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A review on the processing technique, physicochemical, and bioactive properties of marine collagen

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Norizah Mhd Sarbon, Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. Email: norizah@umt.edu.my **Abstract:** Collagens are conventionally derived from bovine and porcine sources. However, these sources were commonly associated with infectious diseases such as bovine spongiform encephalopathy, foot and mouth disease, autoimmune and allergic reactions, and religious constraints. The significant amount of collagen available in marine species, especially fish skins, scales, fins, and bones, shows that marine species can be a potential alternative source to mammalian collagen. Therefore, this review aims to give a clearer outlook on the processing techniques of marine collagen and its physicochemical and bioactive properties as a potential alternative to mammalian collagen. The two most suitable extraction methods for marine collagen are pepsin-soluble extraction and ultrasound-assisted extraction. Additionally, marine collagen's physicochemical and bioactive properties, such as antioxidants, wound healing, tissue engineering, and cosmetic biomaterial have been thoroughly discussed in this review.

KEYWORDS

antioxidants, collagen extraction, marine collagen, tissue engineering, wound healing

Practical Application: Collagen extracted from marine sources showed its potential in physicochemical and bioactive properties, including antioxidants and wound-healing capabilities, as an alternative to mammalian collagen. The significant amount of collagen found in marine species, particularly in fish skins, scales, bones, and sea cucumbers, suggests that marine sources could be a viable alternative to land mammal collagen due to their abundance and accessibility. The ultrasound-assisted extraction technique has improved the extracted marine collagen's physicochemical and bioactivity properties and quality properties.

1 | INTRODUCTION

Collagen is a type of fibrous protein and connective tissue that predominates in animal connective tissue and seems to be present in different forms in tissues of all mul-

ticellular species (Schmidt et al., 2016). Within the body, fibroblast cells spontaneously create collagen. However, as people age and because of bad lifestyle choices, their body's capacity to make collagen decreases. Therefore, collagen from other sources is necessary (Isnaini et al., 2024). It is

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vital in providing structural integrity, strength, and support to various tissues and organs, such as skin, bones, tendons, ligaments, cartilage, and blood vessels (Furtado et al., 2022). Collagen constitutes approximately 25%—30% of the overall protein content for most species, and it performs different functions depending on its position (Arumugam et al., 2018).

Over the years, the global demand for collagen has evolved. Collagen has wide industrial applications, including pharmaceuticals, food industries, cosmetics, and biomedicine, and thus, it is known to be one of the essential biomaterials (Lim et al., 2019; Xu et al., 2021). However, collagen from mammalian sources carries the risk of transmission of zoonotic diseases such as BSE, infectious spongiform encephalopathy (TSE), foot and mouth disease (FMD), and autoimmune and allergic reactions (Jafari et al., 2020; Salvatore et al., 2020). However, there are outbreaks and religious issues associated with these sources. Besides, collagen extraction from poultry slaughter waste has also been investigated, but with less emphasis due to the possibility of avian influenza transmission and the possibility of disease transmission to humans, such as BSE (Schmidt et al., 2016). Due to these issues, the study of marine animals has increased, ranging from various fish species to other marine-derived organisms such as jellyfish, sponges, and mollusks, in light of the growing need for collagen-based treatment techniques and the scarcity of safe supplies of collagen (Diogo et al., 2024).

Therefore, researchers have now sought new sources of collagen to address these concerns. Marine species appeared as potential means due to their accessibility, lack of religious issues, and the prospect of high collagen yields, less likely to spread, are biocompatible, have a lower molecular weight, are less expensive to produce, and are more accessible for the human body to absorb, they provide a viable and sustainable source of alternative collagen (Hadfi & Sarbon, 2019; Prajaputra et al., 2024; Thuy et al., 2014). There is also no evidence of potentially transmissible diseases (Coppola et al., 2020). Additionally, fish waste and some by-catch species, such as small fish, jellyfish, sea cucumber, squid, starfish, and sponges, have been reported to have a significant amount of collagen (Coppola et al., 2020; Sulaiman & Sarbon, 2020). Interestingly, fish collagen is reported to have similar attributes to porcine collagen (Bhagwat & Dandge, 2016), thus making it a potential alternative to mammalian collagen. Generally, fish skin, scales, bones, and fins are abundant in type I collagen, the main structural protein in vertebrates (Bhuimbar et al., 2019). Researchers have extracted collagen from many different marine sources, including the skin of squid (Doryteuthis singhalensis) (Veeruraj et al., 2015), sea cucumber (Shaik et al., 2024; Zhong et al.,

2015), waste materials of fringescale sardinella (*Sardinella fimbriata*) (Hamdan & Sarbon, 2019), and shortfin scad (*Decapterus macrosoma*) (Baderi & Sarbon, 2019).

The extractability of collagen is a measure of the degree to which collagen can be extracted using a specific solvent or method. It highly depends on the type and concentration of solvent used (Bhuimbar et al., 2019; Hadfi & Sarbon, 2019), the extraction method (Ali et al., 2018), the pretreatments applied (Xu et al., 2017), and the source of collagen itself. Marine collagen is typically extracted using acid-soluble collagen (ASC), pepsin-soluble collagen (PSC), and ultrasound-assisted extraction methods.

In addition, the functional properties of collagen are greatly affected by the source from which it is extracted, its molecular structure, molecular weight, and processing conditions (León-López et al., 2019). Interestingly, collagen is known to have a great water absorption capacity, thus making it an ideal component for texturizing, thickening, and gel formation (Felician et al., 2018). Moreover, collagen provides properties associated with its surface behavior, such as emulsifying properties, foam formation, stabilization properties, adhesion and cohesion, and film-forming capacity (Felician et al., 2018). Furthermore, collagen is a great surface-active agent and can penetrate lipid-free boundaries (Ridzwan & Hashim, 2015). However, the use of collagen in the industrial sector commonly depends on the collagen's thermal stability. Unfortunately, marine collagen exhibits a relatively low denaturation temperature, typically ranging from 26.3°C to 35.9°C (Jafari et al., 2020). This lower temperature threshold is associated with the natural habitat of marine species and subsequently imposes limitations on its suitability for use in biomaterials (Hayashi, 2020).

Furthermore, collagen is known to be a promising biomaterial since it also exhibits excellent bioactive properties. "Bioactive" is an alternative to "biologically active" (Ishak & Sarbon, 2018). A bioactive material is a material that somehow affects and induces a reaction or response in living cells, which includes promoting bone formation, promoting or inhibiting cell adhesion in soft tissue, modulating inflammation, and promoting wound healing (Williams, 2022). Previous studies reported that marine collagen's biological properties and amino acid composition are analogous to mammalian collagen and thus suitable to substitute mammalian or human collagen in particular biomedical applications (Lim et al., 2019).

This paper presents a comprehensive review of the possible sources of marine collagen, advancement in extraction processing techniques and its limitations, and properties as a potential alternative to mammalian collagen. It includes a detailed synthesis of marine collagen obtained through various recent extraction methods and a review of

chemical, physical, and bioactive properties from the latest findings.

COLLAGEN

2.1 | Structure of collagen

The basic structural unit of a collagen molecule is composed of three α -helical polypeptide chains that consist of repeating triplets of glycine and two other amino acids, typically proline or hydroxyproline (Coppola et al., 2020). Currently, 28 distinct types of collagens have been identified and classified from type I to type XXVIII based on the order of their discovery (Bou-Gharios et al., 2020). Despite sharing a standard triple-helical structure, these collagen types differ significantly in molecular composition, amino acid composition, and their organization at the molecular and supramolecular levels. These polypeptide chains contain about 1000 amino acids and a molecular weight of around 100 kDa in each chain (Liu et al., 2015a). These chains are arranged into primary, secondary, and tertiary structures, eventually forming fibrils with a unique shape (Coppola et al., 2020). Each collagen molecule is approximately 360 kDa and 28 mm long, and the average molecular weight of collagen is lower than that of other proteins due to the high glycine content, known as the minor amino acid (Liu et al., 2015a; Schmidt et al., 2016). The structure of collagen molecules can be affected by extraction methods, the solvent used, and the source of collagen (Akram & Zhang, 2020).

Moreover, many studies infer that the pepsin hydrolysis method does not affect the secondary structure of the collagen compared with the acid hydrolysis method (Ali et al., 2018). However, all collagen has the same triple-helical structure in the extracellular matrix, but it varies in length, size, function, and distribution of the nonhelical component (Silvipriya et al., 2015). A detailed review of collagen and collagen-like structural proteins from sea sponges was published by Ehrlich et al. (2018). The structural properties of collagen have a direct impact on its functional properties, including strength, stability, solubility, viscosity, and foaming properties. Moreover, the collagen structurefunction relationship refers to the connection between the molecular structure of collagen and its various biological functions and roles within the body. The collagen structure is highly organized. It consists of three polypeptide chains, known as alpha chains, that are tightly twisted to form a triple helix. Each alpha chain is a long sequence of amino acids, primarily glycine, proline, and hydroxyproline, contributing to the distinctive triple-helical structure through their specific arrangements and interactions (Ricard-Blum, 2011). The specific arrangement of amino acids, the formation of triple helices, enzymatic crosslinking, and interactions with other molecules play pivotal roles in determining collagen's mechanical properties, tissue-specific functions, cell signaling, and overall contribution to structural integrity and functionality (Tang et al., 2022).

2.2 Sources of marine collagen

2.2.1 Marine vertebrates

Marine vertebrates, such as fish, are marine creatures with a backbone or spinal column. Marine organisms have been described as the safest and most beneficial alternative resources due to their large quantity, lack of religious restrictions, absence of outbreak risk, and high collagen yields (Ali et al., 2018). Moreover, collagen can be collected from the fish's skins, scales, fins, and bones (Hukmi & Sarbon, 2018). Besides fish, marine collagen can also be extracted from marine reptiles (turtles, crocodiles, etc.) and marine mammals (otters, manatees, whales, etc.). However, until recently, only a few marine vertebrate species, apart from fish, had been studied for collagen extraction: turtles (Yang et al., 2016; Zou et al., 2017b) and crocodiles (Szewczyk & Stachewicz, 2020).

The extraction of collagen from fish is much less complicated, inexpensive, and time-saving than the extraction of collagen from land-origin species (Silvipriya et al., 2015). Based on most findings, collagen extracted from marine vertebrates is mainly known as collagen type I. Type I collagen, the most prevalent subtype, constitutes approximately 70% of the entire collagen family and is abundantly found in connective tissues such as bones, skin, tendons, ligaments, cornea, and blood vessels (Salvatore et al., 2020). Collagen used in the industry is known to be collagen type I and is commonly derived from the bones and skins of mammals, particularly porcine and bovine (Jafari et al., 2020). Collagen type I is derived from most species' skins, tendons, bones, and muscles, whereas type II is from fish cartilage (Benjakul et al., 2012). Countless marine fish have been explored for collagen extraction, including sea bass (Lateolabrax japonicas) (Kim et al., 2012), lizard fish (Saurida spp.) (Jaziri et al., 2022), yellowback seabream (Dentex tumifrons) (Thuy et al., 2014), fringescale sardinella (Hamdan & Sarbon, 2019), clown featherback (Petcharat et al., 2021), amur sturgeon (Zhang et al., 2019), stingray (Ong et al., 2021; Shaik et al., 2021a), and more.

Marine invertebrates 2.2.2

Marine invertebrates are marine creatures that do not have a backbone, such as sea stars, sea urchins, corals, sea cucumbers, jellyfish, and squids. Even though marine

invertebrate species are widespread in the animal kingdom, there are fewer documented findings regarding collagen extraction from these sources compared with marine vertebrates. Moreover, marine invertebrates' collagen is challenging to purify due to its species-dependent complexity and, therefore, becomes a limitation for collagen extraction from these sources (Ehrlich et al., 2018). Until recent times, collagen from marine invertebrates has been extracted from marine sponges (12.6%) (Tziveleka et al., 2017), coral (20%) (Shelah et al., 2021), octopus (1.57%) (Tapia-Vasquez et al., 2020), squid (3.26%) (Coelho et al., 2017; Dai et al., 2018), sea urchins (7%) (Di Benedetto et al., 2014; Ferrario et al., 2020), sea cucumber (Li et al., 2020b; Shaik et al., 2024; Zhong et al., 2015) (72.2%), sea star (2.26%) (Wijanarko et al., 2017), and jellyfish (4.31%) (Felician et al., 2019). However, among most species of marine invertebrates documented, only sea cucumber exhibits a high collagen content, which is more than 70% of collagen (Li et al., 2020b).

In addition, collagens derived from marine invertebrates provide various types of collagens, including type I (coral, sea cucumber, sea urchin, and squid), type II (coral, squid, jellyfish, and octopus), and type IV (sea sponge), depending on the source. Ehrlich et al. (2018) outlined the structural diversity, properties, and application of collagen and collagen-like structural proteins from sponges in biomedical, material science, and technology. In addition, the morphological, biochemical, and biophysical properties of collagen extracted from marine sponges, particularly Axinella cannabina and Suberites carnosus, have been characterized by Tziveleka et al. (2017). Marine collagen is considered a safer and alternative biomaterial than terrestrial sources of collagen. Previously, a study also found a unique collagen fiber from soft coral with hyperelastic behavior similar to human tissue (Orgel et al., 2017; Shelah et al., 2021).

2.3 | Extraction technique of marine collagen

Collagen can be extracted through complex procedures, beginning with preparing raw materials, pretreatment, extraction, purification, and more steps (Figure 1). Generally, there are two ways of obtaining collagen: extraction from animal tissue or artificial synthesis from chemical or biological substances (Yang & Shu, 2014). However, researchers were more engaged in extracting collagen from animal tissue than synthesizing it. As a result, the significant portions of collagen proteins available in the industry come primarily from animal tissues. The commonly used methods for collagen extractions are the neutral-salt-soluble collagen extraction method (also known as the

salting-out method), the ASC extraction method, and the PSC extraction method (Hadfi & Sarbon, 2019). In general, both organic acids (acetic acid, chloroacetic acid, citric acid, and lactic acid) and inorganic acids (hydrochloric acid) can be used in the acid extraction method (Hukmi & Sarbon, 2018). However, 0.5 M acetic acid is the most commonly used solvent for extracting ASC due to its higher yield and affordability. Besides pepsin, papain enzymes are also applicable to extracting collagen (Hadfi & Sarbon, 2019; Ran & Wang, 2014; Wahyuningsih et al., 2018).

Moreover, studies on ultrasound-assisted collagen extraction methods are drawing more attention from researchers due to the higher yield and time savings (Ali et al., 2018; Shaik et al., 2021b). According to Josephin et al. (2024), ultrasonication can also help shorten the salt extraction process. The yield percentages of extracted collagen have commonly defined the extractability of collagen. The extractability of collagen is highly dependent on the type (Bhuimbar et al., 2019) and concentration of solvent used (Hadfi & Sarbon, 2019), the extraction method applied (Baderi & Sarbon, 2019), the source (raw material and tissue source) (Ahmed et al., 2019), and the extraction period (Ali et al., 2018). However, due to the extreme diversity of collagen types, it is challenging to develop a standard extraction method for all types of collagens from various tissues (El Blidi et al., 2021).

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2.3.1 | Preparation and pretreatment of raw material

Preparation usually involves cleaning, isolation of animal components, and reduction of size. A reduction in raw material size effectively facilitates the removal of contaminants and the extraction of collagen (Silva et al., 2014). The raw material for collagen extraction typically contains a variety of contaminants, such as non-collagen proteins, ashes, lipids, and pigments, which will affect collagen extractability (Szewczyk & Stachewicz, 2020). Therefore, one or multiple pretreatments, such as removal of non-collagenous protein, demineralization, and defatting, are applied to eliminate contaminants, boost purity, and improve the yield of the extracted collagen. Despite their species, these preparations and pretreatment steps are practical for all collagen sources.

Removal of non-collagenous protein

Removal of non-collagenous proteins (albumins, globulins, and glycoproteins) can be achieved by applying neutral salt (NaCl) (Silva et al., 2014) and alkaline and acid pretreatments (Schmidt et al., 2016). The acidic pretreatment using hydrochloric acid (HCl) was more compatible with raw materials with fewer entangled collagen fibers,

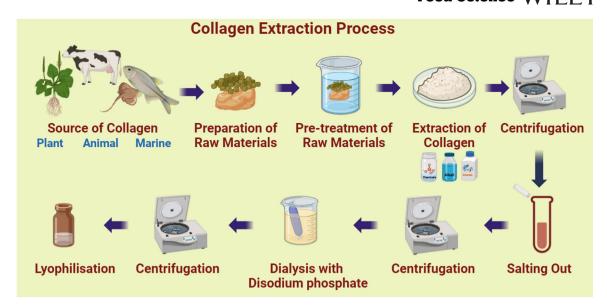


FIGURE 1 Collagen extraction process.

such as pig and fish skins (Schmidt et al., 2016). However, based on various studies, researchers were more engaged in alkaline pretreatments than acidic pretreatments due to better efficiency. Sodium hydroxide (NaOH) and calcium hydroxide (Ca (OH)₂) are the practical solutions for alkaline pretreatment (Schmidt et al., 2016). Even so, many researchers have been found to use a 0.1 M NaOH solution to remove non-collagenous proteins before collagen extraction. These were because NaOH is better at inducing substantial swelling, which enhances collagen extraction by improving the transfer rate of mass in the tissue matrix (Schmidt et al., 2016).

NaOH solution at 0.1 M concentration has been used to remove non-collagenous protein from Shortfin Scad (Decapterus macrosoma) waste materials (Sulaiman & Sarbon, 2020), Miiuy Croaker (Miichthys miiuy) scales (Li et al., 2018), squid outer skin (Doryteuthis singhalensis) (Veeruraj et al., 2015), and Hybrid Sturgeon skin (Wei et al., 2019). A study indicated that pretreatment with NaOH at 0.05 to 0.1 M efficiently removes non-collagen protein without disrupting ASC at temperatures 4°C, 10°C, 15°C, and 20°C (Liu et al., 2015b). In contrast, pretreatment with NaOH at higher concentrations of 0.2-0.5 M resulted in substantial losses to ASC, whereas pretreatment with 0.5 M NaOH caused structural changes to ASC at temperatures of 15°C and 20°C (Liu et al., 2015b). Additionally, applying a NaOH solution with a 0.3 M concentration has been documented to remove non-collagenous protein from the skin of sole fish (Arumugam et al., 2018). A 0.1 M NaOH solution is the most preferred solvent for removing non-collagenous protein from raw material before collagen extraction to improve yield and boost collagen purity.

Demineralization

Demineralization is the process of removing mineral salts, such as calcium, from raw materials. Raw materials with significant mineral content, such as bone, cartilage, and scales, usually need to undergo this process to boost collagen extraction efficacy and purity (Jafari et al., 2020). In addition, the demineralized raw material will exhibit a porous structure with an improved surface area, facilitating the collagen extraction process (Silva et al., 2014). Ethylenediaminetetraacetic acid (EDTA) (Hadfi & Sarbon, 2019), hydrochloric acid (HCl), and acetic acid (Silvipriya et al., 2015) are applicable to remove the minerals from the sample. Most researchers were found to be using a 0.5 M EDTA solution as the demineralization solvent. Due to its chelating function, EDTA is efficient in calcium or mineral removal (Josephin et al., 2024; Meyer, 2019). A study reported that a 0.5 M EDTA solution has been used to demineralize Shortfin Scad (Decapterus macrosoma) waste material (Sulaiman & Sarbon, 2020), Fringescale Sardinella (Sardinella fimbriata) waste material (Hamdan & Sarbon, 2019), squid cartilage (Dai et al., 2018), and Miiuy Croaker (Miichthys miiuy) scales (Li et al., 2018).

Defatting

The defatting process is a method of fat removal from raw material with high fat content, which can be performed by soaking the raw material in an alcohol solution. Therefore, it is vital to perform a defatting process to obtain high-purity collagen. For example, it was reported that collagen extracted from shortfin scad waste material possesses a more significant amount of fat (0.38%) as compared with commercial collagen (0.04%) due to the absence of a

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defatting process (Sulaiman & Sarbon, 2020). Butyl alcohol, ethanol, hexane, and acetone are suitable solvents for this process.

However, several studies have reported the use of 10% butyl alcohol and ethanol for the defatting process. Approximately 10% butyl alcohol has been employed to remove fat from the skin of puffer fish (Lagocephalus inermis) (Iswariya et al., 2018), hybrid sturgeon skin (Wei et al., 2019), and the outer skin of squid (Doryteuthis singhalensis) (Veeruraj et al., 2015). However, the material-to-solvent ratio (typically 1:10) and treatment period vary across these studies. Another documented example involves 20% butyl alcohol on sole fish skin (Arumugam et al., 2018). Moreover, ethanol has frequently been utilized in various concentrations for fat removal from different sources. The study reported that 10% ethanol concentration was employed for removing fat from salmon skin (Alves et al., 2017), whereas 99.5% ethanol concentration was used for Bester sturgeon skin (Zhang et al., 2014). In comparison, 20% ethanol concentration was applied to duck feet (Kim et al., 2016) and quail's (Coturnix japonica) feet (Yousefi et al., 2017). Hexane has been reported to be used in the defatting process for loach skin (Misgurnus anguillicaudatu) (Wang et al., 2018), and Amur sturgeon cartilage (Acipenser schrenckii) (Zhang et al., 2019). Conversely, acetone was used for fat removal from porcine skin (Li et al., 2020b).

2.3.2 | Salting-out method

The salting-out method, also known as salt solubilization extraction, is a widely used approach for the recovery of collagen molecules (Josephin et al., 2024). Adding salt ions to the solution neutralizes the collagen molecules' surface charge, reducing their electrostatic interactions and causing them to precipitate. This process elevates the ionic strength, increasing the affinity of the hydrophobic regions within the protein chains (Hiransuchalert et al., 2021). Standard neutral salt solutions such as NaCl, Tris-HCl, phosphate, citrate, or alkali solutions are employed in this method (Noorzai & Verbeek, 2021). The collagen extracted from bigeye tuna (Thunnus obesus) skins, the salting-out method with NaCl (1.5 M), resulted in a yield of 14.14% (Lin et al., 2019a). Another study on collagen extracted from the heads of Atlantic cod (Gadus morhua) and Pollock (Pollachius virens) showed low yields of 1.9% and 2.3%, respectively (Grefstad, 2022). Despite being regarded as the least preferred collagen extraction method, different types of collagens can be separated using the relationship between the salt concentration and collagen sources (Noorzai & Verbeek, 2021).

2.3.3 | ASC extraction process

Collagen extracted using an acid solution is known as ASC. In general, either organic (acetic acid, chloroacetic acid, citric acid, and lactic acid) or inorganic acid (hydrochloric acid) can be used in the acid extraction method (Sukeri et al., 2021). Among these acids, acetic acid with a concentration of 0.5 M has been widely employed in research for extracting collagen from marine organisms, primarily due to its high extractability. Acetic acid accelerates the hydrolysis process in the acid method by disrupting salt bonds and Schiff bases between molecules, leading to the expansion and dissolution of collagen fibers while also breaking the hydrogen bonds that initially stabilize the triple helix structure of collagen (Isnaini et al., 2024). It was found that the concentration, type, and pH value of the acid used affect collagen extractability. The extraction of the ASC process involves denaturing collagen fibrils and matrix components due to acidity, causing tissue swelling and easier access to collagen fibers. Acid breaks down collagen via hydrolysis, creating soluble gelatin fragments. Adjusting pH or adding salts makes these fragments precipitate, allowing separation through centrifugation or filtration. This method uses acidic conditions to extract and recover collagen for various uses (Oslan et al., 2022).

A study reported that collagen yield increased gradually with the increase in acetic acid concentration. The optimum collagen output (19.27%) was observed at a concentration of 0.54 M and then declined when the acetic acid concentration exceeded 0.6 M (Arumugam et al., 2018). In contrast, Baderi and Sarbon (2019) found that the yield of extracted collagen from shortfin scad (*Decapterus macrosoma*) bone using 0.7 M acetic acid was comparatively higher (1.31%) than that of extracted collagen using 0.5 M acetic acid (1.01%). However, in Sukeri et al. (2021), the yield of collagen extracted from cobia (*Rachycentron canadum*) skin using a higher concentration of lactic acid (1.0 M) was found lower (22.23%) than the collagen extracted using 0.5 M lactic acid (36.70%).

Another study reported that lactic acid with a concentration of 0.5 M exhibited the most potent effect on collagen extractability, resulting in a yield of 45% (wet basis). This was followed by formic acid (32%), tartaric acid (31%), acetic acid (31%), and citric acid (25%) at the same concentration (Bhuimbar et al., 2019). Additionally, hydrochloric acid and sulfuric acid with the same concentration demonstrated the least efficiency, resulting in a negligible yield (Bhuimbar et al., 2019). However, these findings conflicted with another research study, which observed that the highest yield of collagen extraction was achieved using hydrochloric acid at pH 2.4 (42.36%), followed by acetic acid at pH 2.7 (39.45%). In contrast, according to that study,



TABLE 1 Extraction parameters, solvent utilized, and yield of ASC from different sources.

Source	Tissue source	Extraction parameters	Extraction solvent	Yield (%) (weight-based)	Reference
Cobia (Rachycentron canadum)	Skin	Temperature: 4°C Period: 24 hours Ratio (w/v): 1:10	0.5 M lactic acid 1.0 M lactic acid	36.70 22.23	Sukeri et al. (2021)
Shortfin scad (Decapterus macrosoma)	Bone	Temperature: 4°C Period: 24 hours Ratio (w/v): 1:5	0.5 M acetic acid 0.7 M acetic acid	1.01 1.31	Baderi and Sarbon (2019)
Fringescale sardinella (Sardinella fimbriata)	Waste material	Temperature: 4°C Period: 24 hours Ratio (w/v): 1:2	0.5 M acetic acid	7.48	Hamdan and Sarbon (2019)
Bigeye tuna (Thunnus obesus)	Skin	Temperature: 4°C Period: 3 days	0.5 M acetic acid	13.50	Ahmed et al. (2019)
Medusa fish (Centrolophus niger)	Skin	Temperature: 4°C Period: 72 hours Ratio (w/v): 1:25	0.5 M lactic acid	45.00	Bhuimbar et al. (2019)
			0.5 M formic acid	32.00	
			0.5 M tartaric acid	31.00	
			0.5 M citric acid	31.00	
			0.5 M acetic acid	25.00	
Sole fish (Aseraggodes umbratilis)	Skin	Temperature: 10°C Period: 32 hours Ratio (w/v): 1:9	0.5 M acetic acid	19.27	Arumugam et al. (2018)
Loach (Misgurnus anguillicaudatus)	Skin	Temperature: 4°C Period: 24 hours Ratio (w/v): 1:30	0.5 M acetic acid	22.42	Wang et al. (2018)
Golden carp (<i>Probarbus</i> Jullieni)	Skin	Temperature: 4°C Period: 48 hours Ratio (w/v): 1:15	0.5 M acetic acid	51.90	Ali et al. (2018)
Channel Catfish (Ictalurus punctatus)	Skin	Temperature: 4°C Period: 48 hours Ratio (w/v): 1:50	pH 2.4 hydrochloric acid	42.36	Tan and Chang (2018)

lactic acid exhibited the most minor efficacy in collagen extraction (Tan & Chang, 2018). This issue may have been due to the differences in acid concentrations and the pH mixtures, which affect collagen's solubility (Tan & Chan, 2018). Table 1 summarizes the ASC extraction method based on different parameters, solvents, and yields from various sources.

2.3.4 | PSC extraction process

Collagen extracted using pepsin extraction is known as PSC. This enzyme has an optimum pH between 1.5 and 2.5 (Yang & Shu, 2014). Pepsin hydrolysis does not affect the secondary structure of collagen, particularly the triple helix structure, because this enzyme only acts on non-helix peptide chains of collagen protein (Schmidt et al., 2016). In general, other proteolytic enzymes such as trypsin, papain, alkaline protease, bromelain, pancreatin, and alcalase are

also applicable for collagen extraction due to their tendency to assist in the solubilization process by specifically cleaving peptides in the telopeptide area of collagen (Lim et al., 2019). However, pepsin is the most preferred enzyme for collagen extraction. Generally, collagen isolated using pepsin yielded significantly higher than acid-extracted collagen (Schmidt et al., 2016).

However, collagen extraction using an acetic acid and pepsin mixture yields higher yields and purity than extraction using acetic acid and pepsin alone (Delgado et al., 2017). The use of 0.5 M acetic acid in conjunction with pepsin at different concentrations (0.05%–10.0%) has been extensively used in the extraction of PSC from various species such as jellyfish (Khong et al., 2018), shortfin scad (*Decapterus macrosoma*) waste material (Coppola et al., 2020; Sulaiman & Sarbon, 2020), silver catfish (*Pangasius sp.*) skin (Hukmi & Sarbon, 2018), and golden carp (*Probarbus jullieni*) skin (Ali et al., 2018). PSC extraction involves pepsin's targeted enzymatic breakdown of

TABLE 2 Extraction parameters, solvent utilized, and yield of PSC from different sources.

		Extraction			
Source	Tissue source	parameters	Extraction solvent	Yield (%)	Reference
Shortfin scad (Decapterus macrosoma)	Waste material	Temperature: 4°C Period: 30 hours	0.5 M acetic acid with 1.5% (w/w) pepsin	0.10	Sulaiman and Sarbon (2020)
Sharp nose stingray (Dasyatis zugei)	Skin	Temperature: 4°C Period: 30 hours Ratio (w/v): 1:40	0.5 M acetic acid with 1.5% (w/w) pepsin	34.84	Ong et al. (2021)
Bigeye tuna (Thunnus	Skin	Temperature: 4°C	0.5 M acetic acid with	16.70	Ahmed et al. (2019)
obesus)	Scales	Period: 48 hours	0.5% (w/v) pepsin	4.60	
Golden carp (<i>Probarbus</i> Jullieni)	Skin	Temperature: 4°C Period: 48 hours Ratio (w/v): 1:15	0.5 M acetic acid with 1.0% (w/w) pepsin	79.27	Ali et al. (2018)
Miiuy croaker (Miichthys Miiuy)	Scales	Temperature: 4°C Period: 48 hours Ratio (w/v): 1:15	0.5 M acetic acid with 1.0% (w/w) pepsin	3.87	Li et al. (2018)
Silver catfish (<i>Pangasius</i> sp.)	Skin	Temperature: 4°C Period: 30 hours	0.5 M acetic acid with 1.5% (w/w) pepsin	2.27	Hukmi and Sarbon (2018)
Lumpfish	Skin	Temperature: 4°C Period: 24 hours	0.5 M acetic acid with 0.05% (w/v) pepsin	Not evaluated	Zhuang et al. (2018)
Jellyfish (Acromitus hardenbergi)	Oral arm	Temperature: 4°C Period: 48 hours	0.5 M acetic acid with 10% (w/v) pepsin	19.00	Khong et al. (2018)
Loach (Misgurnus anguillicaudatus)	Skin	Temperature: 4°C Period: 24 hours	0.5 M acetic acid with 5.0% (w/w) pepsin	27.32	Wang et al. (2018)
Catla (Catla catla)	Skin	Temperature: 4°C Period: 48 hours Ratio (w/v): 1:60	0.5 M acetic acid with 0.2% (w/v) pepsin	69.53	Pal et al. (2015)

collagen, disrupting its structure and exposing collagen fibers by removing non-collagenous parts. This process forms soluble collagen fragments, or gelatin, separable from undigested material using centrifugation or filtration. PSC extraction uses pepsin's specificity to effectively extract collagen for diverse uses (Kim et al., 2016).

In general, the factors that affect the extractability of the PSC extraction process are the concentration of pepsin used, the treatment period, the ratio of solid to liquid used, and the source of collagen itself. Recently, Yu et al. (2018) reported that the yield of PSC improved from 66.35% to 79.93% when the pepsin concentration increased from 0.08\$ to 0.12%. Similarly, the collagen yield extracted with 1% pepsin increased significantly compared with the sample treated with a lower pepsin level (value not specified) (Ali et al., 2018). Moreover, collagen yield increased from 62.505 to 80.00% with the increase in solid-to-liquid ratio and treatment time from 1:25 to 1:55 and between 4 and 8 hours (Yu et al., 2018). However, when a high volume of pepsin is used for an extended period, the yield of PSC might be lower as the collagen is prone to cleavage, affecting the integrity of the triple helix (Coppola et al., 2020). Table 2 summarizes the PSC extraction method based on different parameters, solvents, and yields from various sources.

2.3.5 | Ultrasound-assisted collagen extraction process

Ultrasound is a mechanism that uses the energy of sound waves induced at a greater frequency than the hearing range of humans (16-20 kHz) but still below microwave frequency (20-10 MHz) (Schmidt et al., 2016). Ultrasound waves induce fast oscillations, causing specific alterations in pressure and temperature, and these physical phenomena can result in the breakdown of collagen structures and promote the liberation of collagen molecules (Quarato et al., 2023). This involves creating and collapsing tiny bubbles through ultrasound, generating shockwaves, and microstreaming that disrupt tissue structure. This enhances solvent and solute movement, aiding collagen extraction. The impacts of ultrasound on liquid systems are primarily due to the cavitation process (Schmidt et al., 2016; Shaik et al., 2021a). Cavitation's mechanical forces physically disrupt tissues, promoting collagen release. Cavitation occurs when tiny bubbles form, expand, and collapse within a liquid medium. The implosion of these bubbles produces shock waves and microjets, generating mechanical forces that have the potential to disrupt collagen fibers and facilitate their release (Moyano et al., 2022). This ultrasound-assisted extraction method is effective due

to improved extraction efficiency, shorter extraction times, and higher collagen yields achieved through enhanced mass transfer and tissue disruption caused by ultrasound-induced effects (Bhargava et al., 2021).

Recently, a study concluded that the yield of collagen was directly proportional to the amplitude (20%-80%) and ultrasound treatment time (10-30 minutes) (Ali et al., 2018). The maximum yield of ultrasound ASC (UASC) (81.53%) was recorded when ultrasound at an amplitude of 80% and a treatment time of 30 minutes was subjected to a collagen solution, and an extraction time of 48 hours was used (Ali et al., 2018). Another study concluded that ultrasound treatment at 80% amplitude resulted in the highest collagen yield, ranging from 42.17% to 57.35%, and preferred to be applied for 10 minutes as the optimal treatment time (Petcharat et al., 2021). The latter was due to the changes in the molecular structure of collagen whenever a more prolonged ultrasound treatment was applied (Petcharat et al., 2021). However, the yield of collagen was also significantly improved (27.18%-57.35%) with the increasing treatment time (10-30 minutes) (Petcharat et al., 2021). Another study reported that the highest yield of collagen (84.14%) was obtained with 36 minutes of ultrasound treatment, and some collagen structure alteration was observed when the more extended ultrasound treatment was applied (Akram & Zhang, 2020).

Generally, this method is frequently employed together with acid to obtain UASC or pepsin to obtain UPSC, as it has been claimed by many researchers to improve collagen extractability and shorten extraction time as compared with the conventional method (Song et al., 2018). This improvement in the extractability must be due to cavitation caused by ultrasound treatment, which disrupts the raw material's cell wall and forces the solvents, such as acetic acid and pepsin, to enter the cavity and solubilize the collagen. Many studies have proven ultrasound treatment's effectiveness in improving collagen yield. For example, a study observed that the yield of collagen using the ultrasound-assisted extraction method was significantly higher (UASC: 81.53%; UPSC: 94.88%) than that of the conventional method (ASC: 51.90%; PSC: 79.27%) (Ali et al., 2018). Similar results have been documented by Zou et al. (2017a), who found an improvement in collagen yield of up to 50.75% in UASC compared with the conventional ASC extraction method (43.62%). These findings also follow another study, which found that the yield of UASC was significantly higher (8.19%–15.47%) than the ASC (5.12%) of the same source (Akram & Zhang, 2020). Table 3 shows the extraction parameters, collagen type, and yield from various sources based on ultrasound-assisted and conventional extraction methods. However, the ultrasound-assisted collagen extraction method has limitations, including the breakdown of hydrogen bonds between collagen chains.

Continuous use of ultrasound leads to a rise in temperature, which can damage the collagen (Furtado et al., 2022).

However, applying a single extraction method for collagen presents a few areas for improvement, including low yield, time consumption, and high costs. Additionally, single extraction methods may not improve the thermal stability of marine collagen. They can lower the bioactive efficiency, as some methods are effective only in extracting collagen from some parts of marine sources. Applying multiple methods for extracting collagen from marine sources offers several advantages, enhancing the final product's yield and quality (Barzkar et al., 2023). Different extraction techniques, such as enzymatic hydrolysis, acid and alkaline extraction, and ultrasound-assisted extraction, each target specific types of collagens and maximize overall recovery (Prajaputra et al., 2024). This multifaceted approach improves collagen's purity and functional properties, making it suitable for diverse applications in pharmaceuticals, cosmetics, and food industries (Tang et al., 2022).

2.4 | Properties of extracted marine collagen

2.4.1 | Chemical properties

Chemical compositions

The chemical composition of a substance refers to the amount or concentration of each chemical component that constitutes the substance itself. Collagen is mainly made up of protein and contains other elements such as moisture, ash, and fat, but in small amounts. Therefore, it is essential to identify collagen composition to measure collagen's purity. The chemical composition of a substance is typically measured through proximate analysis based on AOAC methods (AOAC, 2000). The chemical compositions of collagen were affected by their origins, the extraction method applied, and the solvent used. Table 4 illustrates the chemical composition of extracted collagen based on different sources and extraction methods.

Most extracted marine collagen showed a low moisture content of 0.5% to 7.50%, slightly lower than that of commercial collagen (10.11%) (Sulaiman & Sarbon, 2020). However, higher moisture content was reported in cobia (*Rachycentron canadum*) skin collagen (14.7%–18.2%), and it was believed to be associated with the application of lactic acid as a solvent with hygroscopic properties (Sukeri et al., 2021). Next, most of the extracted marine collagen contains a significant amount of protein (79%–95%) regardless of its sources, following the commercial collagen protein content (93.68%). However, lower

TABLE 3 Extraction parameters and yield of ultrasound-assisted and conventional extraction collagen from different sources.

Source	Source tissue	Extraction parameters	Type of collagen	Yield (%)	Reference
Clown featherback (Chitala ornata)	Skin	Frequency: 20 kHz Amplitudes: 20%–80% Pretreatment time: 10–30 minutes Extraction period: 48 hours	UASC	42.17–57.35	Petcharat et al. (2021)
Sharp nose stingray (Dasyatis zugei)	Skin	Frequency: 20 kHz Amplitudes: 20%	UASC	42.34	Shaik et al. (2021a)
		Pretreatment time: 30 minutes Extraction period: 48 hours	UPSC	61.50	
Golden carp (Probarbus Jullieni)	Skin	Frequency: 20 kHz Amplitudes: 20%–80%	UASC	81.53	Ali et al. (2018)
		Pretreatment time: 10–30 minutes Extraction period: 48 hours	UPSC	94.88	
		Conventional extraction	ASC	51.90	
		method	PSC	79.27	
Flatfish (Paralichthys olivaceus)	Skin	Frequency: 20 kHz Amplitudes: 60% Pretreatment time: 1.5 hours and 3 hours	UASC	30.30 and 40.20	Song et al. (2018)
Jellyfish (Acromitus hardenbergi)	Oral arm	Ultrasound-assisted extraction method Pretreatment time: 15 minutes	UASC	40.20	Khong et al. (2018)
		Conventional extraction	ASC	8.00	
		method	PSC	19.00	

Abbreviation: UASC = ultrasound acid-soluble collagen, UPSC = ultrasound pepsin-soluble collagen, ASC = acid-soluble collagen, PSC = pepsin-soluble collagen.

protein content was reported in shortfin scad waste collagen (22.86%–26.67%) and sharpnose stingray (*Dasyatis zugei*) skin collagen (36.64%–61.77%) (Shaik et al., 2021a; Sulaiman & Sarbon, 2020). This low protein content might be due to a less effective pretreatment procedure before the collagen extraction (Suptijah et al., 2018).

The fat content of most of the extracted marine collagens was moderately lower (0.1%–1.4%) than that of commercial collagen (2.94%) (Table 4). The low fat content of the extracted collagen indicated the effectiveness of the defatting process before collagen extraction (Hukmi & Sarbon, 2018). However, a more significant amount of fat was identified in sharpnose stingray (*Dasyatis zugei*) skin collagen (3.96%–4.16%), and it was associated with the absence of the defatting process during the pretreatment of raw material (Shaik et al., 2021a). Moreover, most of the extracted marine collagen also exhibited a low percentage of ash, below 7%, and significantly lower than commercial collagen's ash content (16.24%). These findings might be due to the efficient demineralization pretreatment before collagen extraction. However, a substantial

amount of ash was reported in shortfin scad waste collagen (60.9%), which is inferred to be due to the inefficient demineralization process in the pretreatment stage (Hukmi & Sarbon, 2018; Sulaiman & Sarbon, 2020). These, therefore, showed the importance of defatting and demineralization pretreatment before the collagen extraction process.

Interestingly, substantially less ash and fat content was found in collagen treated with ultrasound than those without ultrasound treatment (with ultrasound: ash 5.52%, fat 0.06%; without ultrasound: ash 13.03%, fat 1.02%) (Akram & Zhang, 2020). This result agreed with Khong et al. (2018), who claimed that collagen treated with ultrasound contains a smaller amount of ash (2.22%) compared with those without ultrasound treatment (2.76%—29.27%). The lower ash content might be due to the mechanical forces of ultrasound waves that can disintegrate mineral formations associated with collagen, facilitating their removal during subsequent purification steps. As a result, the overall ash content of the collagen sample decreases (Lueyot et al., 2022). Understanding the composition and interactions between these components and collagen is crucial

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TABLE 4 Chemical compositions of extracted collagen based on different sources and extraction methods.

	Extraction	Chemical c	omposition (%)			
Source	method	Moisture	Protein	Fat	Ash	Reference	
Commercial collagen (Tilapia (Oreochromis niloticus) scales)	_	10.11	93.68	2.49	16.24	Sulaiman and Sarbon (2020)	
Shortfin scad (<i>Decapterus macrosoma</i>) waste material	ASC	5.23	22.86	0.38	60.90		
	PSC	_	26.97	_	-		
Cobia (Rachycentron canadum) skin	ASC	14.7–18.2	25.2–33.4	0.60-7.28	10.9–26.0	Sukeri et al. (2021)	
Sharpnose stingray (Dasyatis zugei) skin	UASC	4.04	41.77	4.16	6.12	Shaik et al.	
	UPSC	3.87	36.64	3.96	6.25	(2021)	
Miiuy croaker (Miichthys Miiuy) scales	ASC	5.18	93.19	0.50	1.15	Li et al. (2018	
	PSC	4.37	94.87	0.34	0.92		
Jellyfish (Acromitus hardenbergi) bell	UASC	7.43	78.39	_	2.22	Khong et al. (2018)	
	ASC	7.14	54.94	_	29.27		
	PSC	6.29	77.59	-	2.76		
Croceine croaker (<i>Pseudosciaena crocea</i>) scales	ASC	4.52	93.56	0.43	1.03	Wu et al. (201	
	PSC	3.76	94.66	0.15	0.97		
Redlip croaker (<i>Pseudosciaena polyactis</i>) scales	ASC	5.63	92.05	0.54	1.09		
	PSC	4.02	93.66	0.21	1.13		
Skipjack tuna (Katsuwonus pelamis) skulls	ASC	5.07	92.73	0.74	2.38	Yu et al. (201	
	PSC	5.06	92.77	0.66	1.94		
Scalloped hammerhead (Sphyrna lewini) cartilages	ASC	0.50	93.91	1.05	4.58	Chi et al. (20)	
Red stingray (Dasyatis akajei) cartilages	ASC	4.86	79.34	1.35	14.60		

for bioactive peptide production applications, as bound components can impact the peptides' yield, purity, and bioactivity.

Amino acid compositions

Collagen has been inferred to comprise 19 different types of amino acids. It has a unique composition due to the inclusion of hydroxyproline (Hyp), which does not exist in other proteins (Gauza-Włodarczyk et al., 2017). Total hydroxyproline (Hyp) is also known to indicate collagen presence and is used to determine collagen yield (Jafari et al., 2020; Tapia-Vasquez et al., 2020). It is crucial to determine the amino acid compositions of collagen, as they depict the structural properties and significantly affect the physicochemical characteristics of the collagen (Luo et al., 2020). Diverse amino acid compositions of different collagen types and origins can influence the physicochemical properties of collagen (Zhang et al., 2020b).

The amino acid compositions of collagen are based on glycine (Gly)-hydroxyproline (Hyp)-proline (Pro) and typically consist of about 1000 residues of amino acids (Song et al., 2018). Based on many findings, all extracted marine

collagen had glycine as the primary amino acid (215–354 residues/1000 residues), followed by alanine (89–141 residues/1000 residues), proline (110–132 residues/1000 residues), and hydroxyproline (62–100 residues/1000 residues) (Table 5). These trends were similar to the amino acid composition of mammalian collagen (Gao et al., 2018). Glycine is a significant amino acid, accounting for about one-third of the total amino acid in collagen. Collagen has a high glycine content because all the collagen family members are described by glycine-rich tripeptide (Gly-X-Y) repeated domains involved in triple helix formation (Fidler et al., 2018). In addition, glycine plays a significant role in minimizing steric hindrance and forming interchain hydrogen bonds perpendicular to the helix axis (Wei et al., 2019).

The spot of X and Y on the Gly-X-Y peptide chain was typically taken up by hydroxyproline and proline (Sotelo et al., 2015). Therefore, some researchers deduced that the sum of hydroxyproline and proline (imino acids) contributed to the collagen's thermal stability and structural integrity because the zones rich with Hyp and Pro are most likely to engage in the formation of junction zones

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Amino acid compositions and denaturation temperature of extracted collagen based on different method and sources.

	Siberian sturgeon cartilage	Hybri Sturge skins	eon	scales	ys miiuy)	Golden Pompano (Trachinotus ovatus) skins		Skipjack tuna (Katsuwonus pelamis) spines	Calf skin	Buffalo skin
Amino acids	-	ASC	PSC	ASC	PSC	ASC	PSC	ASC	-	-
Asparagine/ aspartic acid	45	50	51	40	41	44	45	44	45	43
Threonine	22	24	24	26	27	23	25	25	18	19
Serine	36	52	51	31	26	28	31	33	33	31
Glutamine/glutami	92	83	81	62	63	75	75	67	75	76
Glycine	346	338	341	342	345	324	320	339	330	332
Alanine	89	119	116	122	120	135	132	126	119	112
Cysteine	1	-	1	2	3	2	2	_	_	_
Valine	16	17	17	22	24	23	24	26	21	23
Methionine	7	3	2	14	14	16	15	15	6	4
Isoleucine	12	12	12	13	12	9	12	13	11	12
Leucine	32	19	18	23	25	18	20	26	23	19
Tyrosine	3	4	4	6	5	2	3	3	3	4
Phenylalanine	25	15	15	14	15	13	14	14	3	9
Hydroxylysine	-	5	5	6	7	6	6	5	7	5
Lysine	14	26	26	26	25	27	27	30	26	25
Histidine	-	5	5	8	9	6	7	5	5	6
Arginine	46	51	51	46	47	53	52	48	50	51
Hydroxyproline	100	64	65	86	85	64	62	74	94	97
Proline	114	114	115	112	110	132	129	104	121	128
Imino acid	214	178	180	198	195	196	191	178	215	225
T_d (°C)	22.50	26.83	26.54	32.20	29.00	31.80	30.00	17.60	40.80	51.20
References	Lai et al. (2020)	Wei et (2019)	al.	Li et al. (2	2018)	Wang et al.	. (2017)	Yu et al. (2014)	Subhan et al. (2015)	

Abbreviations: ASC = acid-soluble collagen, PSC = pepsin-soluble collagen.

stabilized by hydrogen binding (Chi et al., 2013). Therefore, higher thermal stability was associated with high imino acid content (Rizk & Mostafa, 2016). In general, marine collagen was reported to have lower total imino acids (178-214 residues/1000 residues) in comparison with mammalian collagen (215-225 residues/1000 residues) (Table 5). In addition, warm-water fishes such as the croaker and golden pompano were observed to have higher imino acid content (191-198 residues/1000 residues) (Li et al., 2018; Wang et al., 2017), than that of the skipjack tuna (178 residues/1000 residues) (Yu et al., 2014), which is a coldwater species. Thus, warm-water fish collagen had higher thermal stability than cold-water fish collagen (Ahmed et al., 2019). However, Nile tilapia (Oreochromis niloticus) skin extracted ASC and PSC amino acid composition indicated that the imino acid content was 172.10 and 164.18 per 1000 residues, respectively (Abdelaal et al., 2021). Overall, mammalian collagen had a higher imino acid content and

thus higher thermal stability and denaturation temperature than marine collagen due to the different habitat and body temperatures. Moreover, the extraction method has little impact on the amino acid compositions of collagen (Table 5).

2.4.2 Physical properties

Solubility

Solubility is a crucial functional property of collagen that influences other functional properties, such as emulsifying and foaming abilities (Chi et al., 2016). Solubility refers to the measure of collagen's ability to dissolve in a solvent, and collagen solubility can be determined by dissolving it in acid solutions with different pH levels or salt (NaCl) solutions at different concentrations. However, researchers are more engaged in determining the

solubility of collagen using an acid solution. Understanding the solubility properties of collagen at different pH levels holds significant importance in its isolation and application across various fields (Kumar et al., 2017). The recent research about ultrasonication as an alternative technique in food technology has indicated there is a wide range of pH values at which different types of collagens become most soluble (Lu et al., 2023). Notably, collagen from different sources can exhibit distinct molecular properties, resulting in diverse solubility characteristics. The solubility of collagen escalates as the pH value deviates from the isoelectric point (pI). Therefore, the solubility of collagen is closely tied to the pH and pI values.

Many studies have investigated the solubility of collagen across different pH ranges. The highest solubility rate of marine collagen, sourced from sharpnose stingray (Dasyatis zugei) skin (Shaik et al., 2021b), fringescale sardinella (Sardinella fimbriata) waste materials (Hamdan & Sarbon, 2019), soft-shelled turtle (Pelodiscus sinensis) calipash (Zou et al., 2017b), red drum fish (Sciaenops ocellatus) (Chen et al., 2016), and seabass (Lates calcarifer) scales (Chuaychan et al., 2015), was observed at lower pH levels ranging from 1 to 3. In comparison, the lowest solubility was observed at higher pH values within the range of 6 to 10, with slight increments in solubility observed over these values. The lowest solubility of collagen is associated with the isoelectric point (pI), where hydrophobic-hydrophobic interaction increases and collagen molecules aggregate and precipitate when the total net charges of protein molecules are zero (Singh et al., 2011). A similar trend in solubility rate was observed for mammalian collagen (Vidal et al., 2020).

However, collagen from different sources exhibited varying pI values. For instance, Acaudina molpadioides displayed a pI value of 4.25 (Li et al., 2020b), striped catfish skin collagen had a pI of 4.27 (Singh et al., 2011), spotted golden goatfish scales collagen had a pI of 4.96 (Matmaroh et al., 2011), and bamboo shark skin collagen had a pI of 6.12 (Kittiphattanabawon et al., 2010). Collagen has been reported to have isoelectric points ranging from pH 6 to 9 (Singh et al., 2011). This showed no significant difference in marine and mammalian collagen solubility except for the pI values. Overall, the solubility of collagen extracted is generally suitable for applications in beverages such as juices and yogurt drinks due to the pH range of these products. Yogurt typically has a pH of 4.0, whereas the pH of fruit juices typically ranges from 2.0 to 5.6 (Hamdan & Sarbon, 2019).

Viscosity

Viscosity is a measure of fluid flow consistency. For example, the viscosity of collagen can be measured using a viscometer in the unit of Pa.s (Pascal-second). Typically,

collagen will be dissolved into water and heated to a specific temperature, and then, the viscometer will evaluate the viscosity of the samples with suitable spindle and speed (Liu et al., 2015b). However, the viscosity of collagen has commonly been evaluated based on its denaturation temperature (T_d). The T_d of collagen represents the temperature at which the structure of the collagen triple helix dissolves further into irregular coils in a solvent or solution. Marine collagen denaturates at a lower temperature of 25°C-30°C than that of mammalian collagen, which denatures around 39°C-40°C (Jafari et al., 2020). Generally, the viscosity of collagen decreases with the increase in temperature due to the destroyed hydrogen bond in collagen molecules, causing the triple helix structure to dissolve and change into random coils (Chen et al., 2019b). Additionally, T_d is the primary indicator in measuring the thermal stability of collagen (Thuy et al., 2014). Moreover, collagen's T_d was said to correlate with the imino acid

The T_d of collagen was found to vary significantly depending on their sources. The variations of T_d between collagens from different species were associated with different concentrations of imino acids, body temperature, and habitat (Sukkon et al., 2020). Warm-water fish collagen exhibited higher imino acid content than the T_d of cold-water fish collagen (Table 5). For instance, golden pompano (Trachinotus ovatus) skin collagen, a warmwater fish species, exhibited 196 residues/1000 residues of imino acids and had a T_d of 30.0°C-31.8°C (Wang et al., 2017). A similar range of imino acid content and T_d were observed for some other warm-water marine fishes such as croceine croaker (Pseudosciaena crocea) scales collagen (imino acid = 198 residues/1000 residues; $T_d = 27.5^{\circ}C$ -30.7°C) (Wu et al., 2015), and leather jacket fish collagen (imino acid = 192 residues/1000 residues; $T_d = 29.3$ °C) (Li et al., 2020b). A significantly lower imino acid content and T_d were observed in Antarctic ice fish collagen (imino acid = 147 residues/1000 residues; $T_d = 6$ °C) (Zhang et al., 2020a) and tuna skipjack collagen (imino acid = 178 residues/1000 residues; $T_d = 17.6$ °C) (Yu et al., 2014), which were cold-water species. However, mammalian collagen had a noticeably higher imino acid content and T_d than marine collagen. Particularly, calf skin collagen (imino acid = 215 residues/1000 residues; $T_d = 37^{\circ}C$) (Wu et al., 2015), buffalo skin collagen (imino acid = 225residues/1000 residues; $T_d = 51.20^{\circ}C-61.90^{\circ}C$) (Rizk & Mostafa, 2016), sheep bones collagen (imino acid = 220 residues/1000 residues; $T_d = 38.91^{\circ}C-42.31^{\circ}C$) (Gao et al., 2018). Marine collagen was less stable than mammalian collagen because losing viscosity at lower temperatures was easier. A detailed review of factors affecting collagen thermal stability was performed by Zhang et al. (2020b).

Foaming properties

Foaming is a condition that creates a volume of tiny bubbles or frothing. It is known that proteins, including collagen, are capable of foaming. Because of that, to produce drinks from fresh fruit, collagen hydrolyzes and works well as a food system stabilizer and foaming agent (Dzyuba et al., 2017). Proteins reduce the surface tension at the air/water interface, forming foam (Zou et al., 2016). Therefore, foam formation has been recognized as another valuable element for food formulations, such as mousse processing, beverages, and whipped toppings (Zou et al., 2016). The foaming properties of collagens are typically represented in percentage (%) of foaming capacity (FC) and foam stability (FS). FC measures the capability of collagen to foam at certain conditions (typically at different pH), while FS evaluates the stability of foam produced, usually based on different pH or time (Meenmanee et al., 2022).

There are only a few recent findings regarding the foaming properties of collagen. Red stingray skin collagen was observed to have the highest FC at pH 10 (152%) and the highest FS (72%) at pH 4 (Chen et al., 2019b). Similar findings were reported on collagen extracted from soft-shelled turtle calipash (FC = 110%; FS = 85%) (Zou et al., 2017a) and chicken sternal cartilage (FC = 197%; FS = 166%), which also had the highest FC and FS at pH 10 (Akram & Zhang, 2020). The lowest FC and FS were observed at pH 6 for red stingray skin collagen (FC = 48%; FS = 5%), pH 7 for soft-shelled turtle calipash collagen (FC = 45%; FS = 15%) (Zou et al., 2017a), and pH 4 for chicken sternal cartilage collagen (FC = 117%; FS = 105%) (Akram & Zhang, 2020). Most collagen has high FC and FS at alkaline conditions (pH 10) but lowers FC and FS in low acidic to neutral conditions despite their sources. These foaming property variations might be due to the isoelectric point (Akram & Zhang, 2020). It was discovered that collagen's FC and stability significantly increased when it shifted away from its isoelectric point (Zou et al., 2017a). This is because at the isoelectric point, the solubility of collagen is at the lowest, and the electrostatic repulsion among collagen molecules is weak; thus, it interrupts the interactions between protein and water during foam formation (Zou et al., 2017a).

In addition, collagen's FC and stability are also known to be influenced by the concentration of protein (collagen) (Vidal et al., 2020). A study reported that FC and stability were improved from 5% and 0 to 31% and 21.5% with the increase of collagen concentration from 1.5% to 4.5%, respectively (Bhuimbar et al., 2019). However, calf skin collagen exhibited the highest FC and FS values (31% and 21.5%), even at a 1% concentration (Bhuimbar et al., 2019). This shows that calf skin collagen has better foaming properties than fish skin collagen. In addition, high-molecular-weight protein molecules assemble more resistant films, leading to more excellent FS (Vidal et al.,

2020). Overall, collagens' foaming properties might have been linked with the sources, intrinsic properties, and collagen structure.

2.4.3 | Bioactive properties

Interestingly, marine collagen from fish skin, bone, and scales has been frequently used as a scaffold and carrier owing to its outstanding bioactive properties, such as biocompatibility, low antigenicity, excellent biodegradability, and cell growth potential (Rizk & Mostafa, 2016; Subhan et al., 2015). Furthermore, studies reported that marine collagen replaces mammalian collagen in biomedical engineering (Lin et al., 2021; Subhan et al., 2015). Moreover, the application of marine collagen with enhanced bioactive properties, including antioxidants, wound healing, tissue engineering, and cosmetic biomaterials, is discussed here (Figure 2).

Antioxidant properties

The collagen, sourced from marine organisms such as fish, exhibits antioxidant properties due to its unique composition. Antioxidants are substances that can discharge reactive oxygen molecules and prevent the oxidation process, which can create free radicals (Nurilmala et al., 2020). Interestingly, marine collagens and collagen-active peptides have been used in skincare formulation and biomedical to enhance wound recovery owing to their antioxidant property and some other good qualities (Chen et al., 2019c). Marine collagen exhibits multiple antioxidant mechanisms contributing to its protective effects against oxidative stress. These mechanisms include scavenging free radicals, inhibiting lipid peroxidation, metal ions chelation, electron donation, enhancing antioxidant enzyme activities, regulating gene expression, repairing oxidative damage, and reducing inflammation (Chaudhary et al., 2023). Scavenging free radicals and inhibiting lipid peroxidation prevent cellular damage, while chelating metal ions such as iron and copper reduces oxidation reactions. Donating electrons helps stabilize the free radicals (Xu et al., 2024). In addition, activities of antioxidant enzymes such as superoxide dismutase and catalase are enhanced. Regulation of gene expression strengthens antioxidant defense mechanism and facilitates the repair the oxidative damage to cellular components including, proteins, lipids, and DNA (Gulcin & Alwasel, 2023). The antioxidant property of collagen can be evaluated by DPPH radical, ABTS radical, hydroxyl radical, and superoxide anion radical scavenging assays (Zhang et al., 2019). The rate of scavenging is the primary indicator in determining antioxidant activity. Collagen contains specific peptide sequences that scavenge free radicals and

FIGURE 2 Collagen structure-function relationship and mechanism of bioactivity.

combat oxidative stress. Amino acids in marine collagen, such as glycine, proline, alanine, aspartic acid, and hydroxyproline, contribute to its antioxidant potential by supporting antioxidant enzyme production (Nurilmala et al., 2020). The collagen's metal-chelating ability prevents metal-triggered oxidative damage. Additionally, marine collagen directly scavenges reactive oxygen species, reducing oxidative stress and inflammation. It also stimulates the body's antioxidant enzymes, such as SOD and catalase, enhancing natural defense against oxidative stress (Meyer et al., 2023).

Many studies have been done regarding the antioxidant properties of collagen from marine species (Table 6). It was inferred that the antioxidant activity of collagen is proportional to the collagen concentration (Li et al., 2020b). For instance, the DPPH radical scavenging activity of sea cucumber (Acaudina molpadioides) body wall collagen increases from 14.6% to 66.5% when collagen concentration increases from 0.5 to 10 mg/mL (Li et al., 2020b). Parang-parang fish (Chirocentrus dorab) skin collagen also exhibited the same trend of DPPH scavenging activity, which increased from 7.43% to 53.48% when the collagen concentration increased from 0.1 to 1.0 mg/mL (Ardhani et al., 2019). However, the radical scavenging activity of all collagen was significantly lower (only up to 70%) in comparison with the DPPH scavenging activity of ascorbic acid (up to 95%) and glutathione (up to 80%) at most concentrations (Chen et al., 2019c; Yang & Shu, 2014). For this case, the scavenging activity for the DPPH radical of ascorbic acid (4 mg/mL) was 90%. In comparison, the scavenging activity for DPPH radical of PSC of giant croaker (Nibea japonica) swim bladders at the same concentration (4 mg/mL) was only 52% (Chen et al., 2019c). A similar trend of scavenging activity was observed for ABTS, hydroxyl, and superoxide anion radicals (Li et al., 2018; Zhang et al., 2019).

Diverse antioxidant activities have been observed even when collagen concentrations are similar, and these variations are influenced by factors such as amino acid composition, structure, hydrophobicity, and collagen molecular weight (Ardhani et al., 2019). For instance, when sea cucumber (Acaudina molpadioides) collagen was present at a concentration of 1.0 mg/mL, it displayed a scavenging activity of 19.60% against DPPH radicals (Li et al., 2020b). Similar observations were reported for collagen derived from Miiuy Croaker (Miichthys miiuy) scales (20%) (Li et al., 2018), Lophius litulon skin (25%) (Wen et al., 2019), and significantly higher DPPH scavenging activity was observed for parang-parang fish (Chirocentrus dorab) skin collagen (53.48%) at a comparable concentration (Ardhani et al., 2019). In comparison, calfskin PSC exhibited lower scavenging activity for DDPH (15%), ABTS (18%), hydroxyl (19%), and superoxide anion radicals (15%) at concentration of 1.0 mg/mL when compared with Miiuy Croaker (Miichthys miiuy) scales collagen at same concentration: ABTS (30%), hydroxyl (25%), superoxide anion radicals (28%) (Li et al., 2018). Overall, calfskin collagen showed lower radical scavenging activity compared with marine collagen.

Wound-healing properties

The wound-healing process consists of four major phases: hemostasis, inflammation, proliferation, and maturation. Collagen is believed to have an essential role in all these

TABLE 6 Antioxidant properties of collagen from marine sources.

	Antioxidant activity					
Source of Collagen	ABTS	DPPH	Units	Reference		
Yellowfin tuna (Thunnus albacares) skin	313.29 ± 0.15	560.51 ± 0.02	μg protein/mL	Nurilmala et al. (2020)		
Parang-parang fish (<i>Chirocentrus dorab</i>) skin	-	926.25	ppm	Ardhani et al. (2019)		
Tilapia (Oreochromis niloticus) Skin	3178.22 ± 11.85	10.92 ± 0.84	μmol TE/mg protein	Jantaratch et al. (2022)		
Redlip croaker (<i>Pseudosciaena polyactis</i>) scales	4.24 ± 0.18	-	mg/mL	Wang et al. (2020)		
Yellowfin tuna (Thunnus albacares) skin	-	1.90 ± 1.03	mg/mL	Nurilmala et al. (2019)		
Crimson snapper (Lutjanus erythropterus)	-	39.57 ± 0.99	mg/mL	Ahmed et al. (2019)		
Silver pomfret (Pampus argenteus)	_	40.89 ± 02.22	mg/mL			
Mackerel scad (<i>Decapterus macarellus</i>) skin	-	148.55 ± 3.14	ppm	Herawati et al. (2022)		
Marine sponge (Stylissa flabelliformis)	-	61.5 ± 2.13	ppm	Sunarwidhi et al. (2021)		
Skipjack tuna (Katsuwonus pelamis) bone	9.49	3.15	mM	Ding et al. (2019)		
Lamuru fish (Caranx ignobilis)	_	485.90	μg/mL	Nur et al. (2021)		
Silver carp (Hypophthalmichthys molitrix) skin	38.00 ± 2.10	1.67 ± 0.09	mg/mL	Ilie et al. (2022)		
Yellowfin tuna (Thunnus albacares) skin	11.46 ± 3.77	13.56 ± 4.58	mg/mL	Wardani et al. (2023)		

 $Abbreviations: ABTS = 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic\ acid); DPPH = 2, 2-diphenyl-1-picrylhydrazyl.$

processes primarily because of its chemotactic attributes (Felician et al., 2019). Marine collagen's wound-healing properties arise from its bioactive peptides, unique amino acids, and tissue-regenerating traits. It acts as a scaffold, supporting cell attachment, migration, and proliferation at the wound site, fostering tissue regeneration and closure. The various mechanisms, including cell migration and proliferation, epithelization, angiogenesis, keratinization, and collagen fiber deposition, make marine collagen an effective wound-healing agent (Barzkar et al., 2023). Marine collagen significantly enhances cell migration and proliferation, crucial processes that facilitate the rapid closure and regeneration of damaged tissue (Lim et al., 2019). Additionally, it promotes epithelization, the formation of new epithelial tissue, which is vital for restoring the skin barrier. In the angiogenesis process, the development of new blood vessels ensures an adequate supply of nutrients and oxygen to the wound site, thereby accelerating healing potency (Zhou et al., 2023). The process of keratinization further strengthens the newly formed skin by promoting the production of keratin, a key structural protein. Moreover, marine collagen aids in the deposition of collagen fibers, providing structural integrity and strength to the repaired tissue (Deng et al., 2022). Glycine, proline, and hydroxyproline are amino acids found in marine collagen that are notably linked to wound-healing properties (Geahchan et al., 2022). Marine collagen peptides interact with cell receptors, initiating pathways that boost cell migration and proliferation, expediting wound-

healing and tissue regrowth. The incorporation of marine collagen influences the wound's extracellular matrix, aiding new collagen deposition, tissue reorganization, and strengthening (Alam et al., 2022).

Additionally, it promotes angiogenesis, enhancing blood vessel formation for improved nutrient and oxygen supply to the wound area. Its anti-inflammatory qualities create an optimal healing environment, curtailing inflammation and excessive scarring. Many studies regarding the wound-healing properties of marine collagen have been documented to determine the effectiveness of collagen application in the wound-healing process. The wound-healing property of collagen can be evaluated in vivo and in vitro (scratch test) by determining the percentage of wound closure. Both native collagen sponges and collagen peptides apply to wound-healing properties (Zhang et al., 2019).

In vivo, the wound-healing effect of collagen was typically observed and determined based on the wound closure percentage in a mouse model. Recently, wound closure on a mouse model treated with *Lophius litulo* collagen sponges was significantly higher (90%) than the wound without collagen treatment (58%) after 12 days of observation (Wen et al., 2019). A similar positive observation was reported for wounds treated with jellyfish (*Rhopilema esculentum*) collagen peptides and giant croaker (*Nibea japonica*) swim bladders collagen (Chen et al., 2019c; Felician et al., 2019). In addition, results for wound tests using tilapia skin collagen scaffold were similar to conventional

pig or bovine collagen (mammalian collagen) wound dressing, which promoted wound closure in a mouse excisional wound model (Huang et al., 2024). Moreover, no epithelial necrosis or wound deterioration occurred with applying collagen, indicating that this marine collagen is biocompatible and has no toxic effect (Chen et al., 2019c; Zhang et al., 2019). Furthermore, wounds treated with fish collagen showed an accelerated wound-healing process similar to bovine collagen (Chen et al., 2019a). It was reported that scaffolds derived from marine species exhibit high biodegradability, low immunogenicity, and high biocompatibility (Davison-Kotler et al., 2019).

In vitro, the wound-healing rate of collagen was observed and determined based on the scratch test. Based on a previous study, it was found that the wound-healing effect depended on collagen concentration. The wound closure rate was reported to increase from 70% to 90% when the concentration of PSC (giant croaker) increased from 12.5 to 50 µg/mL after 24 hours (Chen et al., 2019c). Similarly, Felician et al. (2019) deduced that wound closure percentage for the cells treated with collagen peptides increased as the concentration increased from 1.56 to 6.25 µg/mL but remained constant above 6.25 µg/mL. Moreover, the closure rate for scratch treated with giant croaker (Nibea japonica) fish collagen at 50 µg/mL concentration was similar to that treated with 50 µg/mL of bovine collagen (Chen et al., 2019c). This showed that marine collagen had similar wound-healing properties to mammalian collagen and could become a potential source for tissue engineering and scaffolds.

Tissue engineering

Collagen from various sources has been widely utilized in various biomedical applications, including tissue engineering (Yoon, 2023). Due to its bioactivity, marine collagen is gaining prominence as a suitable biomaterial and a safe alternative to mammalian collagen in tissue engineering (Liu et al., 2022). Marine collagen scaffolds and sponges can be developed through various methods such as freeze drying, electrospinning, hydrogelation, 3D bioprinting, and decellularization. These scaffolds have been used in tissue engineering applications for bone, skin, and cartilage regeneration. Marine collagen involves various tissue engineering mechanisms, including promoting osteogenesis, inhibiting inflammation, inducing cartilage differentiation, and improving bone mineral density (Lin et al., 2021).

Marine collagen derived from the *Gadiformes and Pleu*ronectidae, when used in the treatment of osteoblastic MC3T3-E1 cell culture, accelerates matrix mineralization and collagen deposition and upregulates collagenmodifying enzymes, highlighting its potential for bone tissue engineering (Yamada et al., 2013). A composite disk

was synthesized using marine sponge collagen, which promoted cell viability in mouse preosteoblastic cells (MC3T3-E1) and murine fibroblasts (L929), indicating its suitability as the organic component of an artificial bone graft with significant bioactivities (Parisi et al., 2019). Additionally, Hsu et al. (2016) found that human mesenchymal stem cells (hMSCs) cultured with marine collagen derived from tilapia exhibited increased expression of chondrogenic markers and decreased expression of osteogenic markers, suggesting that marine collagen provides appropriate signals for chondrogenic differentiation in vitro. Similarly, the 3D scaffolds developed from tilapia fish collagen significantly promoted cartilage repair in an in-situ rabbit articular defect model (Li et al., 2020a). Moreover, type II collagen extracted from jellyfish shows promise as a therapeutic implant for cartilage repair mechanisms when combined with human mesenchymal stem cells (hMSCs) and transforming growth factor-β3 (TFG-β3) (Pugliano et al., 2017). Highly porous sponge scaffolds developed from grass carp (Ctenopharyngodon idella) fish scalederived collagen have proven to be potential burn wound dressing materials in a rabbit model (Shi et al., 2020).

Cosmetic material

Collagen is vital in many cosmetic formulations due to its moisturizing, regenerating, and film-forming properties. Its excellent water-binding ability helps to maintain the skin's hydration throughout the day, keeping the skin moisturized and softened (Sionkowska et al., 2020). Collagen is used in various cosmetic industries, including hair, oral, mucous membranes, and skin care. The film-forming properties of collagen can be enhanced by collagen binding with other polymeric or biopolymer molecules (Lin et al., 2019b). Collagen derived from marine sources has shown great promise as a cosmetic material. Its unique properties, including high biocompatibility and low immunogenicity, make it an excellent ingredient for skincare products (Rahman et al., 2024). Therefore, marine collagen has become an exciting new biomaterial for cosmetic applications to improve skin health and appearance (Makgobole et al., 2024).

Jellyfish (*Rhizostoma pulmo*) skin yields approximately 60% collagen. It is a valuable resource in the cosmetics industry due to its high biocompatibility, low allergic reactions, and low risk of zoonotic illnesses (Sionkowska et al., 2020). A recent study on the oral administration of marine collagen to women aged 20–60 for 12 weeks significantly reduced skin wrinkle depth and number, increasing skin elasticity (Lee et al., 2022). Collagen derived from sea cucumber (*Holothuria cinerascens*) exhibited better moisture-retention and moisture-absorption capacity due to its rich hydrophilic groups, resulting in a potential biomaterial for cosmetic applications (Li et al., 2020c).



3 | FUTURE TRENDS

The extraction of collagen from marine sources has been raised recently due to its promising advantages and wide range of applications compared with other sources. Several marine organisms still need to be explored for possible collagen sources. In addition, more research is required to determine the most appropriate conditions for extracting marine collagen. Therefore, finding the best methods to improve marine collagen extractability is crucial as these sources have real potential in the collagen market. Furthermore, applications of multiple extraction methods in different combinations must be established to improve extractability, reduce processing time, and enhance the quality of the collagen obtained. Additionally, future studies should focus on overcoming the shortcomings and limitations of standard extraction methods. Moreover, marine collagen is said to have lower denaturation and melting temperature than mammalian collagen, which limits its application. Hence, further studies are needed to identify marine collagen's physicochemicals, including molecular structure, amino acid composition, functional groups, as well as biological properties, including wound healing, tissue engineering, and cosmetic formulations. This will help address the industry's limitations of marine collagen application. Thorough studies are also required to evaluate collagen's functional and bioactive properties from various marine species to identify potential alternatives to mammalian collagen in various applications.

4 | CONCLUSION

In summary, this review highlights the potential of marine collagen as a viable alternative to traditional sources such as bovine and porcine collagen. Collagen from marine species offers the safest choice without health and religious issues compared with land mammals and poultry sources. Besides, the significant amount of collagen available in marine species, especially fish skins and sea cucumber, shows that marine species can be a potential alternative source to land mammal collagen due to its abundance and availability. Based on the extraction methods and parameters discussed, it was found that the most suitable extraction methods for marine collagen are PSC and ultrasound-assisted collagen extraction methods. However, the exact parameters still need to be defined. For physicochemical properties, marine collagen was found to have lower denaturation temperature, water and oil absorption capacity, and foaming properties than mammalian collagen. However, marine collagen exhibited potential antioxidant, wound-healing, tissue engineering,

and cosmetic biomaterial properties similar to mammalian collagen. Overall, collagen derived from marine species exhibited favorable functional and bioactive properties and seemed a possible alternative to mammalian collagen.

AUTHOR CONTRIBUTIONS

Mannur Ismail Shaik: Methodology; data curation; investigation; validation; visualization; formal analysis; writing—original draft. Siti Hajar Abdul Rahman: Writing—original draft; methodology; investigation; data curation; validation; formal analysis. Anis Syafiqah Yusri: Writing—original draft; methodology; writing—review and editing; investigation. Mohammad Rashedi Ismail-Fitry: Writing—review and editing; methodology; validation; visualization. Nune Satya Sampath Kumar: Methodology; writing—review and editing; validation; visualization. Norizah Mhd Sarbon: Conceptualization; investigation; writing—review and editing; methodology; supervision; validation; visualization; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

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