagen films change color in response to spoilage compounds, ensuring consumer awareness [18, 47]. This positions collagen as a key player in intelligent packaging design.

5. Biomedical Industry

Tissue Engineering

Collagen scaffolds closely mimic extracellular matrix architecture, supporting regeneration of bone, cartilage, and skin tissues [48, 52]. Blends with chitosan or hydroxyapatite enhance mechanical strength and osteogenic capacity.

Medical Implants and Devices

Due to high biocompatibility, collagen is used in vascular grafts, corneal implants, and artificial valves. Crosslinking with agents such as genipin or EDC-NHS improves durability and reduces degradation. These devices integrate with host tissues and minimize immune reactions.

Neural and Skin Repair

Collagen scaffolds facilitate neural regeneration in spinal cord injuries by bridging axonal gaps and promoting growth. In wound repair, collagen matrices regulate fibroblast infiltration and improve dermal regeneration, confirming their clinical potential.

Conclusion

Collagen remains an indispensable biomaterial, valued for its structural, regenerative, and functional properties across diverse industries. Traditionally sourced from bovine and porcine tissues, its extraction now faces ethical, safety, and cultural challenges. Fish by-products have therefore gained prominence as sustainable, cost-effective, and biocompatible alternatives. This review explored the major

extraction methods—acid-soluble, pepsin-soluble, hydro-extraction, neutral salt, enzymatic digestion, and gelatin processing—each presenting unique benefits and limitations. Among these, PSC and enzymatic methods offer the most promising balance between yield, bioactivity, and reduced immunogenicity, while gelatin remains dominant in large-scale applications due to its versatility in food, pharmaceutical, and packaging industries. Biomedical uses demand high-purity collagen with intact structural integrity, favoring enzymatic or PSC methods. In contrast, food and cosmetic industries utilize hydrolysates and gelatin for their functional properties, such as gelling, antioxidation, and moisture retention. Emerging technologies—including ultrasound-assisted extraction, membrane filtration, and green-solvent approaches—are addressing challenges of low yield, long processing times, and environmental impact. Continued interdisciplinary research into optimized extraction, purification, and application strategies will enhance collagen's role as a high-value biomaterial. Fish-derived collagen thus represents both a scientific opportunity and a sustainable industrial solution with global relevance.

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