

RECENT PROGRESS IN SUSTAINABLE FISH BYPRODUCT UTILIZATION: UNVEILING FISH COLLAGEN AS A POTENTIAL WOUND HEALING AGENT

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Abstract

Utilizing fish byproducts is a strategic focus on reducing waste from fish processing, garnering significant global attention from researchers and fish processors aiming for sustainable practices in line with Sustainable Development Goal 12 by 2030. One highly effective approach involves converting these byproducts into valuable commodities, such as collagen and its hydrolysates. Collagen is an important protein in living organisms that serves as fibrous structural component in the extracellular matrix. Over the last decade, fish collagen has gained interest due to its superior physicochemical properties, biocompatibility, biodegradability, safety, and high absorption by the human body. These attributes make fish collagen a promising area of research, particularly for wound healing applications. This review covers the extraction procedures and characteristics of collagen derived from fish byproducts, providing a comprehensive overview of the methods used to obtain high-quality collagen. Additionally, it highlights recent research progress on the use of collagen as a wound healing agent, evaluated through both *in vitro* and *in vivo* studies. The review also addresses the challenges associated with applying collagen for wound healing, such as optimizing extraction methods, ensuring consistent quality, and overcoming potential regulatory hurdles. Furthermore, it presents a future perspective on the potential of fish collagen as an efficient and effective wound healing product, emphasizing its role in advancing biomedical research and applications. By offering new insights and stimulating further development in the utilization of fish collagen, this review aims to contribute to waste reduction in fish processing and enhance the economic value of byproducts. Thus, fish collagen holds significant promise as a sustainable and innovative solution in the biomedical field, aligning with global efforts to achieve sustainability and improve human health.

Key words: fish waste, valorisation effort, high-value product, physicochemical properties, wound repair product

Traditionally, fresh seafood has been the most consumed food globally, but over the years, the demand for processed seafood products has increased. Production of frozen fish fillets, peeled shrimp, surimi, shucked shellfish, fish balls, fish cakes, and ready-to-eat seafood products has surged. The Food and Agriculture Organization (FAO) reported that global fish and seafood consumption rose from 71.8 million tons in the 1980s to 157.4 million tons in 2020 (FAO, 2022). To meet this demand, the fish processing industry has diversified its products while maintaining quality. However, increased fish processing has resulted in more fish byproducts, which constitute 75% of total fish weight and pose environmental challenges if discarded untreated (Benjakul et al., 2020). Proper management of these byproducts can enhance resource use and profitability while reducing environmental impact. This practice is in line with the main concern of the United Nations Sustainable Development Goals (SDGs), specifically under Sustainable Consumption and

Production (Goal 12). Fish byproducts, rich in nutrients, have conventionally been converted into low-value products such as silage, fishmeal, fish oil, animal feed, and fertilizer (Jaziri et al., 2021). Advances in science and technology now offer methods to transform these byproducts into high-value products.

Collagen is a fibrous protein that provides structural support to the extracellular matrix of connective tissues. Due to its rigidity and resistance to stretching, it is the perfect matrix for skin, tendons, bones, and ligaments, and it makes up around thirty percent of the protein composition (León-López et al., 2019 a). Up to now, approximately 29 types of collagens have been explored in different tissues, and each type is classified based on a unique protein structure and amino acid sequence (Soroushianova et al., 2019). Among the types studied, type I collagen is the most predominant collagen observed in bones and skin tissue (Jafari et al., 2020). In the industrial setting, this type is often used in food and beverages, cosmetics,

pharmaceuticals and health care (Lim et al., 2019). Interestingly, the global market of type I collagen was estimated at 936.5 tons in 2020 and is projected to increase at approximately 5.6% due to an increase in health-awareness consumer preferences (Pulidindi and Ahuja, 2024). In general, collagen derived from land-based animals such as cows, pigs and chickens' bone and skin are excellent sources for collagen production. However, the use of animal-based collagens is no longer sought due to concern regarding the transfer of infectious diseases, such as bovine spongiform encephalopathy, foot-and-mouth disease and avian influenza. In addition, some religions, such as Hindus and Sikhs, prohibit the usage of bovine-based products, while porcine-originated collagen is not permissible for Muslims and Jews (Kittiphattanabawon et al., 2019). As an alternative, in recent years, several studies have been drawn on fish collagen. Fish collagen has similar and even better characteristics than collagen derived from terrestrial animals after modification of its structure (Zhang et al., 2020).

Collagen and its hydrolysate/peptide have gained a considerable interest amongst the researchers due to its potential applications, especially in the medical sector (Deng et al., 2022). Wounds occur when skin tissue integrity is compromised by injury-causing factors, impairing skin functions and creating deficiencies in the body's defences in the absence of proper repair processes (Shalaby et al., 2023). Acute wounds generally heal within weeks, maintaining normal physiology, while chronic wounds,

marked by persistent inflammation, may take months or years to heal. Chronic wounds pose significant medical and healthcare challenges, including pain, decreased mobility, and a substantial decline in quality of life, along with considerable economic burdens due to medical care and hospitalizations (Landén et al., 2016). In the United States, approximately 5.7 million individuals are affected by wound lesions annually, with healthcare costs estimated at \$20 billion (Childs and Murthy, 2017). In general, the wound healing process is complex and involves phases of haemostasis, inflammation, proliferation, and remodelling, which work synergistically to restore tissue (Cruz et al., 2021). Research has increasingly focused on collagen and collagen composite materials for wound healing (Figure 1). Collagen-based biomaterials, such as hydrogels, sponges, and dressings, have shown beneficial effects on wound healing (Deng et al., 2022). Moreover, many reports showed that fish collagens could accelerate wound healing via oral administration. For example, collagen derived from Atlantic salmon (*Salmo salar*) skin enhanced wound healing in rodents by modifying cutaneous microbiome colonization (Mei et al., 2020); similarly, the collagen from chum salmon (*Oncorhynchus keta*) skin accelerated wound healing and angiogenesis in post-caesarean rats (Wang et al., 2015). Another study noted that oral ingestion of collagen from Alaska pollock (*Theragra chalcogramma*) skin improved cutaneous wound healing and reepithelialisation in excision wound model of rats (Yang et al., 2018).

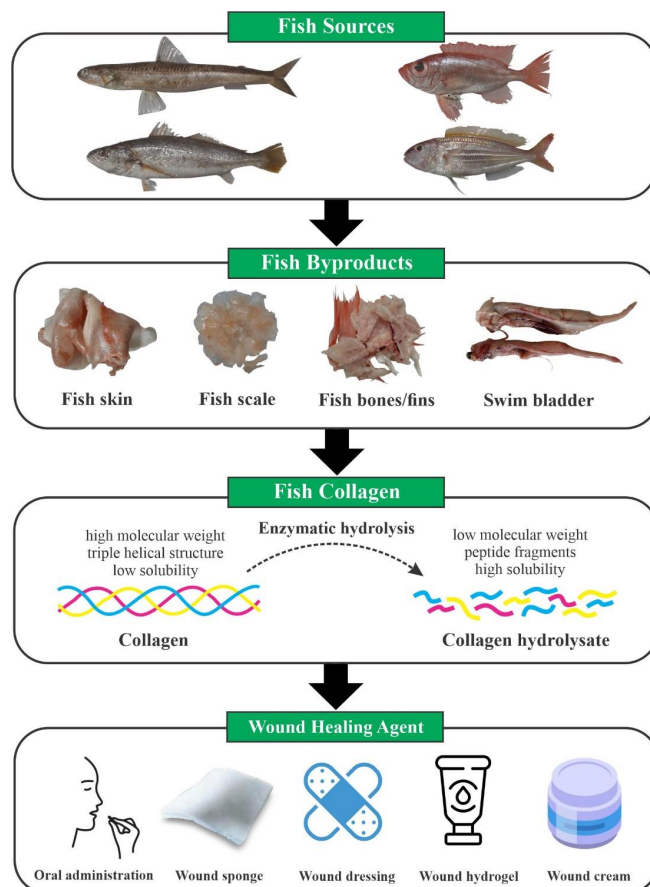


Figure 1. Fish collagen as potential wound healing agent

This review covers the latest progress in the study of collagen derived from fish byproducts and its application also discussed comprehensively, emphasizing its potential as a wound healing agent. It also explores various extraction procedures, such as acid-solubilised collagen, pepsin-solubilised collagen, and other extraction techniques, including optimization strategies to maximize collagen yield. Moreover, the detailed characterizations of collagens from various fish sources are also addressed, composing of their amino acid composition, protein profile, thermal stability properties, colour attributes, infrared spectral absorption, and solubility treated using different pH and sodium chloride concentrations. More importantly, this article highlights the updated research outcomes approached *in vitro* and *in vivo* that demonstrate the effectiveness of fish collagen in wound healing. Lastly, we address the challenges related to the commercial and sustainable utilization of fish collagen, aiming to provide a comprehensive understanding of these issues and stimulate further research and innovation in the wound healing field.

Structure and type of collagen

The name “collagen” originates from Greek words meaning “glue” and “to produce,” initially describing the component of tissues that produces adhesive substances upon boiling. Coined in the 19th century, it specifically designates the constituent of connective tissue yielding gelatine through boiling. Beyond historical contexts, collagen is regarded as a biological adhesive, and crucial for cellular anchorage. At present, collagen is understood as the primary structural protein in the extracellular matrix (ECM) that self-assembles into cross-striated fibrils, and it is found in the body’s various connective tissues (*i.e.*, cartilage, bones, tendons, ligaments, and skin). It not only supports cell growth but also contributes significantly to the mechanical resilience of connective tissues (Jafari et al., 2020).

As the main component of connective tissue, collagen is the most abundant protein in mammals, making up from 25% to 35% of the whole-body protein content. It is characterized by a triple helix structure formed by three polypeptide chains (identical/non-identical), consists of approximately 1000 amino acids. The left-handed supercoiling around a common axis results in a right-handed triple helix conformation. Each chain follows a repeating sequence (Gly-X-Y)_n, with X and Y primarily being proline and hydroxyproline. There are 29 reported collagen types, distributed in various tissues; for instance, type I is found in dermis, bones, cornea, ligaments, and tendons. Type II is located in the vitreous body and cartilage, type III in the reticular fibres of the lungs, blood vessel walls, spleen, and liver, and type IV in the basement membrane. Type V is distributed along with type I, notably in the cornea (Sorushanova et al., 2019). Notably, type I collagen is extensively utilized in biomedicine, including wound healing agents, tissue engineering constructs, and cosmetics, due to its low antigenicity and superior cell adhesion (Senadheera et al., 2020).

The collagen molecule features a triple helical region flanked by two nonhelical regions at each end, defining its structural framework. This collagen triple helix, constituting the tertiary structure, is characterized by a coiled-coil arrangement, with three parallel α polypeptide chains wound around each other to form a rope-like structure of $\approx 300,000$ g mole⁻¹ molecular weight, 280 nm in length, and 1.4 nm in diameter. Intramolecular hydrogen bonds between glycine in adjacent chains and hydroxyl groups of hydroxyproline residues stabilize the triple helix, forming two hydrogen bonds per triplet. The staggered arrangement of left-handed α -chains creates a right-handed superhelix, resulting from twisting by about +30° at each turn. The Gly-Pro-Hyp sequence is the most common, constituting approximately 12% of the molecule. Proline and hydroxyproline, due to their alicyclic nature, impart stability and stiffness to the collagen molecule by preventing rotation around the C-N bond. Post-secretion, specialized enzymes remove pro-peptides at both ends, leaving short, nonhelical telopeptides measuring 9–26 amino acids. These telopeptides are crucial for registering and crosslinking collagen α -chains, adding flexibility to the otherwise rigid molecule. The removal of pro-peptides is essential for the self-assembly of collagen molecules into quarter-staggered arrangements, forming cross-striated fibrils. Collagen type I, the most abundant type, appears as elongated fibrils exceeding 500 μ m in length and 500 nm in diameter, containing over 107 molecules. The fibrils exhibit axial alignment, resulting in characteristic D banding/periodicity due to specific packing arrangements of the collagen molecules, producing an average periodicity of 67 nm in the native hydrated state. However, dehydration during conventional electron microscopy preparation results in periodicities of around 55–65 nm (Sorushanova et al., 2019).

So far, around 39 collagen genes have been documented from the vertebrate organisms, forming 29 distinct homo- and/or heterotrimeric molecules indicated by Roman numerals and Greek letters for type and chain identification, respectively (Sorushanova et al., 2019). The trimeric nature of collagen enables the combination of three identical pro- α -chains or two identical chains and one different one, adhering to fitting length and C-propeptide registration, to form a complete triple helix. Different collagen types arise from the fit of pro- α -chains, their length, registration of C-pro-peptides, and combination, although isoforms within types exist. Group 1 encompasses fibril-forming collagens (type I, II, III, V, XI, XXIV, and XXVII), with triple helices having uninterrupted Gly-X-Y stretches of ≈ 300 nm, often forming heterotypic fibrils. Group 2 includes basement membrane collagens (type IV, VII, and XXVIII), where type IV forms a fibrillar meshwork, and type VII associates antiparallel dimers forming cross-striated fibrils. Group 3 comprises short-chain collagens (type VI, VIII, X, and XXIX) with triple helical regions extending up to 150 nm, forming beaded microfilaments, hexagonal lattices, or having von Willebrand factor A domains. Group 4

consists of collagens with multiple interruptions in triple-helical Gly–X–Y stretches, with type IX, XII, XIV, XVI, and XIX to XXII grouped into the fibril-associated collagens with interrupted triple helices (FACIT collagens), serving specific roles by associating with collagen fibrils. The subgroup of multiple triple-helix domains and interruptions (MULTIPLEXINs) (type XV and XVIII) exhibits the highest number of interruptions. Transmembrane collagens (type XIII, XVII, XXIII, and XXV) possess transmembrane domains, allowing insertion into cell membranes while projecting the triple-helical domains outward into the extracellular matrix (León-López et al., 2019 b).

Fish byproduct-derived collagen

Traditionally, the skin and bones of terrestrial animals, such as cattle and pigs are the main sources of collagen. However, consumers' concerns related to the usage of bovine-based products have increased owing to the outbreaks of various infectious diseases on bovine animals, including foot and mouth disease (FMD), bovine spongiform encephalopathy (BSE), and transmissible spongiform encephalopathy (TSE) (Kittiphattana-bawon et al., 2019). Another reason for not using bovine collagen is that they are allergic to 3% of population (Parenteau-Bareil et al., 2010). The allergic reactions due to bovine collagen are hypersensitivity, conjunctival oedema, periocular angioedema, and throat angioedema. All these are Ig-E mediated inflammatory reactions to collagen whether they are inhaled, ingested, or topically applied (Mullins and Bs, 1996). For porcine source, although collagens produced from the pigs are considered safe because they cannot cause any allergenic response (Silvipriya et al., 2015), these porcine collagens are not allowed due to certain religious laws, and thus collagens from pigs are not widely used. Another source is derived from poultry animals, but due to the rise of avian influenza (H5N1) cases, poultry-based products are not also widely used (Korteweg and Gu, 2008). To tackle these issues, marine source is a great choice because it is considered as safest source of collagen compared to those of bovine, porcine, and poultry sources. Also, the yield obtained from the marine sources is higher than other sources. Furthermore, marine sources of collagen have many advantages over the land-based animals such as: (1) high collagen content, (2) no risk of disease such as BSE, TSE, FMD, H5N1, (3) no religious and ethical conflicts, (4) lesser amounts of biological toxins and contaminants are present, (5) less immunogenic, (6) low inflammatory response, and (7) metabolically suitable (Ahmed et al., 2020).

Byproducts from marine fish, including the skin, scale, bones, cartilages, swim bladders, fins, and head have been converted into potential sources of collagen, and they have drawn a considerable attention of researchers and processors over a decade ago. Many studies have explored the fish collagens, particularly derived from the tropical marine fish species. For examples, threadfin

bream (*Nemipterus japonicus*), spotted golden goatfish (*Parupeneus hetacanthus*) (Matmaroh et al., 2011), purple spotted bigeye (*Priacanthus tayenus*) (Osman et al., 2022), bigeye snapper (*Priacanthus macracanthus*) (Ben-jakul et al., 2010), barracuda (*Sphyrna sp.*) (Matarsim et al., 2023), bigeye tuna (*Thunnus obesus*) (Ahmed et al., 2019), skipjack tuna (*Katsuwonus pelamis*) (Ding et al., 2019), starry triggerfish (*Abalistes stellatus*) (Ahmad et al., 2016), unicorn fish (*Naso reticulatus*) (Fatiroi et al., 2023), sin croaker (*Johnniecop sina*) (Normah and Afiqah, 2018), giant croaker (*Nibea japonica*) (Yu et al., 2018), barramundi (*Lates calcalifer*) (Chuaychan et al., 2015), grouper (*Epinephelus sp.*) (Prihanto et al., 2022), parrotfish (*Scarus sordidus*) (Jaziri et al., 2023), golden pompano (*Trachinotus ovatus*) (Cao et al., 2019), short-fin scad (*Decapterus macrosoma*) (Sulaiman and Sarbon, 2020), mahi mahi (*Coryphaena hippurus*) (Akita et al., 2019), sailfish (*Istiophorus platypterus*) (Tamilmozhi et al., 2013), fringescale sardinella (*Sardinella fimbriata*) (Hamdan and Sarbon, 2019), horse mackerel (*Trachurus japonicus*), grey mullet (*Mugil cephalis*), flying fish (*Cypselurus melanurus*) (Thuy et al., 2014), needle fish (*Tylosurus melanotus*) (Ramle et al., 2022), mackerel (*Scomber japonicus*) (Asaduzzaman et al., 2020), and sharpnose stingray (*Dasyatis zugei*) (Ong et al., 2021). Transforming marine fish byproducts and/or fish wastes into valuable products like collagen contributes to the reduction of aquatic and terrestrial pollutions, as well as increasing financial profit for the processors.

Extraction procedure of fish collagen

General procedure for collagen extraction (particularly derived from marine fish sources) comprises sample preparation, pretreatment, extraction, and recovery (Ahmed et al., 2020). In terms of sample preparation, some activities including washing, separation, cleaning, and cutting tissues of fish are applied to facilitate the further pretreatment process.

In the pretreatment, removal of non-collagenous components and pigments from the fish tissues (*i.e.*, skin, bones, swim bladder, and scales) are initially employed to increase the efficacy of extraction. This step mostly uses the alkaline solutions, such as sodium hydroxide (NaOH) and calcium hydroxide (Ca(OH)₂) with a certain concentration for a period. During the alkaline immersion, the connective tissues of fish swell two- or three-fold leading to the cleavage of the non-covalent inter- and intra-molecular bonds. Nonetheless, using NaOH solution is more effective owing to the greater swelling rate for facilitating the collagen extraction by enhancing the transfer rate of the mass in the tissue matrix (Jafari et al., 2020). After the alkaline pretreatment, the demineralization and defatting of the marine fish byproducts are required to increase efficiency of collagen extraction process. Ethylenediaminetetraacetic acid (EDTA) and n-butanol are widely used to remove inorganic materials like calcium and lipid from the fish's connective tissues, respectively (Jaziri et al., 2022 a).

Table 1. Extraction, yield, type, solubility, and colour of collagens derived from tropical marine fish sources

Source	Portion	Extraction	Time	Yield	SDS-PAGE data				Solubility			Colour attributes			Reference
					$\alpha 1$ (kDa)	$\alpha 2$ (kDa)	Type	NaCl	pH	*L	*a	*b			
1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Threadfin bream (<i>Nemipterus japonicus</i>)	Scale + fin	ASC-Ca	12 h	22%	ND	ND	ND	ND	ND	93.7	-1.84	13.44	(Normah and Afiqah, 2018)		
	Scale + fin	ASC-C	12 h	8.30%	ND	ND	ND	ND	ND	94.82	0.31	0.2			
	Scale	ASC	48 h	0.46%	117	108	I	0-20 g/L	2-4	ND	ND	ND	(Matmaroh et al., 2011)		
	Scale	PSC	48 h	1.20%	117	108	I	0-30 g/L	2-4	ND	ND	ND			
Bigeye snapper (<i>Priacanthus tayenus</i>)	Skin	ASC-A	72 h	5.79%	118	106	I	0-3%	1-5	ND	ND	ND	(Oslan et al., 2022)		
	Skin	ASC-L	72 h	3.19%	118	106	I	0-3%	1-5	ND	ND	ND			
	Skin	ASC-C	72 h	4.15%	118	106	I	0-3%	1-5	ND	ND	ND			
	Skin	PSC	48 h	6.65%	118	106	I	0-3%	1-5	ND	ND	ND			
Barracuda (<i>Sphyraena</i> sp.)	Skin	ASC-A	72 h	6.77%	143.2	136.6	I	0-20 g/L	1-5	78.54	-0.05	0.64	(Matarsim et al., 2023)		
	Skin	ASC-L	72 h	10.06%	143.2	136.6	I	0-20 g/L	1-3	56.88	0.6	5.83			
	Skin	ASC-C	72 h	8.35%	143.2	136.6	I	0-20 g/L	1-3	54.34	0.66	3.78			
	Bone	ASC-A	72 h	0.40%	138	118.3	I	0-10 g/L	1-5	81.44	-0.19	0.79	(Fatiroi et al., 2023)		
Unicorn fish (<i>Naso reticulatus</i>)	Bone	ASC-L	72 h	1.08%	138	118.3	I	0-20 g/L	1-5	82.55	0.4	6.51			
	Bone	ASC-C	72 h	1.36%	138	118.3	I	0-10 g/L	1-3	79.35	0.04	3.26			
	Bone + scale	ASC	72 h	2.74%	120	100	I	0-2%	1-2	ND	ND	ND	(Normah and Afiqah, 2018)		
	Swim bladder	ASC	60 h	1.33%	ND	ND	I	0-2%	1-4	ND	ND	ND	(Li et al., 2018)		
Chu's croaker (<i>Nibea colbor</i>)	Swim bladder	PSC	48 h	8.37%	ND	ND	I	0-2%	1-4	ND	ND	ND			
	Swim bladder	ASC	48 h	7.33%	130	110	I	0-3%	1-5	ND	ND	ND	(Xiao et al., 2023)		
	Swim bladder	ASC	48 h	7.15%	130	110	I	0-2%	1-5	ND	ND	ND	(Xiao et al., 2023)		
Barramundi (<i>Lates calcarifer</i>)	Skin	ASC	48 h	8.12%	ND	ND	I	0-2%	1-5	65.41	0.14	3.16	(Bakar et al., 2013)		
	Skin	PSC	24 h	43.63%	ND	ND	I	0-2%	1-5	61.33	2.59	5.35			
	Scale	ASC	48 h	0.38%	118	105	I	ND	1-4	ND	ND	ND	(Chuaychan et al., 2015)		
	Scale	PSC	48 h	1.06%	118	105	I	ND	1-4	ND	ND	ND			
Seabass (<i>Lates calcarifer</i>)	Skin	ASC	48 h	8.32%	135.8	122.5	I	ND	ND	82.27	4.4	1.9	(Razali et al., 2023)		
	Skin	UAE	48.3 h	56.61%	139.5	127	I	ND	ND	72.45	5.75	7.39			
	Skin	ASC	48 h	7.70%	176	150	I	ND	ND	ND	ND	ND	(Firdayanti et al., 2023)		
Parrotfish (<i>Scarus sordidus</i>)	Scale	ASC	48 h	1.17%	118.1	107.7	I	0-20 g/L	1-5	61.74	2.61	6.15	(Jaziri et al., 2023)		
	Scale	PSC	48 h	1.00%	118.1	107.7	I	0-30 g/L	1-5	74.81	1.09	6.14			

Table 1 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Grouper (<i>Epinephelus</i> sp.)	Swim bladder	PSC	48 h	18.16%	133	123	I	ND	ND	ND	ND	ND	(Dong and Dai, 2022)
Golden pompano (<i>Trachinotus ovatus</i>)	Skin	ASC	48 h	21.81%	105	120	I	0–30 g/L	1–4	ND	ND	ND	(Cao et al., 2019)
	Bone	PSC	48 h	1.25%	105	120	I	0–30 g/L	1–4	ND	ND	ND	
Shortfin sead (<i>Decapterus macrostoma</i>)	Bone + skin	ASC	24 h	3.35%	ND	ND	I	ND	1–10	ND	ND	ND	(Sulaiman and Sarbon, 2020)
	Bone + skin	PSC	30 h	0.10%	ND	ND	I	ND	1–3	ND	ND	ND	
Mahi mahi (<i>Coryphaena hippurus</i>)	Skin	ASC	ND	5.90%	120	110	I	ND	ND	ND	ND	ND	(Akita et al., 2019)
	Skin	PSC	ND	4.00%	120	110	I	ND	ND	ND	ND	ND	
Fringescale sardinella (<i>Sardinella fimbriata</i>)	Scale	ASC	24 h	7.48%	ND	ND	I	ND	1–5	ND	ND	ND	(Hamdan and Sarbon, 2019)
	Scale	PSC	30 h	0.94%	ND	ND	I	ND	1–6	ND	ND	ND	
Horse mackerel (<i>Trachurus japonicus</i>)	Scale	ASC	96 h	0.64%	ND	ND	I	0–0.4 M	1–5	ND	ND	ND	(Minh et al., 2014)
Grey mullet (<i>Mugil cephalis</i>)	Scale	ASC	96 h	0.43%	ND	ND	I	0–0.4 M	1–5	ND	ND	ND	(Minh et al., 2014)
Flying fish (<i>Cypselurus melanurus</i>)	Scale	ASC	96 h	0.72%	ND	ND	I	0–0.4 M	1–5	ND	ND	ND	(Minh et al., 2014)
Yellowback seabream (<i>Dentex tumifrons</i>)	Scale	ASC	96 h	0.90%	ND	ND	I	0–0.4 M	1–5	ND	ND	ND	(Minh et al., 2014)
Needle fish (<i>Tylosurus melanotus</i>)	Skin	ASC-A	72 h	3.13%	130	100	I	0–10 g/L	1–3	69.77	71.89	57.14	(Ramle et al., 2022)
	Skin	ASC-L	72 h	0.56%	130	100	I	0–10 g/L	1–3	0.15	0.92	0.73	
	Skin	ASC-C	72 h	1.03%	130	100	I	0–10 g/L	1–5	5.52	5.2	4.69	
Mackerel (<i>Scomber japonicus</i>)	Bone	PSC	72 h	1.75%	123	116	I	ND	ND	ND	ND	ND	(Asaduzzaman et al., 2020)
	Skin	PSC	72 h	8.10%	123	116	I	ND	ND	ND	ND	ND	
Sharpnose stingray (<i>Dasyatis zugei</i>)	Skin	ASC	24 h	20.48%	ND	ND	I	ND	1–6	ND	ND	ND	(Ong et al., 2021)
	Skin	PSC	30 h	34.84%	ND	ND	I	ND	1–5	ND	ND	ND	
Sharpnose stingray (<i>Dasyatis zugei</i>)	Skin	ASC+UEA	1 h	42.34%	ND	100	I	ND	1–6	ND	ND	ND	(Shaik et al., 2021)
	Skin	PSC+UEA	1 h	61.50%	ND	95	I	ND	1–5	ND	ND	ND	
Silvertip shark (<i>C. albigarinatus</i>)	Skeletal	PSC	96 h	ND	ND	ND	II	0–1%	3–6	ND	ND	ND	(Jeevithan et al., 2014)
	Head bone	PSC	96 h	ND	ND	ND	II	0–1%	3–6	ND	ND	ND	

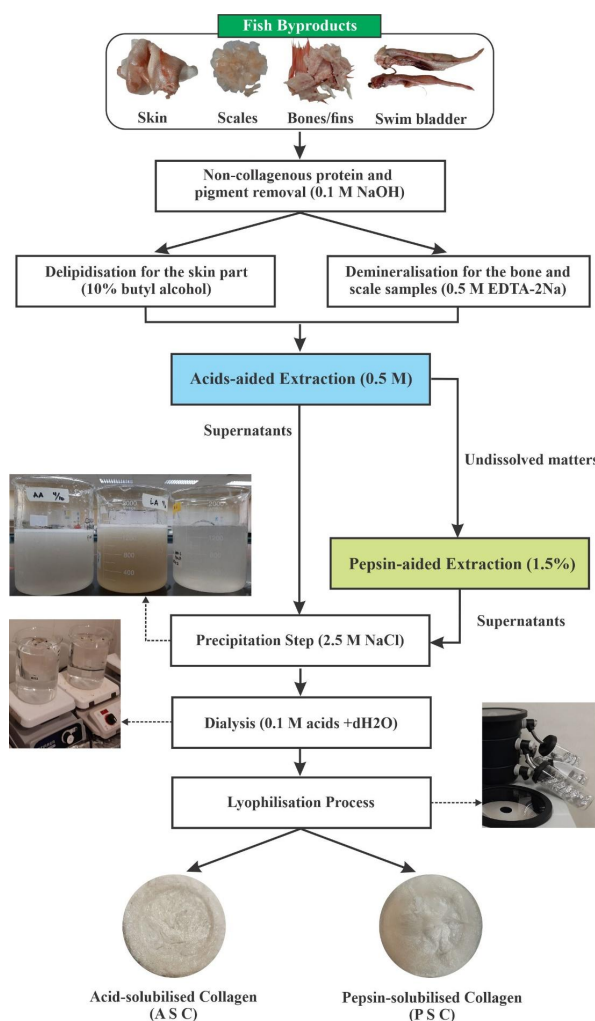


Figure 2. ASC and PSC extraction process

For extraction process, various techniques were widely used to obtain fish collagen from the animals' tissues, as presented in Table 1. Based on these techniques, marine fish collagens are mostly categorized into acid-solubilised collagen (ASC), pepsin-solubilised collagen (PSC), and optimized extraction process. The detailed explanation of each extraction procedure from different sources is further described.

Acid solubilisation extraction

Collagen extracted with the acidic solution, is commonly known as acid-solubilised collagen (ASC) (Figure 2). The use of acids (organic and inorganic acids) during collagen production can cleave bonds in collagen molecules to enable extraction of the tissues (Skierka and Sadowska, 2007). In an acidic condition, the collagen molecules undergo a net positive change and the resulting electrostatic repulsive force between them facilitates molecular separation. There are many acid compounds used to obtain collagen from the marine fish tissues, including organic acids (acetic, lactic, and citric acids) and inorganic acid (hydrochloric acid) (Tan and Chang, 2018). Among

these acids, organic acids are apparently more effective to break some inter-stand crosslinks of collagen molecules, resulting in higher extractability of collagen yield, as compared to the mineral acid. In addition to this, organic acids are capable of solubilising non-crosslinked collagens. However, acetic acid (AcOH) is mostly employed in extracting collagen, in particular collagens from marine fish sources. Collagen extraction from marine fish derived by-products has been effectively achieved by using various parameters such as time, concentration of acetic acid and temperature. The reported ASCs extraction by using various concentrations of acetic acids are from skin, bones, scales, and swim bladders of spotted golden goatfish (*P. heptacanthus*) (Matmaroh et al., 2011), purple-spotted bigeye (*P. tayenus*) (Oslan et al., 2022), barracuda (*Sphyraena* sp.) (Matarsim et al., 2023), bigeye tuna (*T. obesus*) (Ahmed et al., 2019) starry triggerfish (*A. stellatus*) (Ahmad et al., 2016), unicorn fish (*N. reticulatus*) (Fatiroi et al., 2023), sin croaker (*J. sina*) (Normah and Afqah, 2018), seabass (*L. calcalifer*) (Chuaychan et al., 2015), grouper (*Epinephelus* sp.) (Dong and Dai, 2022), shortfin scad (*D. macrosoma*) (Sulaiman and Sarbon, 2020), grey

mullet (*M. cephalis*) (Minh et al., 2014), needle fish (*T. melanotus*) (Ramle et al., 2022), and sharpnose stingray (*D. zugei*) (Ong et al., 2021).

Pepsin solubilised extraction

In general, collagen extraction involves the use of various acids, such as acetic, lactic, citric, and hydrochloric acids, without the addition of protease enzymes. However, this method often yields incomplete dissolution of collagens from different sources, resulting in suboptimal extraction yields (Jaziri et al., 2022 b; Skierka and Sadowska, 2007). To address this limitation and achieve maximum extractability, enzymatic extraction methods have been developed. These methods employ enzymes like pepsin, trypsin, bromelain, papain, and various collagenases under specific environmental conditions (*i.e.*, concentration, pH, temperature, and liquid-solid ratio). Among these enzymes, pepsin is a prominent choice for extracting collagen from marine fish sources (Table 1). Pepsin can be applied either independently or in conjunction with different concentrations of acetic acid. Literally, pepsin can effectively cleave the telopeptide regions within the triple helical structure of collagen molecules, thereby aiding in the release of collagen peptides into the solution and consequently enhancing extraction yields (Benjakul et al., 2010). Collagen obtained through pepsin-assisted extraction are referred to as pepsin solubilised collagen (PSC) (Figure 2). Notably, researchers have found that utilizing limited concentrations of enzymes, particularly pepsin, along with acids, significantly enhances the overall yield of collagen. This underscores the importance of enzymatic extraction methods in optimizing collagen extraction efficiency (Arumugam et al., 2018). Enzymatic approach is a promising technique for collagen extraction, driven by some reasons. For instance, improving extraction efficiency by facilitating the enhanced solubilisation of collagen in acetic medium, reducing collagen antigenicity, and preserving triple helical structure of collagen. Overall, these factors contribute to the increasing applications of enzyme-extracted collagen in both the food and pharmaceutical industries (Ahmed et al., 2020).

Optimization method for collagen extraction

Optimization is a critical aspect of extraction processes, involving the systematic exploration of various factors to determine the conditions that yield optimal results (Arumugam et al., 2018). Particularly in complex processes where the targeted response is influenced by multiple factors, the selection of an appropriate experimental design becomes imperative. In the context of collagen extraction from marine fish byproducts, efficiency is notably influenced by factors such as concentration, extraction time, solid-liquid ratio, and temperature. Thus, a well-designed experiment capable of analysing these multiple factors is essential. Once the region of optimal response is identified through preliminary studies, such as one-factor-at-a-time (OFAT), further characterization of the response within

that region is often necessary. To achieve this, experimental designs such as the Box-Behnken design (BBD) and central composite design (CCD) are commonly applied in response surface methodology (RSM). These designs facilitate the approximation of a second-order polynomial estimation for the response within the identified optimal region (Mohammadi et al., 2016).

RSM employs mathematical and statistical techniques to model situations where variables impact a response of interest, with the primary objective of achieving an optimized response. When the relationship between independent variables and the response is linear, the first-order model is employed as an approximate function. However, if the response surface exhibits curvature, a second-order polynomial, or a higher degree polynomial, is used as the model. Response surface design is a specific approach for fitting response surfaces, and two common types are the one-factor-at-a-time design and factorial design. Factorial design is particularly useful when studying two or more levels, including both full factorial and fractional factorial designs. In cases where the number of runs for a full factorial design becomes impractical, fractional designs offer a viable alternative. Notably, BBD and CCD are examples of widely used fractional factorial designs employed for optimization purposes (Mohammadi et al., 2016). RSM offers several advantages, including a reduction in the number of experimental attempts required to assess multiple parameters and the capacity of the statistical tool to identify interactions. RSM provides the added benefit of generating a comprehensive mathematical model that captures the entirety of the process. Besides that, it proves to be a time-saving method that minimizes errors in assessing parameter effects. Mostly used statistical approaches in RSM are CCD and BBD, both of which have proven successful in optimizing marine collagen extractions, as illustrated in Table 2.

For instance, the effects of NaOH concentration, pretreatment time, pepsin concentration, and hydrolysis time on the extraction yield, a four-factor, five-level CCD for RSM was employed. The CCD encompassed 28 experimental runs, including 8 axial points, 16 factorial points, and 6 replicates of the central point. Using the desirability function approach, the estimation of the R^2 value, multi-factor ANOVA, lack of fit value, and second-order model prediction determinants was employed to attain optimal conditions. Response surface plots, including surface plots and contour plots, were generated to elucidate the impacts of the four factors on the extraction yield. The ANOVA of the estimated model led to the conclusion that the effects of NaOH concentration, pretreatment time, pepsin concentration, and hydrolysis time showed interactive effects on the collagen yield. Consequently, with the assistance of the regression model, the optimal conditions were determined to be the NaOH concentration of 0.92 N, pretreatment time of 24 h, pepsin concentration of 0.98%, and hydrolysis time of 23.5 h (Woo et al., 2008).

Table 2. Optimisation methods for collagen derived from fish byproducts

Source	Fish portion	Optimisation procedure	Yield	Characterization remarks	References
Milkfish (<i>Chanos chanos</i> Forskal)	Scale	CCD with the optimal conditions: extraction time (X1) = 61.30 h; AcOH concentration (X2) = 0.66 M.	0.73%	FTIR, DSC and analysis amino acid show that milkfish scale collagen in this study resembles that of commercial collagen	(Isnainita and Bambang, 2018)
Small-spotted catshark (<i>Scyliorhinus canicula</i>)	Skin	CCD with the optimal conditions: temperature (X1) = 25°C; extraction time (X2) = 34.2 h; AcOH concentration (X3) = 1.0 M.	61.24%	It contained two identical $\alpha 1$ chains (120 kDa) and one $\alpha 2$ chain (110 kDa) in the molecular form of $[\alpha 1(I)]_2 \alpha 2(I)$.	(Blanco et al., 2019)
Yellowfin tuna (<i>Thunnus albacares</i>)	Dorsal skin	CCD with the optimal conditions: NaOH concentration (X1) = 0.92 N; NaOH treatment time (X2) = 24 h; pepsin concentration (X3) = 0.98% (w/v); hydrolysis time (X4) = 23.5 h.	27.10%	The obtained type I PSC had a 20.5% imino acid and the optimal extraction process did not affect the helical structure of collagen.	(Woo et al., 2008)
Sole fish (<i>Aseraggodes umbratilis</i>)	Skin	Initiated with OVAT, and followed by BBD with the optimal conditions: AcOH concentration (X1) = 0.54 M; salt concentration (X2) = 1.90 M; solvent/solid ratio (X3) = 8.97 mL/g; extraction time (X4) = 32.3 h.	19.27%	Extracted collagen was categorised as type I and IR analysis showed the existence of helical arrangements of collagen	(Arumugam et al., 2018)
Cuttlefish (<i>Sepia pharaonis</i>)	Skin	BBD with the optimal conditions: pH value (X1) = 1.5; solid-liquid ratio (X2) = 20 mL/g; pepsin concentration (X3) = 15 U/mg	8.79%	Considered as type I, and it confirmed the presence of collagen fibrils in the cuttlefish skin	(Hou et al., 2022)
Giant croaker (<i>Nibea japonica</i>)	Skin	Initiated with OVAT, and followed by BBD with the optimal conditions: pepsin concentration (X1) = 1389 U/g; solid-liquid ratio (X2) = 1:57; extraction time (X4) = 8.67 h.	84.85%	Characterized as type I collagen, its triple helical structure was maintained, and had a high solubility at pH 1.0–4.0	(Yu et al., 2018)
Nile tilapia (<i>Oreochromis niloticus</i>)	Skin	Two-level 23 factorial experimental with the optimal conditions: AcOH = 0.35 M; temperature: 20°C; extraction time: 65 h.	19.00%	The triple helical structure of ASC from tilapia skin collagen did not change after being confirmed by FTIR.	(Menezes et al., 2020)
Sea eel (<i>Muraenesox cinereus</i>)	Swim bladder	Initiated with OVAT and followed by BBD with the optimal conditions: pepsin concentration (X1) = 2067 U/g; solid-liquid ratio (X2) = 1:83; extraction time (X4) = 10 h.	93.76%	Classified as type I and it displayed a fibrous structure under electron microscopy	(Li et al., 2023)
Grass carp (<i>Ctenopharyngodon idella</i>)	Swim bladder	CCD with the optimal conditions: liquid-solid ratio (X1) = 17.85; AcOH concentration (X2) = 0.54 M; extraction time (X3) = 34 h.	8.21%	–	(Zhang et al., 2010)

Characteristics of marine fish collagen

Colour attributes

Colour is one of the most important parameters of collagen in industrial applications (*viz.* foods, cosmetics, pharmacy, and medicine). Collagen with bright colour is more acceptable because it does not change the original colour of final products, and it does not influence the functional properties of collagen (Liu et al., 2019). Some works related to the tropical marine fish collagen examined the attributes of colour in the final products using CIELAB colour space, which represents the values of L^* (lightness), a^* (red/green), b^* (blue/yellow) described by the International Commission on Illumination (CIE). For instance, the barracuda (*Sphyraena* sp.) and needlefish (*T. melanotus*) skin collagens extracted with different organic acids had a low value of L^* (54.34–56.88 and 57.14–69.77, respectively) (Matarsim et al., 2023; Ramle et al., 2022). In addition to this, the ASC and PSC derived from barramundi (*L. calcalifer*) skin showed the L^* values of 65.41 and 61.33, respectively (Bakar et al., 2013). In contrast, collagens isolated from the bones of unicorn fish (*N. reticulatus*) and parrotfish (*S. sordidus*) exhibited the L^* values of 881.55 and 74.81, respectively (Fatiroi et al., 2023; Jaziri et al., 2023). Besides that, the lightness property observed in the threadfin bream (*N. japonicus*) scale and fins collagen was considered higher ($L^* = 93.70$ – 94.82) (Normah and Afqah, 2018). It can be stated that the byproducts of bones, scale and fins are more effective raw materials in producing collagen rather than fish skins. As described by Sadowska et al. (2003), the existence of pigments in fish skins causes the difficulty of preparing fish collagen entirely devoid of colour. Thus, the colour of collagen depends on the raw materials used and method of extraction. As confirmed by Liu et al. (2019), the L^* value of extracted collagens generated from snakehead (*Channa argus*) skins was 89.49, indicating higher than the aforementioned fish skin collagens due to addition of hydrogen peroxide (H_2O_2) during pretreatment process. The use of H_2O_2 solution might be effective to remove the skin sample's pigment.

Ultraviolet visible (UV-vis) absorption spectrum

Ultraviolet visible (UV-vis) absorption is a common method to evaluate the characteristic of extracted collagen from several sources. It is considered as a simple approach by scanning the collagen sample from 200 nm to 400 nm, because triple helical collagen has maximum peak at 230 nm and a negative peak near 204 nm (Piez and Gross, 1960). In addition, collagen contains a low concentration of chromophore amino acids, such as phenylalanine, tryptophan, and tyrosine, in which these amino acids typically absorb UV light at 250 nm and 280 nm (Liao et al., 2018). The number of studies on the ASC and PSC produced from various tropical marine fish byproducts had different prominent absorption peaks, such as purple-spotted bigeye (*P. tayanus*) skin collagens (230–240 nm) (Oslan et al., 2022), barracuda (*Sphyraena* sp.) skin collagens (230.5 nm) (Matarsim et al., 2023),

puffer fish (*L. inermis*) skin collagens (230 nm) (Iswariya et al., 2018), unicorn fish (*N. reticulatus*) bone collagens (229.8–231.2 nm) (Fatiroi et al., 2023), parrotfish (*S. sordidus*) bone collagens (230–232 nm) (Jaziri et al., 2023), and needlefish (*T. melanotus*) skin collagens (231.5 nm) (Ramle et al., 2022). Overall, the mentioned peaks are closely related to the functional groups of carbonyl ($C=O$), carboxyl ($-COOH$) and amide ($-CO-NH_2$) belonging to the polypeptide chains of collagen (Edwards and O'Brien, 1980).

SDS-PAGE profile

So far, type I collagen is mostly explored and applied in industrial fields. It is characterized by possessing at least two different alpha chains ($\alpha 1$ and $\alpha 2$) and the dimer beta (β) chain (governed by intramolecular crosslinking) (Matmaroh et al., 2011). Using the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), the electrophoretic bands of collagen can be confirmed, and the molecular weights (MW) of collagen may also be determined. Table 1 shows all tropical marine collagens isolated from the skin, bone, scale, fin, and swim bladder byproducts were classified as a type I due to the presence of two identical subunits of $\alpha 1$ chains and one subunit of $\alpha 2$. However, the obtained collagens from the cartilage and head bone of silvertip shark (*Carcharhinus albimarginatus*) have been identified as a type II collagen, indicating one α chain and its dimer (β chains) (Jeevithan et al., 2014). Although most of cartilage-derived collagens are identical to the type II collagen, Kittiphattanabawon et al. (2010) reported that the collagens produced from the cartilages of brownbanded bamboo shark (*Chiloscyllium punctatum*) and blacktip shark (*Carcharhinus limbatus*) were categorized as both type I and type II, as similar to that of *Carcharias acutus* cartilage collagen documented by Rama and Chandrakasan (1984). In terms of MW prediction, the $\alpha 1$ and $\alpha 2$ of type I marine fish collagens were comparable, and almost above 100 kDa, such as starry triggerfish (*A. stellatus*) skin collagen had $\alpha 1$ of 119–121 kDa and $\alpha 2$ of 112–117 kDa (Ahmad et al., 2016), while the purple-spotted bigeye (*P. tayanus*) skin collagens were 118 kDa and 106 kDa (Oslan et al., 2022). Other collagen sources, the spotted golden goatfish (*P. heptacanthus*) and parrotfish (*S. sordidus*) scales showed a similar MW of $\alpha 1$ and $\alpha 2$ (118 kDa and 107 kDa, respectively) (Jaziri et al., 2023; Matmaroh et al., 2011). On the other hand, higher MWs were found in the unicorn fish (*N. reticulatus*) bone collagen ($\alpha 1 = 138$ kDa and $\alpha 2 = 118.3$ kDa) and the grouper (*Epinephelus* sp.) swim bladder collagen ($\alpha 1 = 133$ kDa and $\alpha 2 = 123$ kDa) (Dong and Dai, 2022; Fatiroi et al., 2023), indicating that different fish species and extraction methods resulted in different molecular weight distributions. Besides the different MW distributions, the band intensities of $\alpha 1$ chain in the extracted marine fish collagen are generally twice those of $\alpha 2$ chain. The β - and γ -components of all tropical marine collagens had higher MWs of 200 kDa (Matarsim et al., 2023; Ramle et al., 2022).

Table 3. Amino acid compositions of collagen derived from tropical marine fish byproducts

Source	Portion	Method	Amino acid (residues/1000)																References				
			Ala	Arg	Asp	Cys	Glu	Gly	His	Hyl	Hyp	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val	Imino acid	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Spotted golden goatfish (<i>P. heptacanthus</i>)	Scale	ASC	134	53	43	2	71	336	6	5	78	8	19	27	14	14	108	36	23	3	19	186	(Matmaroh et al., 2011)
	Scale	PSC	134	52	42	2	69	340	6	6	81	7	19	26	14	13	108	35	24	2	19	189	
Bigeye tuna (<i>Thunnus obesus</i>)	Skin	ASC	99	82	40	1	99	227	7	9	82	11	25	33	21	20	150	36	31	4.3	22	232	(Ahmed et al., 2019)
	Skin	PSC	97	80	42	1	99	222	7	9	82	12	27	33	20	20	148	38	33	5.5	23	230	
Skipjack tuna (<i>Katsuwonus pelamis</i>)	Scale	PSC	92	75	44	2	102	222	8	9	87	14	29	32	19	20	142	39	33	6.2	25	229	(Ding et al., 2019)
	Bones	PSC	91	79	44	2	101	216	9	10	87	14	27	39	19	20	140	38	33	6.4	24	227	
Unicorn leatherjacket (<i>Aluterus monoceros</i>)	Skull	ASC	127	41	46	0	67	337	4	4	73	16	27	30	14	15	102	31	26	3.9	26	176	(Ahmad and Benjakul, 2010)
	Skull	PSC	111	50	43	0	77	330	3	5	69	24	30	27	4	20	100	36	27	1.9	32	170	
Starry triggerfish (<i>Abalites stellatus</i>)	Spine	ASC	126	48	46	0	66	339	5	4	73	12	26	29	14	14	104	33	25	2.9	26	178	(Ahmad et al., 2016 a)
	Spine	PSC	110	51	44	0	78	332	2	5	70	21	28	31	0	23	98	39	29	0	28	168	
Puffer fish (<i>Lagocephalus inermis</i>)	Skin	PSC	141	53	47	0	72	325	6	4	83	7	16	27	12	12	109	36	27	2	21	192	(Iswariya et al., 2018)
	Skin	PSC-A	140	53	45	0	74	320	6	4	81	8	18	28	12	12	106	38	27	2	22	187	
Brownstripe red snapper (<i>Lutjanus vitta</i>)	Skin	PSC-Y	134	53	55	0	86	290	8	4	72	12	27	33	13	15	97	39	30	5	26	169	(Jongjaroonrak et al., 2005 b)
	Skin	ASC	144	50	46	0	73	322	6	5	84	10	16	26	13	14	109	35	25	4	18	193	
Seabass (<i>Lates calcarifer</i>)	Skin	PSC	140	52	50	0	75	319	7	4	79	8	19	30	12	12	107	37	26	3	20	186	(Bakar et al., 2013)
	Skin	ASC	139	53	47	0	72	329	6	0	73	10	20	24	13	9	117	36	29	4	19	190	
Seabass (<i>Lates calcarifer</i>)	Skin	PSC	132	51	46	0	70	334	8	0	79	12	22	20	11	11	119	31	27	4	23	198	(Chuaychan et al., 2015)
	Skin	ASC	143	65	50	0	81	252	7	9	81	7	24	33	15	15	131	37	29	4	18	212	
Mahi mahi (<i>Coryphaena hippurus</i>)	Skin	PSC	142	68	49	0	79	235	6	15	86	8	24	34	16	16	135	39	30	2	17	221	(Akita et al., 2019)
	Skin	ASC	70	57	43	0	69	126	4	0	47	10	19	28	15	13	80	17	17	3.8	18	128	
Pompano (<i>Trachinotus ovatus</i>)	Skin	PSC	77	60	48	0	74	129	7	0	56	9	18	30	14	13	83	19	19	2.5	18	140	(Cao et al., 2019)
	Skin	ASC	133	52	44	0	71	327	7	6	85	11	21	27	15	14	108	28	24	5	22	193	
Sailfish (<i>I. platypterus</i>)	Scale	PSC	133	51	42	0	69	337	7	6	89	9	19	26	14	12	106	33	24	3	20	195	(Tamilmozhi et al., 2013)
	Skin	ASC	120	54	45	0	80	331	5	3	69	8	20	26	14	30	110	39	24	2	19	179	
Sailfish (<i>I. platypterus</i>)	Skin	PSC	122	55	45	0	80	338	5	3	72	9	20	27	13	13	113	40	25	2	19	185	(Cao et al., 2019)
	Skin	ASC	137	53	45	1	71	332	5	0	73	8	21	27	9	12	124	31	25	2.1	20	198	
Sailfish (<i>I. platypterus</i>)	Bone	PSC	130	54	43	1	70	343	5	0	77	9	22	26	9	12	120	31	24	2.3	21	197	(Tamilmozhi et al., 2013)
	Skin	ASC	113	58	44	2	76	318	3	0	94	14	25	26	11	13	118	37	23	4	20	212	
Sailfish (<i>I. platypterus</i>)	Skin	PSC	111	58	40	3	72	325	3	0	99	12	23	25	11	12	122	35	23	5	21	221	(Tamilmozhi et al., 2013)
	Skin	ASC	111	58	40	3	72	325	3	0	99	12	23	25	11	12	122	35	23	5	21	221	

Table 3 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Cobia (<i>R. canadum</i>)	Skin	ASC	134	52	46	0	70	329	5	0	94	11	21	25	14	14	109	24	23	4	25	203	(Zeng et al., 2012)
	Skin	PSC	135	51	45	0	69	342	5	0	79	12	21	26	14	14	112	25	23	2	25	191	
Giant grouper (<i>E. lanceolatus</i>)	Skin	ASC	131	54	48	0	71	337	6	0	70	9	20	26	12	14	119	34	24	3	21	189	(Hsieh et al., 2016)
	Skin	PSC	123	50	55	0	71	319	6	0	67	15	26	25	10	16	114	43	30	6	24	181	
Miiuy croaker (<i>Mitichthys miiuy</i>)	Swim blad- der	ASC	96	41	44	0	90	321	9	0	89	11	34	32	6	26	107	28	21	7	23	196	(Zhao et al., 2018)
		PSC	95	55	39	0	84	334	8	0	87	13	26	24	5	23	111	27	21	2	32	199	
Giant croaker (<i>Nibea japonica</i>)	Swim blad- der	ASC	98	46	43	0	46	322	9	0	73	8	16	23	9	9	107	25	16	2	13	181	(Chen et al., 2019)
		PSC	100	47	43	0	45	328	8	0	74	8	15	24	8.4	9	112	25	16	1	13	187	
Brownbanded bamboo shark (<i>C. punctatum</i>)	Cartilages	ASC	104	51	42	1	78	317	7	7	94	18	24	28	13	14	109	41	23	3	25	203	(Kittiphattana- bawon et al., 2010)
	Cartilages	PSC	104	51	43	1	77	317	7	7	94	19	25	27	12	13	110	41	24	3	25	204	
Blacktip shark (<i>C. limbatus</i>)	Cartilages	ASC	119	54	42	1	77	317	8	7	91	19	25	27	14	14	105	30	21	3	26	196	(Kittiphattana- bawon et al., 2010)
	Cartilages	PSC	118	54	43	1	77	316	8	8	91	20	26	26	14	13	106	31	22	3	26	197	
Silvertip shark (<i>C. albigarinatus</i>)	Skeletal	PSC	133	49	45	4	76	320	9	0	49	21	29	29	13	14	106	38	25	7	25	156	(Jeevithan et al., 2014)
	Head bone	PSC	135	40	43	7	71	322	5	0	51	19	40	22	13	16	124	37	23	7	16	175	

Amino acid composition

Since collagen is protein molecules made up of amino acids, understanding amino acid composition of collagen is an essential perspective, particularly type I collagen. In general, the quantification of amino acids is carried out by using chromatographic methods (Jafari et al., 2020). It is well recognized that collagen predominantly contains glycine (about a third of the total amino acid composition), proline and hydroxyproline amino acids. As presented in Table 3, the major amino acids found in the tropical marine fish collagens are glycine, alanine, proline, glutamic acid, hydroxyproline, and arginine, thus describing the presence of type I. These amino acids are also documented in other sources of collagen like calf skin, porcine skin, and human type I (Yu et al., 2018). Among these amino acids, as aforementioned, both proline and hydroxyproline are called an imino acid due to presence of a secondary amine group, and they are often used to identify collagen properties from different sources. Additionally, hydroxyproline content represents the amount of collagen extracted in the final product, and its composition is diverse depending on the raw material of extracted collagen. Based on the existing literature related to collagen isolated from several sources, the imino acid contents are higher in mammalian collagens than those found in fish (e.g., warm-water fish and cold-water fish) collagens. As can be seen in Table 3, the contents of imino acid obtained in different collagen sources are approximately 22% for mammals, 17–20% for tropical marine fish, and 15% for tempered fish (Ahmad and Benjakul, 2010; Ding et al., 2019; Matmaroh et al., 2011; Yu et al., 2018). Moreover, the diverse amino acid compositions of collagen influence the collagen properties. For instance, the thermal stability of mammals' collagen is greater compared to that of collagen derived from fish species, because the sterical side groups of imino acid restrict the conformation of the polypeptide chain, and the hydroxyl group of hydroxyproline plays a major role in intramolecular hydrogen bonds.

FTIR profile

Fourier transform infrared (FTIR) spectroscopy is widely used to examine the collagen extracted from organisms' tissues, including tropical marine fish species. Using this tool, the chemical composition and type classification of collagen can be determined after being isolated with various extraction conditions. As illustrated in Table 4, the characteristic peaks of extracted marine collagen samples were amide a, amide b, amide I, amide II, and amide III (Ahmed et al., 2019; Fatiroi et al., 2023; Matarsim et al., 2023). The absorption parameter of amide A is closely related to N-H stretch vibration, and normally located at the wavenumber of 3400–3440 cm^{-1} (Sai and Babu, 2001), while amide B is associated with CH_2 asymmetric stretching (Abe et al., 1972). For amide I, it is found in the wavenumber range 1600–1700 cm^{-1} and representing the C=O stretching/hydrogen bond coupled with COO- (Payne and Veis, 1988). Amide II is commonly responsible for N-H

bend coupled with C-N stretching (Krimm and Bandekart, 1986), and the rest of amide III is regarded as N-H bend coupled with C-H stretching (Payne and Veis, 1988). By assessing these amides as recognized in the absorption peaks of collagens (Table 4), all extracted collagens were consistent with the wavenumber of amides. Furthermore, especially in the amide I-III, these peaks are involved in the formation of a triple-helical structure of collagen molecules. Other studies by Nikoo et al. (2013) and Plepis et al. (1996), revealed that the triple-helical regions can be examined by measuring the difference in the wavenumber (cm^{-1}) between amide I and II bands using the formula $\Delta\nu (\nu I - \nu II)$, where values <100 , indicating that the collagen structure is preserved. Besides that, the triple helix can be also verified by using the absorption ratio (~ 1.0) of the amide III to the 1450 cm^{-1} band (A_{III}/A_{1450}) (Plepis et al., 1996). Through these approaches, for the resultant collagens from tropical fish species prepared by using acids and enzymatic processes it could be confirmed that the functional groups present in the triple-helical structure were not damaged, suggesting that the treatment conditions during extraction would not influence the final collagen products.

X-ray diffraction study

X-ray diffraction (XRD) method is often used in aquatic and marine sources to evaluate the fibrillar structure of collagen after being extracted by different approaches. XRD uses the Bragg law with an equation: $2d\sin\theta = \lambda$, and was employed to determine the minimum of repeated interval (d), where λ is the wavelength of x-ray ($1.5405 \text{ \AA}$), and θ is the Bragg diffraction angle (Wang et al., 2009). The peaks in the 2θ curves plotted against intensity correspond to features like the spacing between collagen fibres, bonds within polypeptide chains, and/or the diameter of the collagen triple helix. These characteristics could be influenced by the method used for extraction and solubilisation, ultimately indicating the integrity of the extracted collagen (Chen et al., 2016). Basically, XRD pattern of collagen has three prominent peaks (e.g., peak A_1 , A_2 and A_3) with the diffraction angle usually located in $5\text{--}10^\circ$, near 20° and $30\text{--}35^\circ$, respectively (Jaziri et al., 2022 c). Peak A_1 , first peak in XRD, reflects the distance between the molecular diffraction pattern of collagen. Second peak (A_2) is obtained by diffuse scattering, and the last peak of A_3 describes the unit height, typical of the triple helical structure of collagen molecules (Giraud-Guille et al., 2000). Comparably, some researchers have reported the presence of two significant peaks i.e., sharp and broad peaks in the assessment of tropical marine fish collagens isolated from the skin of barracuda (*Sphyræna* sp.) (ASC: $11.6\text{--}12.2 \text{ \AA}$ and $4.4\text{--}4.6 \text{ \AA}$) (Matarsim et al., 2023), the bones of unicorn fish (*A. monoceros*) (ASC: $11.3\text{--}11.4 \text{ \AA}$ and $3.3\text{--}3.4 \text{ \AA}$) (Fatiroi et al., 2023), barramundi (*L. calcalifer*) (PSC: $12.5 \text{ \AA}$ in the first peak) (Liao et al., 2018), and the skin and bone of golden pompano (*T. ovatus*) (PSC: $11.1 \text{ \AA}$ and $11.7 \text{ \AA}$ in the peak 1) (Cao et al., 2019). According to Chen et al. (2016), if the spacing between fibres is higher at first

peak, collagen is more capable of carrying drugs, making it a preferred choice for the pharmaceutical company.

Thermal stability analysis

Differential scanning calorimeter (DSC) assesses collagen enthalpy (ΔH) variations using a thermally inert reference material (usually indium). Under identical temperature conditions, the sample and reference material produce positive and negative peaks, indicating energy removal (exothermic peak) or addition (endothermic peak). The endothermic peak in the sample reflects collagen denaturation, with T_{max} representing the maximum transition temperature and correlating directly with enthalpy (ΔH) and denaturation resistance. Pepsin-soluble collagen (PSC) exhibits lower thermostability than acid-soluble collagen (ASC) due to telopeptide region removal by pepsin. The study establishes a positive correlation between collagen thermostability and imino acids (proline and hydroxyproline) content, indicating enhanced hydrogen bond stabilization (Matmaroh et al., 2011). Many studies have been conducted on the tropical marine fish sources with having a variety of thermal stability, such as the ASC and PSC from spotted golden goatfish (*P. heptacanthus*) scale ($T_{max} = 41.58^\circ\text{C}$, $\Delta H = 12.38 \text{ J/g}$ and $T_{max} = 41.01^\circ\text{C}$, $\Delta H = 15.39 \text{ J/g}$) (Matmaroh et al., 2011), starry triggerfish (*A. stellatus*) skin ($T_{max} = 35.9^\circ\text{C}$, $\Delta H = 2.4 \text{ J/g}$ and $T_{max} = 33.6^\circ\text{C}$, $\Delta H = 2.1 \text{ J/g}$) (Ahmad et al., 2016), and brownbanded bamboo shark (*C. punctatum*) ($T_{max} = 36.73^\circ\text{C}$, $\Delta H = 1.55 \text{ J/g}$ and $T_{max} = 35.98^\circ\text{C}$, $\Delta H = 0.85 \text{ J/g}$) (Kittiphattanabawon et al., 2010).

Microstructural property

Microstructural property and surface area of collagen are important to evaluate its potential applications in biomedicine and biomedical engineering. Its characteristics are normally observed under scanning electron microscopy (SEM) or field emission scanning electron microscopy (FESEM) (Bhuimbar et al., 2019). Typically, fish collagens extracted by using acid medium and pepsin-added process show irregular dense sheet-like film linked by random-coiled filaments and their surface is partially wrinkled due to dehydration during freeze-drying. Moreover, with a certain magnification employed, lyophilized fish collagens present some fibrillar structures. Ogawa et al. (2004) stated that collagens with interconnectivity, fibrillary and sheet-like film structures have the potentiality to be used in new tissue formation, cell seeding, growth, wound healing and mass transport and migration. In general, the microstructural analysis of collagens obtained from the tropical marine sources, such as barracuda (*Sphyræna* sp.) skin (Matarsim et al., 2023), puffer fish (*L. inermis*) skin (Iswariya et al., 2018), unicorn fish (*N. reticulatus*) bone (Fatiroi et al., 2023), giant croaker (*N. japonica*) swim bladder (Chen et al., 2019), silvertip shark (*C. albimarginatus*) skeletal and head bone (Jeewithan et al., 2014), indicated the microstructural characteristics conducive to various medical applications. This cumulative evidence suggests that collagens from these tropical marine sources may indeed serve as promising biomaterials for a range of medical applications.

Table 4 – contd.

1	2	3	4	5	6	7	8	9	10	11
Miuy croaker (<i>Mitichthys miui</i>)	Swim bladder	ASC	3325 cm ⁻¹	2938 cm ⁻¹	1653 cm ⁻¹	1543 cm ⁻¹	1241 cm ⁻¹	109.8	0.9	(Li et al., 2018)
Seabass (<i>Lates calcarifer</i>)	Swim bladder	PSC	3362 cm ⁻¹	2932 cm ⁻¹	1655 cm ⁻¹	1548 cm ⁻¹	1243 cm ⁻¹	107	0.9	
	Scale	ASC	3285 cm ⁻¹	3075 cm ⁻¹	1657 cm ⁻¹	1553 cm ⁻¹	1456 cm ⁻¹	104	1.0	(Chuaychan et al., 2015)
	Scale	PSC	3311 cm ⁻¹	3086 cm ⁻¹	1650 cm ⁻¹	1548 cm ⁻¹	1459 cm ⁻¹	102	1.0	
Seabass (<i>Lates calcarifer</i>)	Skin	ASC	3292 cm ⁻¹	2922 cm ⁻¹	1634 cm ⁻¹	1548 cm ⁻¹	1238 cm ⁻¹	86	0.9	(Razali et al., 2023)
	Skin	UAE	3307 cm ⁻¹	2923 cm ⁻¹	1651 cm ⁻¹	1548 cm ⁻¹	1235 cm ⁻¹	103	0.9	
Seabass (<i>Lates calcarifer</i>)	Skin	PSC	3379 cm ⁻¹	2931 cm ⁻¹	1657 cm ⁻¹	1553 cm ⁻¹	1241 cm ⁻¹	104.5	0.9	(Liao et al., 2018)
Emperor fish (<i>Lethrinus lentjan</i>)	Skin	ASC	3367 cm ⁻¹	2916 cm ⁻¹	1635 cm ⁻¹	1555 cm ⁻¹	1385 cm ⁻¹	80	1.0	(Firdayanti et al., 2023)
Parrotfish (<i>Scarus sortidus</i>)	Scale	ASC	3287 cm ⁻¹	2428 cm ⁻¹	1636 cm ⁻¹	1541 cm ⁻¹	1235 cm ⁻¹	95	0.9	(Jaziri et al., 2023)
	Scale	PSC	3298 cm ⁻¹	2927 cm ⁻¹	1636 cm ⁻¹	1541 cm ⁻¹	1235 cm ⁻¹	95	0.9	
Grouper (<i>Epinephelus</i> sp.)	Swim bladder	PSC	3336 cm ⁻¹	2923 cm ⁻¹	1647 cm ⁻¹	1548 cm ⁻¹	1238 cm ⁻¹	98.8	0.9	(Dong and Dai, 2022 a)
Golden pompano (<i>Trachinotus ovatus</i>)	Skin	ASC	3421 cm ⁻¹	2932 cm ⁻¹	1653 cm ⁻¹	1542 cm ⁻¹	1240 cm ⁻¹	110.5	0.9	(Cao et al., 2019 a)
	Bone	PSC	3423 cm ⁻¹	2926 cm ⁻¹	1656 cm ⁻¹	1548 cm ⁻¹	1241 cm ⁻¹	107.3	0.9	
Puffer fish (<i>Lagocephalus inermis</i>)	Skin	ASC	ND	ND	1640 cm ⁻¹	1546 cm ⁻¹	1247 cm ⁻¹	94.2	0.9	(Iswariya et al., 2018)
	Skin	PSC	ND	ND	1640 cm ⁻¹	1546 cm ⁻¹	1247 cm ⁻¹	94.2	0.9	
Shortfin scad (<i>Decapterus macrostoma</i>)	Bone + skin	ASC	3456 cm ⁻¹	ND	1637 cm ⁻¹	1590 cm ⁻¹	1262 cm ⁻¹	46.9	0.9	(Sulaiman and Sarbon, 2020)
	Bone + skin	PSC	3449 cm ⁻¹	2934 cm ⁻¹	1636 cm ⁻¹	1560 cm ⁻¹	1264 cm ⁻¹	76.4	0.9	
Mahi mahi (<i>Coryphaena hippurus</i>)	Skin	ASC	3325 cm ⁻¹	3083 cm ⁻¹	1654 cm ⁻¹	1543 cm ⁻¹	1240 cm ⁻¹	111	0.9	(Akita et al., 2019)
	Skin	PSC	3326 cm ⁻¹	3078 cm ⁻¹	1656 cm ⁻¹	1534 cm ⁻¹	1235 cm ⁻¹	122	0.9	
Fringescale sardinella (<i>Sardinella fimbriata</i>)	Scale	ASC	3417 cm ⁻¹	ND	ND	1590 cm ⁻¹	1414 cm ⁻¹	ND	1.0	(Hamdan and Sarbon, 2019)
	Scale	PSC	3424 cm ⁻¹	ND	ND	1401 cm ⁻¹	1265 cm ⁻¹	ND	0.9	
Needle fish (<i>Tylosurus melanotus</i>)	Skin	ASC-A	3290 cm ⁻¹	2939 cm ⁻¹	1640 cm ⁻¹	1541 cm ⁻¹	1232 cm ⁻¹	98.8	0.8	(Ramle et al., 2022)
	Skin	ASC-L	3290 cm ⁻¹	2917 cm ⁻¹	1640 cm ⁻¹	1541 cm ⁻¹	1232 cm ⁻¹	98.8	0.8	
	Skin	ASC-C	3290 cm ⁻¹	2920 cm ⁻¹	1640 cm ⁻¹	1541 cm ⁻¹	1236 cm ⁻¹	98.8	0.9	
Sailfish (<i>Istiophorus platypterus</i>)	Skin	ASC	3423 cm ⁻¹	2928 cm ⁻¹	1654 cm ⁻¹	1560 cm ⁻¹	1240 cm ⁻¹	94	0.9	(Tamilmozhi et al., 2013)
	Skin	PSC	3337 cm ⁻¹	2924 cm ⁻¹	1646 cm ⁻¹	1549 cm ⁻¹	1240 cm ⁻¹	97	0.9	
Cobia (<i>Rachycentron canadum</i>)	Skin	ASC	3384 cm ⁻¹	2927 cm ⁻¹	ND	ND	ND	ND	ND	(Zeng et al., 2012)
	Skin	PSC	3333 cm ⁻¹	2924 cm ⁻¹	ND	ND	ND	ND	ND	
Sharpnose stingray (<i>Dasyatis zugei</i>)	Skin	ASC	3424 cm ⁻¹	ND	1627 cm ⁻¹	1553 cm ⁻¹	1239 cm ⁻¹	73.9	0.9	(Ong et al., 2021)
	Skin	PSC	3439 cm ⁻¹	ND	1627 cm ⁻¹	1553 cm ⁻¹	1239 cm ⁻¹	74.1	0.9	
Sharpnose stingray (<i>Dasyatis zugei</i>)	Skin	ASC+UEA	3449 cm ⁻¹	2997 cm ⁻¹	1632 cm ⁻¹	1573 cm ⁻¹	1263 cm ⁻¹	59	0.9	(Shaik et al., 2021)
	Skin	PSC+UEA	3449 cm ⁻¹	2998 cm ⁻¹	1637 cm ⁻¹	1579 cm ⁻¹	1262 cm ⁻¹	58	0.9	
Silvertip shark (<i>Carcharhinus albimarginatus</i>)	Skeletal	PSC	3331 cm ⁻¹	2932 cm ⁻¹	1660 cm ⁻¹	1551 cm ⁻¹	1240 cm ⁻¹	109.1	0.9	(Jeevithan et al., 2014)
	Head bone	PSC	3415 cm ⁻¹	2957 cm ⁻¹	1655 cm ⁻¹	1548 cm ⁻¹	1242 cm ⁻¹	107.4	0.9	

Solubility analysis

Solubility of collagen is extensively treated with various pH levels (1–12), and with various salt (NaCl) concentrations (0–60 g/L and 0–6%), in acetic acid medium, usually initial concentrations used are between 3 mg/mL and 6 mg/mL (Jaziri et al., 2022 d). In general, the protein content in the supernatant (solubilised samples) is determined by the Lowry procedure using the standard curve of bovine serum albumin (BSA). Furthermore, the solubility of collagen can be measured in comparison to that obtained at the pH and/or NaCl concentration yielding the highest solubility, referred to as the relative solubility (%) (Ahmed et al., 2019). The relative solubility range of collagen varies based on the extraction method applied. Table 1 shows that the collagens isolated from the skin, bones, scales and swim bladder of tropical marine fish were soluble within the pH range of 1–4, 1–5, 1–5, and 1–4, respectively. Meanwhile, for the NaCl treatment, the mentioned samples were almost soluble at the salt concentration of below 30 g/mL (Cao et al., 2019; Chen et al., 2019; Fatiroi et al., 2023; Jaziri et al., 2023). The solubility of collagen is affected by its structure and amino acid composition, particularly when subjected to elevated concentrations of NaCl (Ahmed et al., 2019).

Collagen hydrolysis

Collagen hydrolysis, a process involving the cleavage of peptide bonds, can be performed by chemical or enzymatic approaches. Chemical approaches, such as acid or alkaline hydrolysis, are often challenging to control and result in products with altered amino acids. Conventional acid hydrolysis conditions to determine amino acids composition (e.g., 6 M HCl at 110°C for over 24 h), can destroy essential amino acid (tryptophan) (Tsugita and Scheffler, 1982). In terms of alkaline hydrolysis, same amino acids content (arginine, cystine, isoleucine, lysine, serine, and/or threonine) can be chemically reduced, and produce unusual amino acid residues like lanthionine or lysinoalanine. On the other hand, enzymatic hydrolysis can be carried out under mild conditions and can avoid the extreme environments required by chemical treatments. Typically, enzymatic approaches avoid side reactions and do not diminish the nutritional value of the protein source. Within dietary proteins, biologically active peptides are released during digestion or processing, influencing various physiological functions upon separation from the parent protein sequence (Tavano, 2013).

Figure 3 illustrates the reaction of enzymatic hydrolysis (protease), wherein each cleaved peptide bond liberates one mole from both the carboxyl and amino groups. Complete breakdown yields a mixture of all constituent amino acids, while partially broken bonds result in a mixture of polypeptides derived from the original protein (Hajj et al., 2024). In terms of collagen, it can be hydrolysed using proteolytic enzymes such as papain, pepsin, alkaline protease, collagenase, and others. The hydrolysed collagen (HC) contains small peptides with low molecular weight (3–6 kDa) (Hu et al., 2017; León-

López et al., 2019 a). Usually, HC enhances functional properties such as solubility, emulsification, and foaming. It exhibits also lower viscosity in aqueous solution, colourlessness, neutral odour, film forming, dispersibility, wettability, powder compressibility, carrier substance and low allergenicity (Denis et al., 2008; Zhang et al., 2006). Additionally, HC possess several significant functions, such as antioxidative, anti-inflammatory, antimicrobial, and antihypertensive effects. Specifically, HC proves advantageous for digestion, allowing for easy absorption and convenient application in various products, including food, beverage, dietary supplements, and specialty. This has led to a surge in demand for HC in recent years, establishing it as a valuable ingredient in diverse products like functional foods, dietary items, cosmetic and pharmaceutical products (Hajj et al., 2024).

Proteolytic enzymes used in the fish collagen hydrolysates

Proteases (also known as proteinase, peptidase, or proteolytic enzymes) are enzymes that catalyse proteolysis, breaking down proteins into smaller polypeptides or single amino acids, and facilitating the formation of new protein products (López-Otín and Bond, 2008). They belong to a certain group of enzymes that can hydrolyse by catalysing the reaction of hydrolysis of different bonds and they do that with the participation of a water molecule. Each enzyme has its own optimal conditions in relation to temperature, pH and ionic strength that differ between enzymes. The order in which enzymes are added to the reaction mixture can change the effects of each individual enzyme. When the first enzyme has brought on their reaction it becomes the substrate of the second enzyme. The order of the enzyme reactions can influence the degree of hydrolysis (León-López et al., 2019 b). In general, proteolytic enzymes can be divided into two major groups: endopeptidases, which cleave internal peptide bonds, and exopeptidases, which cleave C- or N-terminal peptide bonds, and those enzymes were widely used in industrial applications, accounting for more than 60% of total global enzyme sales with the market size estimated at USD 3.54 billion in 2024.

In terms of marine fish collagen hydrolysates, researchers have employed a variety of proteases in producing collagen hydrolysates, such as papain, alkaline protease, collagenase, pepsin, Alcalase, Neutrase, trypsin, and Flavourzyme (Felician et al., 2019; Hema et al., 2017; Wang et al., 2020). Those proteolytic enzymes were used individually and/or in combination in generating fish collagen hydrolysates, and their properties (physicochemical, functional, and antioxidant) have been evaluated. Among the mentioned proteinases, papain and alkaline protease are often used in hydrolysing collagens from the byproducts of tropical marine fish source, as reported by Chotphruethipong et al. (2020), Felician et al. (2019), Hema et al. (2017), and Wang et al. (2020) for collagen hydrolysates derived from the grouper (*Epinephelus malabaricus*) skin, jellyfish (*R. esculentum*) filaments, redlip croaker (*Pseudosciaena polyactis*), and barramundi (*L. calcarifer*), respectively.

Table 5. UV-vis, XRD, DSC and microstructural properties of collagen derived from tropical marine fish byproducts

Source	Part	Method	UV-vis	XRD		DSC			Microstructural results	References
				Peak A	Peak B	T_{max}	ΔH			
1	2	3	4	5	6	7	8	9	10	
Spotted golden goatfish (<i>P. heptacanthus</i>)	Scale	ASC	ND	ND	ND	41.58°C	ND	ND	(Matmaroh et al., 2011)	
	Scale	PSC	ND	ND	ND	41.01°C	ND	ND		
Bigeye snapper (<i>Priacanthus tayenus</i>)	Skin	PSC	ND	ND	ND	31.3°C	ND	ND	(Benjakul et al., 2010)	
	Skin	PSC	ND	ND	ND	31.15°C	ND	ND	(Benjakul et al., 2010)	
Bigeye snapper (<i>Priacanthus macracanthus</i>)	Skin	ASC-A	ND	ND	ND	31.4°C	ND	ND	(Oslan et al., 2022)	
	Skin	ASC-L	ND	ND	ND	31.7°C	ND	ND		
Bigeye snapper (<i>Priacanthus tayenus</i>)	Skin	ASC-C	ND	ND	ND	31.5°C	ND	ND		
	Skin	PSC	ND	ND	ND	33.2°C	ND	ND		
Brownstripe red snapper (<i>Lutjanus vitta</i>)	Skin	ASC	ND	ND	ND	31.52°C	ND	ND	(Jongiareonrak et al., 2005 a)	
	Skin	PSC	ND	ND	ND	31.02°C	ND	ND		
Barracuda (<i>Sphyræna</i> sp.)	Skin	ASC-A	230.5 nm	7.48°	20.02°	41.29°C	0.13 J/g	A multi-layered form with the irregular sheet-like film connected by random-coiled filaments	(Matarsim et al., 2023)	
	Skin	ASC-L	230.5 nm	7.26°	19.16°	40.69°C	0.08 J/g			
Bigeye tuna (<i>Thunnus obesus</i>)	Skin	ASC-C	230.5 nm	7.64°	19.12°	40.16°C	0.05 J/g		(Ahmed et al., 2019 a)	
	Skin	ASC	ND	ND	ND	32.07°C	-	ND		
Skipjack tuna (<i>Katsuwonus pelamis</i>)	Skin	PSC	ND	ND	ND	33.73°C	-	ND		
	Scale	PSC	ND	ND	ND	31.63°C	-	ND		
Starry triggerfish (<i>Abalistes stellatus</i>)	Bones	PSC	ND	ND	ND	32.26°C	-	ND	(Ding et al., 2019)	
	Skull	ASC	220 nm	ND	ND	ND	-	ND		
Puffer fish (<i>Lagocephalus inermis</i>)	Skull	PSC	220 nm	ND	ND	ND	-	ND		
	Spine	ASC	220 nm	ND	ND	ND	-	ND		
Puffer fish (<i>Lagocephalus inermis</i>)	Spine	PSC	220 nm	ND	ND	ND	-	ND		
	Skin	ASC	ND	ND	ND	35.9°C	2.4 J/g	ND	(Ahmad et al., 2016)	
Puffer fish (<i>Lagocephalus inermis</i>)	Skin	PSC	ND	ND	ND	33.6°C	2.1 J/g	ND		
	Skin	ASC	230 nm	ND	ND	ND	ND	Both ASC and PSC showed fibrous and porous structure under SEM	(Iswariya et al., 2018)	
Puffer fish (<i>Lagocephalus inermis</i>)	Skin	PSC	230 nm	ND	ND	ND	ND			
	Skin	PSC	230 nm	ND	ND	ND	ND			

Table 5 – contd.

1	2	3	4	5	6	7	8	9	10
Unicorn leatherjacket (<i>Aluterus monoceros</i>)	Skin	PSC	ND	ND	ND	31.98°C	0.60 J/g	ND	(Ahmad and Benjakul, 2010)
	Skin	PSC-A	ND	ND	ND	31.73°C	0.75 J/g	ND	
	Skin	PSC-Y	ND	ND	ND	31.68°C	0.76 J/g	ND	
Unicorn fish (<i>Naso reticulatus</i>)	Bone	ASC-A	231.2 nm	7.22°	21.33°	33.51°C	3.9 mJ/g	The PSCs had an irregular and dense flake structure with coiled filaments	
	Bone	ASC-L	229.8 nm	7.24°	21.74°	33.39°C	7.7 mJ/g		
	Bone	ASC-C	230.8 nm	6.66°	20.11°	33.34°C	5.7 mJ/g		
Miuiy croaker (<i>Mitichthys miuiy</i>)	Swim bladder	ASC	226 nm	ND	ND	ND	ND	ND	(Li et al., 2018)
	Swim bladder	PSC	226 nm	ND	ND	ND	ND	ND	
Chu's croaker (<i>Nibea coibor</i>)	Swim bladder	ASC	201–220 nm	ND	ND	79.74°C	ND	The ASC was uniform and porous	(Xiao et al., 2023)
Giant croaker (<i>Nibea japonica</i>)	Skin	PSC	230 nm	ND	ND	ND	ND	ND	(Yu et al., 2018)
Giant croaker (<i>Nibea japonica</i>)	Swim bladder	ASC	ND	ND	ND	ND	ND	All samples showed multi-layered, fibrous, and porous structure	(Chen et al., 2019)
	Swim bladder	PSC	ND	ND	ND	ND	ND	ND	
Sin croaker (<i>J. sina</i>)	Bone	ASC	ND	ND	ND	31.31°C	0.05 J/g	ND	(Normah and Afiqah, 2018)
Blackspotted croaker (<i>Protonibea diacanthus</i>)	Swim bladder	ASC	201–220 nm	ND	ND	85.93°C	ND	The extracted collagen appeared to be an irregular dense sheet-like film	(Xiao et al., 2023)
Seabass (<i>Lates calcarifer</i>)	Skin	PSC	230.3 nm	7.05°	20.30°	109.6°C	ND	The collagen presented like soft white sponge with irregular dense sheet-like film	(Liao et al., 2018)
Seabass (<i>Lates calcarifer</i>)	Scale	ASC	ND	ND	ND	38.17°C	0.72 J/g	ND	(Chuaychan et al., 2015)
	Scale	PSC	ND	ND	ND	39.32°C	0.91 J/g	ND	
Giant grouper (<i>E. lanceolatus</i>)	Skin	ASC	215–230 nm	ND	ND	31.71°C	ND	ND	(Hsieh et al., 2016)
	Skin	PSC	215–230 nm	ND	ND	31.33°C	ND	ND	
Grouper (<i>Epinephelus</i> sp.)	Swim bladder	PSC	234 nm	ND	ND	33.84°C	ND	ND	(Dong and Dai, 2022 b)
Parrotfish (<i>Scarus sordidus</i>)	Scale	ASC	230 nm	7.65°	19.71°	37.78°C	0.35 J/g	ND	(Jaziri et al., 2023)
	Scale	PSC	232 nm	7.59°	19.37°	36.22°C	0.02 J/g	ND	
Golden pompano (<i>Trachinotus ovatus</i>)	Skin	ASC	ND	7.97°	20.8°	37.04°C	ND	ND	(Cao et al., 2019 a)
	Bone	PSC	ND	7.58°	20.6°	38.23°C	ND	ND	
Cobia (<i>Rachycentron canadum</i>)	Skin	ASC	ND	ND	ND	ND	ND	ND	(Zeng et al., 2012)
	Skin	PSC	ND	ND	ND	ND	ND	ND	

Table 5 – contd.

1	2	3	4	5	6	7	8	9	10
Sailfish (<i>Istiophorus platypterus</i>)	Skin	ASC	ND	ND	ND	ND	ND	The samples look like fine globular filaments	(Tamilmozhi et al., 2013)
	Skin	PSC	ND	ND	ND	ND	ND		
Narrow-barred Spanish mackerel (<i>S. commerson</i>)	Skin	ASC	ND	7.6°	19.4°	ND	ND	The extracted collagen had irregular dense sheet-like structure	(Naderi Gharahgheshlagh et al., 2023)
Horse mackerel (<i>T. japonicus</i>)	Scale	ASC	ND	ND	ND	28.1°C	0.59 J/g	ND	(Minh et al., 2014)
Grey mullet (<i>Mugil cephalis</i>)	Scale	ASC	ND	ND	ND	27.1°C	0.28 J/g	ND	
Flying fish (<i>Cypselurus melanurus</i>)	Scale	ASC	ND	ND	ND	29.2°C	0.59 J/g	ND	
Yellowback seabream (<i>D. tumifrons</i>)	Scale	ASC	ND	ND	ND	28.2°C	0.56 J/g	ND	
Needle fish (<i>Tylosurus melanotus</i>)	Skin	ASC-A	231.5 nm	ND	ND	39°C	ND	ND	(Ramle et al., 2022)
	Skin	ASC-L	231.5 nm	ND	ND	38.6°C	ND	ND	
	Skin	ASC-C	231.5 nm	ND	ND	38.15°C	ND	ND	
Sharpnose stingray (<i>Dasyatis zugei</i>)	Skin	ASC	ND	ND	ND	31.94°C	ND	ND	(Ong et al., 2021)
	Skin	PSC	ND	ND	ND	31.76°C	ND	ND	
Sharpnose stingray (<i>Dasyatis zugei</i>)	Skin	ASC+UEA	ND	ND	ND	45.57°C	ND	ND	(Shaik et al., 2021)
	Skin	PSC+UEA	ND	ND	ND	45.55°C	ND	ND	
Silvertip shark (<i>Carcharhinus albimarginatus</i>)	Skeletal	PSC	237.7 nm	ND	ND	58.07°C	ND	The collagens depicted a porous, fibrillary and multi-layered structure	(Jeevithan et al., 2014)
	Head bone	PSC	238 nm	ND	ND	54.64°C	ND		
Brownbanded bamboo shark (<i>C. punctatum</i>)	Cartilages	ASC	ND	ND	ND	36.73°C	1.55 J/g	ND	(Kitiphattanabawon et al., 2010)
	Cartilages	PSC	ND	ND	ND	35.98°C	0.85 J/g	ND	
Blacktip shark (<i>C. limbatus</i>)	Cartilages	ASC	ND	ND	ND	36.28°C	0.70 J/g	ND	(Kitiphattanabawon et al., 2010)
	Cartilages	PSC	ND	ND	ND	34.56°C	0.95 J/g	ND	

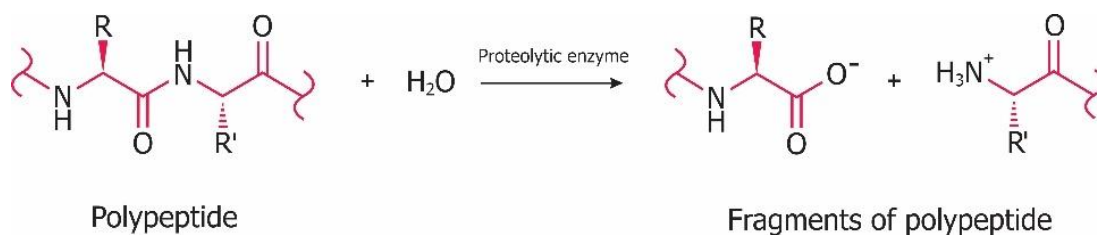


Figure 3. Enzymatic hydrolysis reaction of protein into peptides

Wound healing agents of fish collagen

The skin is the largest and outermost organ of the body that serves as a continuous shield against infections caused by bacteria and other microorganisms. It acts as a primary defence mechanism against external environment, preventing dehydration and protecting against various forms of damage including chemical, mechanical, osmotic, thermal, and photic damage, particularly from exposure to ultraviolet light. It also plays important roles in sensation, regulation, biochemical processes, metabolism, and immunity (Lee et al., 2006). Structurally, the skin comprises three layers: the epidermis, dermis, and hypodermis. The epidermis, the outer layer, consists of four or five sub-layers depending on the body region, and key cell types within the epidermis include keratinocytes, melanocytes, Langerhans cells, and Merkel cells (Man and Hoskins, 2020).

Wounds refer to skin injuries or flaws resulting from various forms of damage or physiological conditions, potentially extending to other tissues and structures, such as subcutaneous tissue, tendons, muscles, bones, blood vessels, and nerves (Boateng and Catanzano, 2015). Wounds lack a standardized grouping but can be classified in various ways to facilitate appropriate medical attention. Recent findings propose a threefold categorization of wounds based on their cause, the healing process's nature, and the wound's clinical appearance (Tabriz and Douroumis, 2022).

The general process of wound healing

The process of wound healing can be divided into four stages: haemostasis, inflammation, proliferation, and remodelling, as depicted in Figure 3.

a) Haemostasis

Following a wound, the haemostasis phase begins immediately, characterized by the initiation of blood vessel constriction to stop bleeding and the formation of a fibrin clot to seal the wound (Dryden et al., 2013). Platelets adhere to exposed collagen, initiating the formation of an unstable fibrin clot. This clot is subsequently reinforced by clotting factors, resulting in fibrin formation. Skin injury triggers the release of growth factors, pro-inflammatory cytokines, and chemokines. Chemokine release stimulates the migration of immune cells, such as neutrophils and monocytes, to the wound site, while the secretion of pro-inflammatory molecules initiates the inflammatory phase (Kurahashi and Fujii, 2015).

b) Inflammation

The inflammatory stage is marked by the presence of neutrophils, lymphocytes, and macrophages at the wound site, aiming to remove pathogens and cellular remnants. Neutrophils, the initial immune cells recruited to the injury location, engage in bacterial elimination through phagocytosis, triggering the release of cytokines, growth factors, reactive oxygen species (ROS), and proteolytic enzymes. These substances originate from various cell types and activate other immune cells (Wilgus et al., 2013). Macrophages, amongst the most abundant immune cells in wounds, can be categorized into M1 and M2 types based on their cytokine secretion patterns. Damage-associated molecular patterns (DAMPs), pathogen-related molecular patterns (PAMPs), and pro-inflammatory cytokines influence macrophages to adopt the M1 phenotype, characterized by the secretion of inflammatory cytokines crucial for recruiting more immune cells to the wound site (Mosser, 2003). In this phase, immune cells function to eliminate intracellular pathogens, and macrophages play a vital role in the clearance of apoptotic neutrophils. Controlled removal of aged neutrophils is critical for the progression of healing, achieved by inhibiting neutrophil degradation to prevent the release of inflammatory substances. Uncontrolled phagocytosis leads to the release of DAMPs by neutrophils, causing additional inflammation and tissue damage (Greenlee-Wacker, 2016). Once neutrophils are cleared, the environment triggers the transformation of M1 macrophages into M2 macrophages, which secrete anti-inflammatory cytokines like IL-10, marking the onset of the proliferation and migration phase (Bratton and Henson, 2011).

c) Proliferation and migration

The proliferation and migration stage can be divided into three main components: re-epithelialisation, neo-vascularization, and granulation tissue formation. This stage is distinguished by the movement and multiplication of keratinocytes, endothelial cells, and fibroblasts, collectively responsible for covering the epithelial layer, restoring blood supply to damaged areas, and replacing tissue through fibroblast mediation. These components occur by overlapping one another (Ellis et al., 2018). Re-epithelialisation occurs beneath the fibrin clot as keratinocytes migration stimulated by epidermal growth factor (EGF). Cell migration involves dynamic processes driven by actin filaments, resulting in cell protrusion,

adhesion, contraction, and detachment. Actin polymerization initiates protrusions at the leading edge, stabilized by adhesive proteins. Cell contraction facilitated by actin-myosin polymerization enables forward movement, facilitated by enzymes like matrix metalloproteinases (MMPs) detaching cells from the matrix (Ronfard and Barrandon, 2000). Adequate angiogenesis is vital for effective wound healing, as oxygen and nutrients are crucial for energy generation needed for processes such as cell proliferation and collagen production. Angiogenesis, an invasive process, comprises matrix degradation, migration towards angiogenic stimuli, endothelial cell proliferation, and reorganization. Sprouting angiogenesis is steered by tip cells responsive to vascular endothelial growth factor (VEGF) stimuli, releasing proteolytic enzymes to facilitate endothelial cell membrane degradation and sprouting. Granulation tissue, formed if the wound extends into the dermis, is characterized by fibroblasts synthesizing extracellular matrix (ECM) and collagen to fortify the tissue. Although collagen III is prominent, newly formed skin remains weaker until remodelling strengthens the tissue (Van Hinsbergh and Koolwijk, 2008).

d) Remodelling

The remodelling phase may extend over several years until new tissue matures to resemble the original tissue (Velnar et al., 2009). This phase is characterized by the rearrangement of collagen and the reduction of blood supply to the new tissue. Moreover, specialized fibroblasts known as myofibroblasts decrease the size of the newly formed tissue by binding collagen at the wound edges and contracting the wound area (Darby et al., 2014). Tissue maturation involves a complex process where the primary components of the extracellular matrix (ECM) are broken down and replaced; for example, collagen III is replaced by collagen I, which enhances tension and fortifies the wound area (Gonzalez et al., 2016). Following wound contraction, myofibroblasts undergo programmed cell death, immune cell populations decrease, while blood vessels remain intact. Newly formed skin differs from uninjured skin, possessing thicker fibres and disorganized collagen. Throughout the remodelling phase, fibres become more structured and interconnected, further bolstering tissue strength.

Recent updates on wound healing evaluation using *in vitro* test

Many collagens from the byproducts of marine fish sources were studied by researchers in terms of their capability of enhancing wound healing under *in vitro* evaluations. As tabulated in Table 6, collagen from the byproducts of bigeye tuna (*T. obesus*) (Lin et al., 2019), unicorn leatherjacket (*A. monoceros*) (Kumar et al., 2019), Nile tilapia (*O. niloticus*) (Zhou et al., 2017), narrow-barred Spanish mackerel (*S. commerson*) (Naderi Gharahgheshlagh et al., 2023), pirapitinga (*Piaractus brachipomus*) (Manjushree et al., 2023), flounder fish (*Paralichthys* sp.)

(Sousa et al., 2023), giant croaker (*N. japonica*) (Zheng et al., 2020), mrigal fish (*Cirrhinus cirrhosus*) (Pal et al., 2016), jellyfish (*Rhopilema esculentum*) (Felician et al., 2019), gurijuba (*H. parkeri*) (Ferreira et al., 2022) increased the growth of normal skin cells (*i.e.*, fibroblasts, keratinocytes, and endothelial cells) during viability and/or scratch tests. Among them, the type I collagen derived from the skin of bigeye tuna (*T. obesus*) showed the increment of NIH-3T3 fibroblasts' growth even though there were no significant differences ($P > 0.05$) on all treated groups; however, the scratch closure rates were better compared to the control (Lin et al., 2019). Other examples of collagen originated from the skins of unicorn leatherjacket (*A. monoceros*) and Nile tilapia (*O. niloticus*) exhibited that at concentration of 0.2 mg/mL, a greater migration of cells was noted in the collagen compared to other treated samples and a higher scratch closure presented in the hydrolysed collagen (50.0 µg/mL) than other treatments, respectively (Hu et al., 2017; Kumar et al., 2019).

Manjushree et al. (2023) investigated that at the highest concentration of the hydrolysed collagen from pirapitinga (*P. brachypomus*) skin could increase the percentage of L292 mouse fibroblast cells, and its hydrolysate became non-toxic when observed in the MTT assay. In addition to this, a remarkable wound closure ability of more than 80% at 12 h and 100% within 24 h was recorded during the scratch evaluation. Besides that, Felician et al. (2019) confirmed that collagen from jellyfish (*R. esculentum*) had significant effects on the scratch closure on human umbilical vein endothelial cells treated with collagen (6.25 mg/mL for 48 h), as compared to the vehicle treated cells, and even reported by Zheng et al. (2020) that the collagen from the swim bladders of giant croaker (*N. japonica*) could significantly minimize the oxidative stress damage caused by H_2O_2 in HUVEC.

Fish collagen incorporated with other components also supported the growth of skin cells, as documented from Afzali and Boateng (2022), Ferreira et al. (2022), Muthukumar et al. (2014 a), Ramanathan et al. (2017 b), and Selvaraj et al. (2023). Barramundi (*L. calcarifer*) scale collagen incorporated with mupirocin and extracts of *M. uniflorum* exhibited a great biocompatibility agent against the NIH-3T3 fibroblast cells and human keratinocyte cells (HaCaT) (Muthukumar et al., 2014 b). In line with previous example, a high biocompatibility with NIH-3T3 murine fibroblast cells was observed in the swim bladder collagen of gurijuba (*H. parkeri*) incorporated with chitosan (Ferreira et al., 2022), and a good biocompatibility of the scaffolds (*Arothron stellatus* skin collagen loaded with extract of *Coccinia grandis* and drug ciprofloxacin) was noted owing to the presence of grooved pattern of the collagen matrix with cellular growth and adhesion (Ramanathan et al., 2017 b). Furthermore, collagen-coated silk fibroin nanofibers increased production of collagen shown in the BALB/3T3 clone A31 fibroblast cells (Selvaraj et al., 2023).

Table 6. *In vitro* studies on fish collagens and other components towards skin wound repair

Source	Sample concentration	Cell lines used	<i>In vitro</i> assay	Results	References
1	2	3	4	5	6
Type I collagen from the skin of bigeye tuna (<i>Thunnus obesus</i>)	0–100 µg/mL	NIH-3T3 fibroblasts	MTT test: 1×10^5 cells/well Scratch test: 2×10^5 cells/well	Enhanced the growth of NIH-3T3 fibroblasts, albeit no significant cytotoxic effect ($p > 0.05$); Better scratch closure rates in the type I PSC treated group, compared to the control.	(Lin et al., 2019)
Collagen peptide (CP) from the swim bladders of giant croaker (<i>Nibea japonica</i>)	0–100 µg/mL	HUVECs	MMT test 1×10^6 cells/well	CP sample significantly minimized the oxidative stress damage caused by H_2O_2 in HUVECs	(Zheng et al., 2020)
Barramundi (<i>Lates calcarifer</i>) scale collagen incorporated with mupirocin and <i>Macrotyloma uniflorum</i>	ND	NIH 3T3 cells and HaCaTs	MTT test: 8×10^3 to 7×10^6 cells/well	A great biocompatibility agent against the fibroblasts and keratinocyte cell lines	(Muthukumar et al., 2014 c)
Scaffold from scale of mrigal fish (<i>Cirrhinus cirrhosus</i>)	ND	Primary fibroblasts and keratinocytes from human	MTT test: 5×10^6 cells/scaffold	A highly efficient cell growth and proliferation found in the treated collagen scaffold; Enhanced development of stratified epidermal layer <i>in vitro</i> .	(Pal et al., 2016)
Hydrolysed collagen from the skin of unicorn leatherjacket (<i>A. monoceros</i>)	0.2 mg/mL	3T3-L1 cells 0.05×10^6 cells/well	Scratch assay (0.05×10^6 cells/well)	At concentration of 0.2 mg/mL, a greater migration of cells was noted in the collagen peptide 5 (CP-5) compared to other treated samples.	(Kumar et al., 2019)
Hydrolysed collagen from the skin of Nile tilapia (<i>Oreochromis niloticus</i>)	6.25–50.0 µg/mL	HaCaT cells	Scratch test: 5×10^5 cells/well	A higher scratch closure presented in the hydrolysed collagen (50.0 µg/mL) than other treatments.	(Hu et al., 2017)
Nanofibres of collagen from Nile tilapia (<i>Oreochromis niloticus</i>) skin	ND	HaCaTs	MTT test (2×10^4 cells/well)	The fabricated type I collagen nanofibres enhanced the adhesion, proliferation, and migration of HaCaTs.	(Zhou et al., 2017)
Narrow-barred Spanish mackerel (<i>S. commerson</i>) skin collagen-based films	ND	3T3 cells	MTT assay: 5×10^3 cells/well	Collagen-based films could increase the growth of 3T3-cell and proliferation, as compared to control; Enhanced cell attachment due to its hydrophilicity	(Naderi Gharahgheshlagh et al., 2023)
Hydrolysed collagen from the skin of pirapitinga (<i>Piaractus brachipomus</i>)	0–3 mg/ml	L292 mouse fibroblast cells	MTT test: 1×10^4 Scratch test: 2×10^5 cells/mL	At the highest concentration, increased % cell viability, and became non-toxic; A remarkable wound closure ability of more than 80% at 12 h and 100% within 24 h.	(Manjushree et al., 2023)
Type I ASC from flounder fish (<i>Paralichthys</i> sp.) skin	25–100%	HFF-1 and L929 cell lines	MTT test (3×10^3 cells/well)	A minimum (70%) of cell viability was presented in the ASC groups for both HFF-1 and L929 cells	(Sousa et al., 2023)
Hydrolysed collagen from salmon skin	0–1000 µg/mL	HaCaT cell lines	MTT assay: 1×10^4 cells/well Scratch test: 3.5×10^5 cells/well	Enhanced proliferation and migration of keratinocytes; Promoted the second phase of wound healing process, and enhanced the KSC properties	(Woonnoi et al., 2021)

Table 6 – contd.

Source	Sample concentration	Cell lines used	In vitro assay	Results	References
1	2	3	4	5	6
Collagen from jellyfish (<i>Rhopilema esculentum</i>)	0–50 mg/mL	HUVECs	Scratch test: 1×10^5 cells	Significant effects on the scratch closure on cells treated with collagen (6.25 mg/mL for 48 h), as compared to the vehicle treated cells.	(Felician et al., 2019)
<i>Arothron stellatus</i> skin collagen scaffold loaded with extract of <i>Coccinia grandis</i> and drug ciprofloxacin	ND	NIH 3T3 fibroblast and HaCaT cell line	MTT test: 5×10^4 cells/mL	A good biocompatibility of the scaffolds was noted owing to the presence of grooved pattern of the collagen matrix with cellular growth and adhesion	(Ramanathan et al., 2017 b)
Collagen from the bones of silver carp (<i>Hypophthalmichthys molitrix</i>)	0.03–1.5 mg/ml	HaCaTs	MTT and Scratch tests: 6×10^4 cells/ml	Collagen could increase cell viability, proliferation and migration on MTT test; A high efficiency in supporting wound healing, especially stimulation of keratinocytes metabolism.	(Iosageanu et al., 2021)
Swim bladder collagen of gurjuba (<i>H. parkeri</i>) incorporated with chitosan	7.5–15,000 µg/mL	NIH-3T3 cell line	MTT test: 5×10^5 cells/well	A high biocompatibility with NIH-3T3 murine fibroblast cells was observed in the collagen product	(Ferreira et al., 2022)
Fish collagen scaffolds (SA*:FCOL:HA)	ND	HDFs and KCs	MTT test (1×10^5 cells/well)	No inhibition in the proliferation of HDFs and KCs after being treated with collagen scaffolds and considered as biocompatible scaffolds.	(Afzali and Boateng, 2022)
Collagen-coated silk fibroin nanofibers	ND	NIH-3T3 cells	MTT assay: 5×10^4 cells/mL	Increased biocompatibility was noted in the collagen coated silk fibroin nanofibre, and fibroblast viability over the uncoated scaffolds.	(Selvaraj et al., 2023)
Polysaccharides from jellyfish (<i>Rhizostoma pulmo</i>)	0.125 mg/mL per well	BALB/3T3 clone A31	MTT test: 1×10^4 cells/well; Scratch test: 1.25×10^5 cells/well	An effective scratch repair in 2 days (80%) was noted in the treated groups, and supporting in the cell migration and proliferation	(Migone et al., 2022)
Fibrous star poly(ε-caprolactone) melt-electrospun scaffolds	ND	BALB/3T3 clone A31 fibroblast cells	WST-1 cell proliferation reagent: 2×10^4 /cm ²	Increased production of collagen shown in the 3T3 fibroblasts, and supported adhesion, proliferation, and spatial organization of cells	(Gazzarri et al., 2013)
Hyaluronic acid/chitosan/bacterial cellulose-based membrane	ND	BALB-3T3 clone A31 cells	MTT method: 10^4 cells/well	Better biocompatibility was observed in the film membranes, and the cell viability was around 94% at 5 days, suggesting a safe material for wound dressing development	(Dechojarassri et al., 2023)
Aloe vera and copaiba oleoresin-loaded chitosan films	ND	Balb/c 3 T3 clone A31 fibroblast cells	MTS test: indirect (1.2×10^4 cells/well) and direct cytotoxicity (5×10^4 cells/well)	Films obtained with either 0.5% chitosan or 0.5% copaiba oleoresin induced cell proliferation which anticipate their potential for closure of wound and for the healing process	(Genesi et al., 2023)

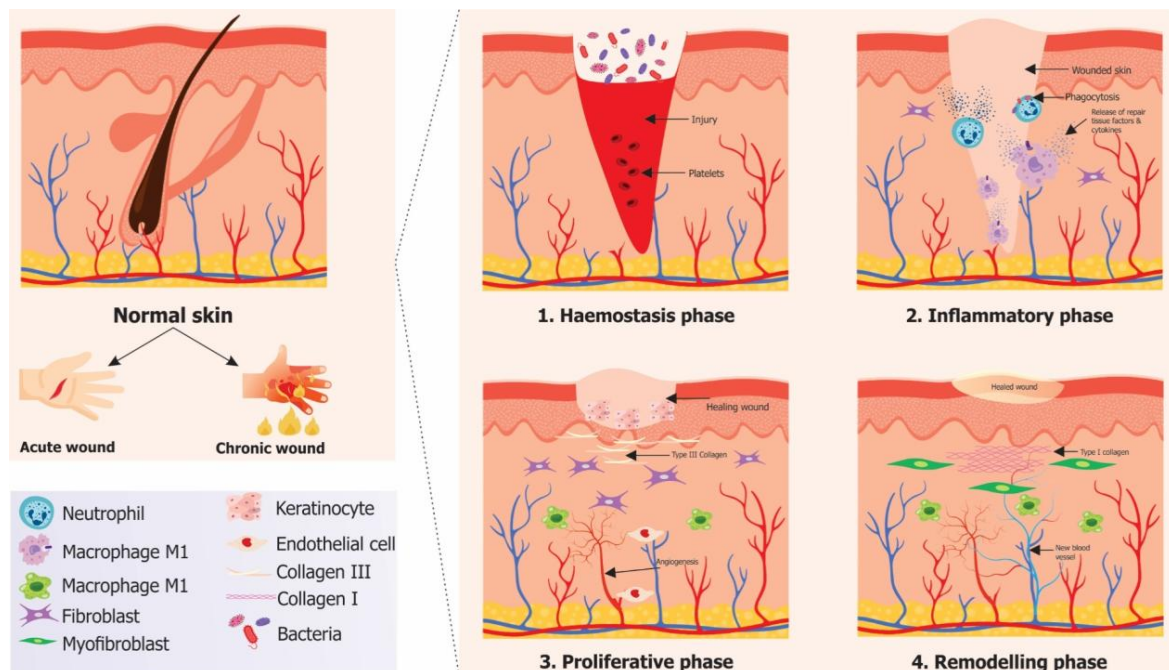


Figure 4. Schematic diagram of four phases in the wound healing process

Latest reports on the wound healing treatment derived from fish collagens

The existing investigations documented that the collagens from the byproducts of aquatic fish sources had potential wound healing agents, as presented in Table 7. The collagen products were widely treated via oral/intragastrical administration in rodent models (rats and mice) in a dose-dependent manner. Zhang et al. (2011) initially observed the collagen from the skin of chum salmon (*Oncorhynchus keta*) administered orally started 1 day before wounding (2 g/kg/day) in male Sprague-Dawley rats (230–250 g) and the results showed twelve percent ($p < 0.01$) increase in the % coverage of wound found in the treated group as compared to the control group (excision wound type), increased fibroblast infiltration, vascularization, epithelialisation and collagen deposition in the wounds treated with collagen peptides (incision type), and strongest upregulation in the VEGF and FGF-2 genes expression. Another finding was reported by Yang et al. (2018), wherein collagen peptides with low-molecular weight from Alaska pollock (*Theragra chalcogramma*) gavaged with collagen samples daily (0.5–2.0 g/kg) in male Sprague-Dawley rats (160–170 g) had faster wound healing rate in the treated group with collagen peptides on day 4–12 post-surgery with a near-normal epidermis structure, increased rates of EGF, bFGF, TGF- β 1 and T β RII, and decreased levels of Smad7 in TGF/Smad signaling pathway. Mei et al. (2020), moreover, updated the collagen from *Salmo salar* and tilapia skins could accelerate significant wound healing, with a complete healing on day 12 post-injury, decrease TNF- α , IL-6 and IL-8, upregulate BD14, NOD2, IL-10, VEGF, and β -FGF, alter cutaneous microbiome colonization through upreg-

ulated NOD2 and BD14 genes expression. The collagen samples were previously prepared through daily oral administration of collagen peptides (2 g/kg body weight) to male Sprague-Dawley rats.

Besides that, some researchers evaluated the wound healing process treated intragastrically by the collagen derived from fish byproducts in mice model. Felician et al. (2019) revealed that smaller wound closure on mice (excisional wound = 8.5 mm²) after 6 days post-injury was obtained from the collagen from jellyfish (*R. esculentum*) administered intragastrically every morning for 6 days (0.3–0.9 g/kg b.w.), resulting in remarkable sign of reepithelialisation, tissue regeneration and increased collagen deposition. Another study from Xiong et al. (2020) reported that tilapia collagen peptide administered via drinking water (15–45 g/L) could enhance wound healing rate after 5 days post-wounding on male C57BL/6 mice, and it could increase collagen deposition, hydroxyproline level, as well as improve IGF-1, FGF2, mRNA expression of growth factors, serum cytokine, NO, SOD and CAT. Yang et al. (2019) also reported that the active peptides with low dose (0.5 g/kg) exhibited a shortened epithelialisation time by inhibiting inflammatory response, enhanced the proliferation of FGF, CD31 and EGF, and increased collagen synthesis via the TGF- β /Smad signalling pathway. Furthermore, latest research by Hou and Chen (2023) confirmed that sturgeon fish (*Acipenser baerii* $\times$ *Huso huso*) skin collagen peptide-based nanoemulsion was the most effective in decreasing fasting blood glucose (46.75%) obtained from the treated group with low-MW and high-dose nanoemulsion, as well as the low-MW and high-dose nanoemulsion also enhanced wound healing area (95.53%) compared to other treated groups.

Table 7. Latest reports on wound healing treatment derived from fish collagens

Source	Animal model used	Type and size of wound	Wound treatment	Findings	References
1	2	3	4	5	6
Collagen peptide from jellyfish (<i>Rhopilema esculentum</i>)	Male mice (26 g)	Excision wound (d = 8.5 mm ²)	Collagen peptide administered intragastrically every morning for 6 days (0.3–0.9 g/kg b.w.)	Smaller wound closure on mice after 6 days post-injury was obtained from the collagen from jellyfish; showed remarkable sign of re-epithelialisation, tissue regeneration and increased collagen deposition; Significantly increased levels of β -FGF and TGF- β expression.	(Felician et al., 2019)
Sturgeon fish (<i>Acipenser baerii</i> × <i>Huso huso</i>) skin collagen peptide-based nanoemulsion	Male mice (8-week-old)	Excision wound (d = 0.85 cm ²)	Tube feeding initiated at day 2 to day 14 post-traumatic (100 and 300 mg/kg/day)	Most effective in declining fasting blood glucose (46.75%) obtained from the treated group with low-MW and high-dose nanoemulsion; The low-MW and high-dose nanoemulsion also enhanced wound healing area (95.53%) compared to other treated groups.	(Hou and Chen, 2023)
Collagen sponge from the swim bladders of giant croaker (<i>N. japonica</i>)	Male ICR mice (6–8 weeks old, 22–24 g)	Excision wound model (d = 8 mm ²)	Collagen sponge dressing	Quicker wound closure in the wounds treated with collagen sponges compared to the control group; significantly decreased the levels of IL-1 β , IL-6 and TNF- α	(Chen et al., 2019)
Type II collagen from cartilage of <i>Acipenser baerii</i>	Male C57BL/6J mice (8–10 weeks old)	Excision wound model (d = 8 mm ²)	Wound dressing, the dressings were changed every 2 days	Accelerated wound healing, associated with reducing inflammation, increasing granulation, tissue formation and collagen deposition; Upregulated the production of growth factors.	(Lai et al., 2020)
Tilapia collagen peptide mixture TY001	Male C57BL/6 mice (3 months old, 25 g)	Excision wound model (d = 8 mm ²)	Administered via drinking water (15–45 g/L)	Enhanced wound healing rate after 5 days post-wounding; Increased collagen deposition and Hyp level; Improved IGF-1, FGF2, mRNA expression of growth factors, serum cytokine, NO, SOD and CAT.	(Xiong et al., 2020)
Swim bladder collagen from sea eel (<i>Muraenesox cinereus</i>)	Male ICR mice (22–24 g)	Excision wound model (1 cm ²)	Collagen sponge dressing	Rapid wound healing was exhibited in the treated group; Enhanced activities of SOD, CAT, T-AOC and GSH-Px; Decreased the levels of MDA, IL-1 β , IL-6, and TNF- α ; Prevented scar formation in the later stage.	(Li et al., 2023)
Hydrolysed collagen from the skin of chum salmon (<i>Oncorhynchus keta</i>)	Male Sprague-Dawley rats (230–250 g)	Excision wound model (d = 2 cm ²) and incision wound model (two 4-cm long, parallel)	Administered orally started 1 day before wounding (2 g/kg/day)	Twelve percent ($p < 0.01$) increase in the % coverage of wound found in the treated group as compared to the control group (excision wound type); Increased fibroblast infiltration, vascularisation, epithelialisation, and collagen deposition in the wound treated with collagen peptides (incision type); Strongest upregulation in the VEGF and FGF-2 genes expression.	(Zhang et al., 2011)
<i>Pinctada martensii</i> active peptides	Mice	Full cortical wound (d = 0.8 cm ²)	Gavaged with samples daily (0.5–2.0 g/kg b.w.)	The active peptides with low dose (0.5 g/kg b.w.) exhibited a shortened epithelialisation time by inhibiting inflammatory response; Enhanced the proliferation of FGF, CD31 and EGF; Increased collagen synthesis via the TGF- β /Smad signalling pathway; Inhibited scar formation and improved healing quality.	(Yang et al., 2019)
Collagen peptides from the skin of chum salmon (<i>Oncorhynchus keta</i>)	Sprague-Dawley rats	Incision wound (2.0 cm ²)	Administered intragastrically every morning after the surgery day (0–1.15 g/kg b.w.)	Higher values of the skin tensile strength, uterine bursting pressure, and Hyp in the collagen treated group; Increased formation of capillary, fibroblast, and collagen fibre; Enhanced TGF- β 1, bFGF, and CD31 expression.	(Wang et al., 2015)
Collagen peptides with low MW from Alaska pollock (<i>Theragra chalcogramma</i>)	Male Sprague-Dawley rats (160–170 g)	Excision wound model (d = 1 cm ²) and incision wound model (2 cm long)	Gavaged with samples daily (0.5–2.0 g/kg b.w.)	Faster wound healing rate was recorded in the treated group with collagen peptides on day 4–12 post-surgery; Presented a near-normal epidermis structure; Increased the rates of EGF, bFGF, TGF- β 1 and T β RII; Decreased the levels of Smad7 in TGF/Smad signalling pathway.	(Yang et al., 2018)

Table 7 – contd.

1	2	3	4	5	6
Collagen peptides from <i>Salmo salar</i> and tilapia skin	Male Sprague-Dawley rats (150–170 g)	Incision wound model (4.0 cm ²) and excision wound (1.0 cm ²)	Daily oral administration with collagen peptides (2 g/kg b.w.)	Accelerated significantly wound healing, with a complete healed on day 12 post-injury; Decreased TNF- α , IL-6 and IL-8; Showed upregulated BD14, NOD2, IL-10, VEGF, and β -FGF; Altered cutaneous microbiome colonization through upregulated NOD2 and BD14.	(Mei et al., 2020)
Biomimetic tilapia skin nanofibres	Male Sprague-Dawley rats (6–8 weeks old; 200–250 g)	Full-thickness skin/excision wound (d = 1.8 cm ²)	The wound area was covered with tilapia collagen nanofibres as a wound dressing for 14 days	Complete healing of the wound observed in the treated collagen nanofibres after 14 days; The lowest level of inflammatory response in the treated group; Enhanced the growth of new epidermis throughout the wounded skin.	(Zhou et al., 2017)
Starry puffer (<i>Arothron stellatus</i>) skin collagen coated nanofibrous scaffold	Wistar albino male rats (180–220 g)	Open excision-type wound (2 $\times$ 2 cm ²)	Dressing the wound after wounding, and changed periodically at an interval of 4, 8 and 12 days	The collagen coated nanofibrous scaffold with addition of <i>Coccinia grandis</i> extract showed with rapid wound closure after 16 days of injury; Increased levels of Hyp, hexosamine and uronic acid; Higher expression of VEGF, EGF, and TGF- β .	(Ramanathan et al., 2017 a)
Starry puffer (<i>Arothron stellatus</i>) skin collagen film incorporated with <i>Coccinia grandis</i>	Wistar albino male rats (200–220 g)	Full thickness excision wound (2 $\times$ 2 cm ²)	The wounds were dressed at day 0, and changed periodically at an interval of 4 days	A faster healing was detected in the treated group after 8th day up to the complete healing (at day 18); Increased collagen synthesis and re-epithelialisation; The highest expression levels of EGF, VEGF and TGF- β on day 4–8 in the treated group.	(Ramanathan et al., 2017 b)
Biocomposite of fish scale collagen and fibrin with <i>Macrophyloma uniflorum</i>	Wistar albino male rats (180–200 g)	Full thickness excision wound (2 $\times$ 2 cm ²)	Wound dressing was replaced at an interval of 4 days	Complete healing was recorded at day 13 post-injury and considered faster compared to other samples; Enhanced expression levels of growth factors (i.e., VEGF, EGF, FGF, and TGF- β); Increase collagen synthesis and down regulated MMPs.	(Muthukumar et al., 2014 a)
Collagen sponge of mrigal fish (<i>Cirrhinus cirrhosus</i>) scale	Wistar rats (200 g)	Excision wound (2 $\times$ 2 cm (4 cm ²))	Wound dressing	Significant reduction in wound area was observed in the collagen-treated group (day 10 = 80.51%; day 15 = 98.97%); Especially at day 10, a new epithelium layer with well-grown hairs was noted in the treated group.	(Pal et al., 2016)
Nile tilapia (<i>Oreochromis niloticus</i> L.) skin collagen	Wistar male rats (110 g)	Full thickness skin wound excision (1.5 $\times$ 1.5 cm)	Wound covered with dressing, and changed every 2 days	Smaller wound areas on days 9, 12 and 15 post-wounding were noted in the Nile tilapia skin collagen-treated group. The adjacent skin of rats treated by the collagen gels became smoother compared to the control. Upregulation in the bFGF, VEGF and α -SME genes expression.	(Elbially et al., 2020)
Collagen-chitosan from <i>Scomberomorus guttatus</i> and shrimp skin	Male Sprague-Dawley rats (300–350 g)	A second degree of burn injury (2 $\times$ 4 cm ²)	Hydrogel dressing	A significant reduction in wound size in the group treated with collagen-chitosan (3:1) compared to silver sulfadiazine treated group on days 15 and 25	(Fatemi et al., 2021)
Hydrolysed collagen from Nile tilapia (<i>Oreochromis niloticus</i>) skin rabbits	New Zealand White rabbits	Deep partial-thickness scald model (d = 4 cm ²)	Hydrolysed collagen treated once daily for 4 weeks	Wound treated with collagen was almost healed (95.9%) after 21 days, while the control group was 72.1% healing rate.	(Hu et al., 2017)

Table 7 – contd.

1	2	3	4	5	6
Tilapia scale collagen nanoparticles	Rabbits (5–6 months old; 1.5–2 kg)	Full thickness opens wound excision (d = 2.5 cm ²)	Dressing gel of collagen nanoparticles	Effective sealing was noted after postoperative days 7 to 14; Better and quicker re-epithelisation, compared to the control and positive groups; Remarkably decreased exudation, inflammation, and microbial contamination	(Shalaby et al., 2023)
Collagen sponge from small-spotted catshark (<i>Scyliorhinus canicula</i>) incorporated with plant extract	BALB/c mice	Excisional wound model (d = 6 mm ²)	Collagen sponge dressing	Increased significantly healing rate in the wounds treated with sponge collagen scaffolds plus plant extracts after 15 days post-injury.	(Lahmar et al., 2022)

Unless oral gavage approach, collagens from the by-products of aquatic species were also applied in the form of wound dressings, including sponge, film, nanofiber, and hydrogel dressings. For instance, Lin et al. (2019) observed collagen sponges from the swim bladders of giant croaker (*Nibea japonica*) had quicker wound closure in the wounds compared to the control group (untreated sample), and the sponges significantly decreased the levels of IL-1 β , IL-6 and TNF- α in male ICR mice (6–8 weeks old, 22–24 g). Similarly, Li et al. (2023) used swim bladder collagen from sea eel (*Muraenesox cinereus*) as a sponge dressing in the wounded male ICR mice (excision wound model = 10 mm²), and the results show rapid wound healing was exhibited in the treated group and prevented scar formation in the later stage. Another example using type II collagen from the cartilage of *Acipenser baerii*, the findings showed that the wounded male C57BL/6J mice (8–10 weeks old) were repaired after treated by the sponge dressing. This treatment was associated with reduced inflammation, increasing granulation, tissue formation and collagen deposition, upregulating the production of growth factors (Lai et al., 2020). In terms of film dressing, Ramanathan et al. (2017 b) explored starry puffer (*Arothron stellatus*) skin collagen film incorporated with *Coccinia grandis* had a faster healing of the wounded Wistar albino male rats (2 $\times$ 2 cm²) that was detected in the treated group after 8th day up to the complete healing (at day 18). Besides film dressing, Fatemi et al. (2021) studied the collagen-chitosan from *Scomberomorus guttatus* and shrimp exhibited a significant reduction in wound size of wounded Sprague-Dawley rats compared to silver sulfadiazine treated group on days 15 and 25.

Challenges with wound healing applications of fish collagen

The application of fish collagen in wound healing presents several challenges in both *in vitro* and *in vivo* models. *In vitro*, ensuring consistent cellular compatibility is crucial, as variations in collagen composition can affect cellular attachment, proliferation, and differentiation. Standardizing extraction methods to produce uniform collagen quality is essential for reproducible results. Additionally, fish collagen's susceptibility to enzymatic degradation can impact the longevity and efficacy of collagen-based scaffolds and dressings. Maintaining the biological activity of extracted collagen, crucial for promoting cell signalling pathways involved in wound healing, is another significant challenge. Furthermore, developing scaffolds that mimic the natural extracellular matrix while maintaining structural integrity and porosity suitable for cell infiltration and nutrient exchange adds to the complexity. In the case of *in vivo* viewpoints, fish collagen can still elicit immune responses, necessitating understanding and mitigating potential immunogenic reactions. The mechanical properties of fish collagen-based materials must match the wound environment's requirements, ensuring adequate tensile strength and elasticity

to withstand physiological stresses. Effective integration with host tissue is necessary for successful wound healing, as poor integration can lead to scaffold rejection or inadequate tissue regeneration. Controlling the degradation kinetics of fish collagen *in vivo* is also crucial; rapid degradation can result in premature loss of structural support, while slow degradation can impede tissue remodelling. Additionally, achieving regulatory approval involves demonstrating safety and efficacy through comprehensive preclinical and clinical studies. The variability in collagen quality due to different fish sources, the high cost of production, and the need for scalable methods further complicate the widespread adoption of fish collagen in wound healing applications.

Conclusions and future perspective

Fish collagen has significant promise as a wound healing agent due to its biocompatibility proven scientifically through *in vitro* and *in vivo* studies. Despite these advantages, challenges such as standardizing extraction processes, ensuring consistent biological activity, and addressing immune responses and mechanical property requirements *in vivo* persist. To fully harness its potential, future research should optimize extraction and purification methods, advance scaffold fabrication techniques, and conduct extensive studies to understand biological interactions and degradation kinetics better. Collaborative efforts between researchers, industry stakeholders, and regulatory bodies will be essential for regulatory approval and commercialization. Exploring genetic engineering and biotechnology to enhance fish collagen's performance, alongside sustainable sourcing and cost-effective production methods, will further support its viability. By overcoming these challenges and leveraging its unique advantages, fish collagen can play a crucial role in developing advanced wound healing therapies, leading to improved patient outcomes and more sustainable healthcare practices.

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