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Review

# Marine collagen and its derivatives: Versatile and sustainable bio-resources for healthcare

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ABSTRACT

In the last two decades, marine collagen has attracted great scientific and industrial interest as a 'blue resource', with potential for use in various health-related sectors, such as food, medicine, pharmaceutics and cosmetics. In particular, the large availability of polluting by-products from the fish processing industry has been the key factor driving the research towards the conversion of these low cost by-products (e.g. fish skin and scales) into collagen-based products with high added value and low environmental impact. After addressing the extraction of collagen from aquatic sources and its physicochemical properties, this review focuses on the use of marine collagen and its derivatives (e.g. gelatin and peptides) in different healthcare sectors. Particular attention is given to the bioactive properties of marine collagen that are being explored in preclinical and clinical studies, and pave the way to an increased demand for this biomaterial in the next future. In this context, in addition to the use of native collagen for the development of tissue engineering or wound healing devices, particularly relevant is the use of gelatin and peptides for the development of dietary supplements and nutraceuticals, specifically directed to weight management and glycemic control. The marine collagen market is also briefly discussed to highlight the opportunities and the most profitable areas of interest.

## 1. Introduction

Collagen is the most abundant structural protein in animals, and in humans it accounts for about 30% of the total body protein content [1]. It is ubiquitously found in the extracellular matrix (ECM) of tissues, where it not only provides strength and structural stability but it also performs highly specialized regulatory functions, especially during development and repair [2,3].

'Collagen' is actually a quite generic term that stands for a large family of proteins, all sharing a peculiar structural feature: each protein is composed of three left-handed polypeptide chains (called α helices), which self-assemble to form at least one right-handed triple-helical domain [4,5]. To date, 28 types of collagen have been identified and described [7], called type I to type XXVIII based on the chronological order of their discovery. Despite their common triple-helical arrangement, the collagen types greatly differ in terms of molecular composition (e.g. amino acid composition, identity of the three α chains), molecular and supramolecular organization (e.g. occurrence and length of triple helical domains, packing of the triple helices, etc.), as well as function and distribution in tissues [8–11].

Fibril-forming type I collagen represents about 70% of the whole collagen family, being it the most abundant subspecies in connective tissues, such as bones, skin, tendons, ligaments, cornea and blood vessels [4,12,13]. Looking into its molecule (Fig. 1), each α chain is composed of about 1000 amino acid residues, and is characterized by the repetition of the Gly-X-Y triplet, where the X and Y positions are usually occupied by proline and hydroxyproline [5]. Glycine plays a crucial role in the packing of the three α helices [14], while proline and hydroxyproline, have a fundamental role in stabilizing the triple helical structure in physiological conditions, through the formation of hydrogen bonds that prevent free rotation [6,15]. However, the hydrogen bonds that stabilize the triple helix can be broken upon denaturation, a process (activated by thermal or chemical treatments) that transforms collagen into a random coil form known as gelatin. The high concentration of hydroxyproline residues is a unique characteristic attributable only to type I collagen, and is thus commonly used for the

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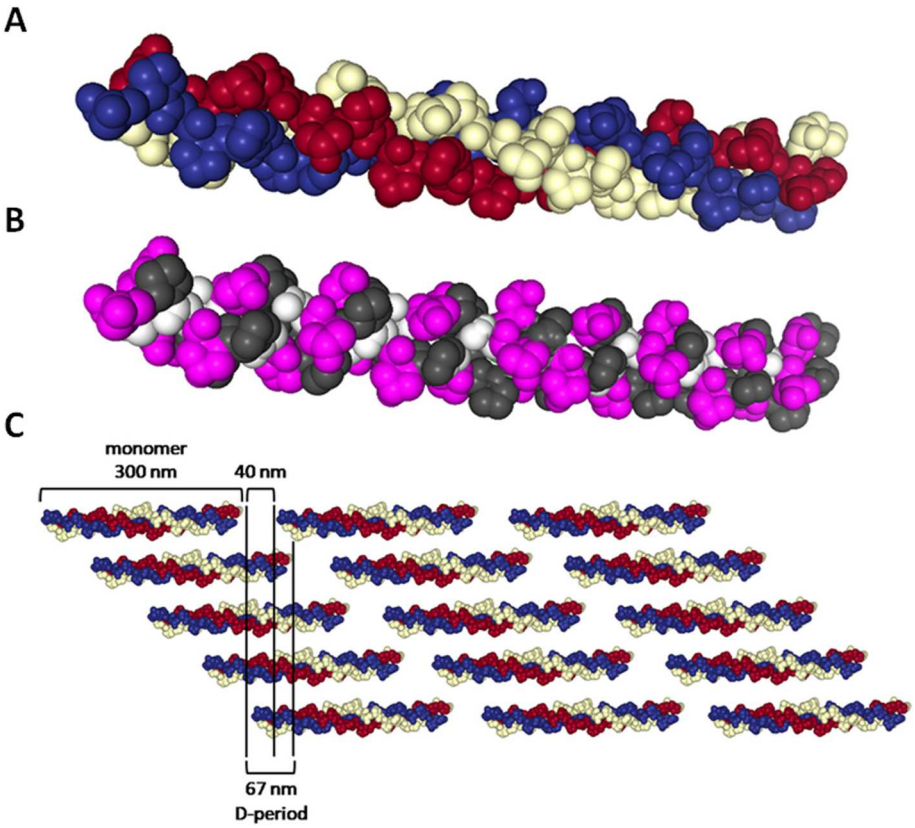


**Fig. 1.** Structure of type I collagen. Colored models of the triple helix (about 300 nm long) are provided in (A) and (B), to highlight the single chains and the amino acid composition $(Gly\text{-}X\text{-}Y)_n$, respectively. Glycine (blank) is a small amino acid that fits perfectly inside the helix, while most of the remaining positions are filled by proline (grey) and hydroxyproline (purple). A model of collagen microfibrils is also provided in (C): five molecules pack in parallel and stagger by one D-period to form microfibrils. Images were created with the PDB ID (http://www.rcsb.org/) and associated publications [29,30], NGL Viewer [31], and RCSB PDB. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recognition and quantification of this specific protein [16].

Thanks to its biodegradability, biocompatibility and bioactivity [2,17–19], collagen is one of the most widely used biomaterials in health-related sectors [20,21], including medical care, pharmaceutics and cosmetics. Its large exploitation is also due to the ease in processing to obtain a variety of product types, such as films, sheets, beads, meshes, fibers and sponges [17,19,22–26]. In addition, it is worth mentioning that collagen, especially in its denatured form, finds extensive application in the food sector as an additive, and in the development of nutraceuticals as an active compound [27,28].

The industrial production of collagen mostly relies on the extraction from animal tissues and, to a lesser extent, on the purification from recombinant production systems. With regard to the latter, recombinant human collagen has been expressed in both prokaryotic and eukaryotic hosts (i.e. yeast, bacteria, mammalian cells, insects or plants) with great degrees of success. However, the impassable limitation of recombinant collagen across all hosts is the inability to reproduce the full-length collagen molecule with the native amounts of post-translational modifications. In particular, hydroxylation remains challenging in prokaryotic systems, which lack native post-translational modification mechanisms. Even in eukaryotic systems, native hydroxylation modules are unable to adequately hydroxylate collagen molecules, making it necessary to incorporate non-native hydroxylases in ad hoc engineered hosts. Despite the potential of recombinant collagens, these unresolved issues make them expensive and non-biological, thus less attractive than animal-derived collagen, which is still the gold standard for use [32].

In particular, the best animal source for collagen extraction is represented by terrestrial mammals like cows, pigs and sheep, due to the high sequence homology with human collagen. However, the use of mammal-derived collagen is constrained by the risks of triggering an immune reaction (in about 3% of the population) and transferring zoonosis, e.g. the foot and mouth disease (FMD), and the group of the bovine spongiform encephalopathies (BSE), among which the most dangerous for humans is the transmissible spongiform encephalopathy (TSE) [22]. In addition, there are cultural or religious concerns associated with the use of porcine and bovine collagen (for Muslim, Jewish and Hindu faiths), which further restrict the applicative potential of mammal-derived collagen [33–35].

In this perspective, marine organisms (e.g. fish, jellyfish, sponges) have attracted much interest in the last two decades as safe, abundant and alternative sources for collagen extraction (Fig. 2). Marine collagen (MC) shows an intrinsically lower threat of transmissible diseases and is free from religious concerns. Furthermore, every year the fish processing industry produces a number of discards or byproducts (e.g. skin, bones, fins, heads, guts and scales) that account for about 70–85% of the total weight of catch and raise significant environmental pollution [36]. Therefore, the possibility to valorize the fish byproducts as sources of collagen makes fish collagen eco-friendly and particularly attractive in terms of profitability and cost effectiveness [37].

The aim of this article is to provide a glimpse into the most recent health-related uses of MC and its derivatives that are contributing to expand the related research (Fig. 2) as well as the market. The sources and the extraction methods of MC are discussed, along with the properties of MC that justify its use as a 'blue biomaterial'. With specific regard to fish, which is currently the main marine source exploited in the industry, it is worth noting that the expression 'fish collagen' reported in the literature usually includes collagen derived from both freshwater and saltwater fish. Accordingly, this article provides some information on freshwater fish collagen (e.g. collagen from carp), although not strictly belonging to the MC family.

## 2. MC extraction

### 2.1. Sources

MC can be divided into two categories, depending on the source type: invertebrates and vertebrates. Since the majority of marine

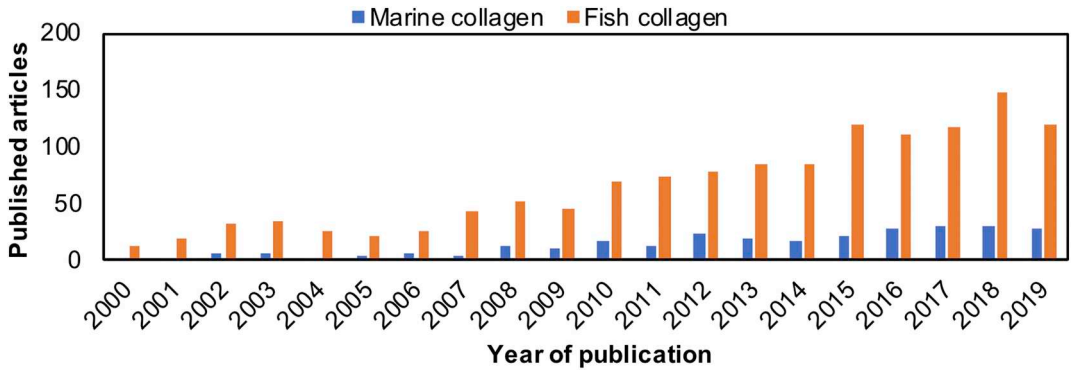


**Fig. 2.** The increasing research interest in collagen derived from aquatic animals. Articles indexed in Scopus (www.scopus.com) with the keywords 'marine collagen' and 'fish collagen' and published from 2000 to 2019 (last accessed on November 10th, 2019).

animals are invertebrates, this category offers an almost inexhaustible availability of potential collagen sources, such as cuttlefish, sea anemone, prawn, starfish, jellyfish, sponges, sea urchin, octopus, squid and mollusks [27,38–45]. Some pioneering studies on the extraction and characterization of collagen from marine invertebrates were already reported between the 70s and 80s [46–48], and constituted the basis for more recent investigations, showing the similarities between marine invertebrate collagens and their human counterparts [49]. In agreement with these findings, the scientific and industrial interest towards invertebrate MC has rapidly increased in the last two decades [50]. In particular, marine sponges [38,51] and jellyfish [52] nowadays represent the most widely used sources of MC among invertebrates. Marine sponges have been long studied and exploited as an invaluable source of pharmacologically active compounds (predominantly terpenoids and alkaloids) [53–56], thus their further usage for collagen extraction has been promptly addressed. Various sponge species have been studied for MC extraction [38,57,58], among which it is worth mentioning *Chondrosia reniformis* as an example of type I collagen source, suitable for biomedical and cosmetic applications [59–63]. As for jellyfish, increasingly frequent jellyfish outbreaks (leading to environmental and economic problems) have recently encouraged the utilization of this biological resource for MC isolation [49,64]. Different jellyfish species have been investigated [43,47,49,65,66], with *Rhizostoma pulmo* already shown to provide a type I collagen suitable for in vitro and in vivo applications [67–69].

The aquatic vertebrate category includes fish and marine mammals (e.g. whales). While various ethical concerns limit the exploration of collagen derived from marine mammals, fish has emerged in the last 20 years as an optimal source for collagen extraction [70], due to the possibility to reuse and add value to the abundant waste byproducts of the fish processing industry [71]. In this regard, Nile tilapia (*Oreochromis niloticus*), an African cichlid, appears as an appealing collagen source. Tilapia is indeed one of the world's most representative species of the fisheries and aquaculture food sector, with a global aquaculture production increasing 11% per year [72]. Tilapia processing generates a large volume of waste, about 62.5% of the raw material, making it a major environmental problem due to its high potential for polluting water, soil and air [73]. Therefore, there is large scientific and industrial interest in developing waste recovery technologies to obtain high added value products, such as collagen and its derivatives, with low environmental impact [73].

It is worth highlighting that collagen from aquatic sources and currently employed in healthcare and food sectors is predominantly type I collagen, mostly derived from fish scales and skin. Type II collagen can be obtained from fish cartilage (e.g. shark cartilage), while type IV collagen can be extracted from marine sponges and some jellyfish [74].

For both invertebrate and vertebrate sources, research efforts are prevalently directed to optimize the isolation protocols, in order to increase the yield, improve the batch-to-batch consistency and enhance the potential of MC for healthcare use (in addition to the large use in the food industry).

### 2.2. Methods

Depending on the biological source, different protocols have been developed to purify MC and its derivatives (gelatin or peptides) [34,75–78]. Moreover, within a single marine source, the extraction methodology must be diversified, based on the composition of the specific site. For example, fish bones and scales, that are bio-composites consisting of hydroxyapatite and type I collagen fibers, require an additional demineralization step to isolate collagen [79,80]. The scale source greatly affects the extraction process, since fish species are characterized by different complexity of both structural conformation and chemical composition [81].

Beyond process optimizations and special precautions aimed at increasing the extraction yield and consistency and reducing the extraction time and complexity for a potential scale-up (as addressed in the section below), all protocols share five main procedural steps or phases (Fig. 3), which can be identified in the following: (a) separation and cleaning; (b) size reduction; (c) removal of non-collagenous components; (d) collagen extraction via acidic and/or enzymatic treatment;

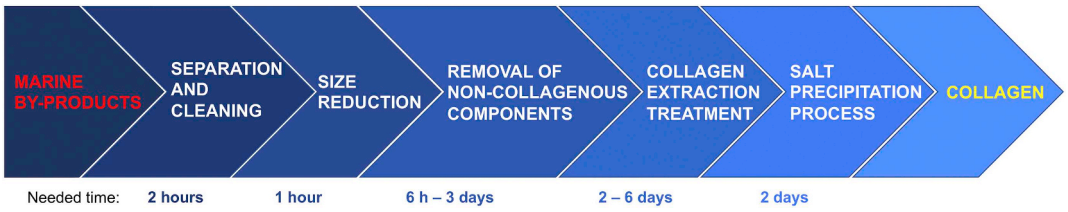


**Fig. 3.** Schematic procedure for native collagen extraction from marine sources. All extraction processes include an initial step of separation of fish components from discards, and the removal of unnecessary parts. Marine byproducts are then reduced in size to facilitate the following step that includes the removal of non-collagenous proteins, fats, pigments, cell remnants and minerals (pre-treatment). After that, an acidic treatment (ASC) followed by an optional enzymatic treatment (PSC) is performed to extract collagen molecules that are finally recovered by salt precipitation, dialyzed and lyophilized.

(e) salt precipitation and recovery. Moreover, they also share the same working temperature, which must be kept around 4–10 °C in all steps, in order to avoid collagen denaturation.

Marine sources are firstly cleaned and reduced in size by cutting or mincing to facilitate next purification steps. Then, non-collagenous components are removed through chemical pre-treatments. Since the antigenicity of collagen is related not only to its telopeptides (i.e. non-helical regions) [27,74] but also to the presence of non-collagenous proteins, fats and pigments, as well as cells and cell remnants [18], these pre-treatments are very important for the removal of all these sources of antigenicity. The stripping of non-collagenous proteins is usually based on the use of sodium hydroxide (NaOH). The efficacy of the removal is dependent on time, temperature and concentration of NaOH solution [82,83]. A sample-to-solvent ratio of 1:10 to 1:20 (*w/v*), with a solution of 0.1 M NaOH, is recommended for a period of time that may vary from 6 h to 3 days, under continuous stirring at 4 °C [82,84,85]. In this process, the alkali solution is periodically changed every 12–24 h [86]. Then, washings with cold distilled water are performed until the pH stabilizes at 7.0–8.0. The use of a 10% solution of sodium chloride (NaCl) at 1:10 (*w/v*) sample-to-solvent ratio has been also proposed [87]. However, this treatment demonstrated a lower efficacy in removing albumins and globulins than the NaOH one. The removal of fats and pigments can be achieved through the use of alcohols or oxygen peroxide. Butyl alcohol is commonly used at a concentration of 10–20% at a sample-to-solvent ratio of 1:10 (*w/v*) for 24 h, and then discarded by repeated washing with distilled water [71,86]. Isopropanol is alternatively used at 10% (w/v) at a sample-to-solvent ratio of 1:80 (w/v) for 36 h under stirring, with no remarkable differences with butyl alcohol [84]. The removal of cell remnants (decellularization) can be performed by the use of sodium dodecyl sulfate (SDS) for approximately 12 h, followed by washing [88].

As previously mentioned, bone and scales samples require an additional demineralization step, which usually involves the use of 0.5 M ethylenediaminetetraacetic acid (EDTA), due to its chelating action over calcium ions [86]. Alternatively, 0.4–1.0 M hydrochloric acid (HCl) can be used at a sample-to-solvent ratio of about 1:3–1:10 (*w/v*) for 24 h [89]. The samples are then washed in distilled water for 24 h [84].

Following these pre-treatments, the collagen extraction phase can take place. Solubility of collagen in water is poor, due to the presence of strong cross-links in the triple helix structure. Thus, an acidic treatment is performed to cleave non-covalent inter- and intra-molecular bonds [90]. Among acids, acetic acid is the most widely used one, followed by citric acid and lactic acid [27]. Acetic acid is usually used at a concentration of 0.5–0.7 M [82,84,91,92] with a sample-to-solvent ratio of 1:6 (*w/v*) for 2–4 days, with continuous shaking [82,84]. The mixture is then centrifuged (at about 15,000–20,000*g* for 20–30 min at 4 °C) to separate the viscous liquid phase that contains the so-called acid soluble collagen (ASC) [92,93].

It is widely known that collagen has strong intermolecular covalent bonds in the telopeptide regions, which cannot be cleaved by acidic treatment alone. Therefore, enzymes can be also used in combination with acids, in the same processing step [36,94] or right after acidic treatment [95], in order to obtain higher collagen yields. To this aim, non-specific collagen enzymes such as pepsin, trypsin, papain, alkaline protease, bromelain, alcalase have been employed [34,76]. Among these, pepsin is the most widely used enzyme for MC extraction (in this regard, it is worth noting that pepsin is commonly of porcine origin, which renders the *halal/kosher* nature of pepsin-extracted MC highly debatable [96,97]). Pepsin solution is usually prepared with a concentration of 0.1–1%, and employed with a sample-to-solvent ratio of 1:6 (*w/v*) for 1–2 days [92,93]. Pepsin treatment is very useful to remove telopeptide regions and related non-collagenous proteins. The obtained collagen, which is named pepsin soluble collagen (PSC) or atelo-collagen, thus shows increased purity and reduced antigenicity compared to ASC [70].

At the end of either the acidic or the enzymatic treatment, collagen is recovered from the liquid phase with a salt precipitation process. NaCl is added up to a final concentration that can vary between 0.8 M and 2.6 M in buffered tris-hydroxymethyl-aminomethane (Tris-HCl) (pH 7.5) for 24 h [82,84,92,98]. The precipitate is then collected by centrifugation at approximately 15,000–20,000*g* for 30–60 min at 4 °C and dissolved in 0.5 M acetic acid [91,98]. Finally, the suspension is dialyzed against 0.1 M acetic acid [92], 0.02 M sodium phosphate ($Na_2HPO_4$) [84] or distilled water [34,99] until neutral pH is obtained (usually over 24 h), and then freeze-dried [85,100].

In the case of gelatin, a combined acid-alkali-acid treatment is performed to achieve extraction [73]. This consists of an acid treatment in 0.1 M acetic acid solution at 1:10 (*w/v*) for 1 h, followed by an alkali treatment with 0.1 M NaOH solution at 1:3 (w/v) for 1 h, and then another acid treatment with 0.1 M sulfuric acid ($H_2SO_4$) at 1:3 (w/v) for 1 h. At the end of each treatment, the material is neutralized and washed with distilled water. The final step is the effective extraction of gelatin: at this step, distilled water is added at 1:4–1:10 (w/v) ratio and kept under stirring at 60 °C for 1 h, or at 45 °C for 12 h [93]. The obtained gelatin solution is finally lyophilized and milled to obtain a powder.

When considering marine species different from fish, collagen extraction may vary from the protocol described above, although the five main procedural steps remain. Being jellyfish and sea sponges the most largely adopted MC sources among invertebrates, some peculiar details on the respective extraction protocols are provided in the following. With regard to MC isolation from other invertebrates (e.g. sea urchin, mollusks, etc.), the reader is directed to specific research articles for further information [39,101–104].

Collagen from jellyfish is commonly obtained from the mesoglea by solubilization in acetic acid solution, typically for three days [43,77]. Since jellyfish contains a huge amount of water (in the range 95–99%, depending on the species [88]), in order to improve the collagen solubility in dilute acids, and thus achieve a better yield, it is necessary to freeze-dry and filter the jellyfish raw material, prior to the extraction phase [43,47,77]. Extracts are then dialyzed against $Na_2HPO_4$ solution (for about 2–3 days), resulting in precipitated collagen, which can be separated by centrifugation. The obtained collagen can be then purified by a re-precipitation method, i.e. by a re-solubilization in acetic acid followed by a precipitation in NaCl.

In the case of marine sponges, different extraction methodologies have been set up, because of the sponge mineralization and insolubility in acid solutions [38,59,61,105]. In a typical protocol, fresh sponges sea water-conserved [57,62] or alcohol-conserved sponges (e.g. stored in ethanol [59,61] or in methanol [106]) are extensively washed with tap and distilled water, cut into small pieces and homogenized in an alkaline denaturing buffer (100 mM Tris-HCl: pH 9.5, 10 mM EDTA, 8 M urea, 100 mM 2-mercaptoethanol). The pH of the dispersion is adjusted to 9 by using NaOH. After 24 h of continuous stirring at room temperature, the viscous extract is centrifuged (at about 5000*g* for 5 min); the pellet is discarded, while the collagen in the supernatant is precipitated by adjusting the pH to 4 with acetic acid and then collected by centrifugation (at about 20,000*g* for 30 min). The pellet is finally re-suspended in distilled water, centrifuged and freeze-dried.

Overall, independently of the specific protocol used for a selected MC source, the extraction procedure can be very laborious, time consuming (Fig. 3) and quite difficult to scale up, mainly due to the multiple processing steps and the required amounts of water and other solvents. Moreover, the final MC yield for given sources can be very low, thus making the extraction particularly expensive.

### 2.3. Yields and process optimizations

The extraction yield is usually expressed as the percentage weight of collagen per unit weight of the source material, and can be calculated on either a wet or a dry weight basis [65]. The yield is clearly affected

by several parameters that are both species-related and process-related [34,71,75,107]. Firstly, the species, age, and type of tissue have a strong influence on the extraction efficacy. In addition, the collagen yield is determined by multiple extraction parameters or conditions, such as initial state of the biomass (e.g. fresh, frozen, freeze-dried), pH, ionic strength, time, temperature, sample-volume ratio, types of acid and enzyme used.

The temperature of the process affects all the extraction steps, from the tissue separation to the final collagen precipitation, due to its effect on protein unfolding. Animal species living in aquatic environment have characteristic body temperatures due to their evolutionary adaptation to the cold and wide marine environment. For this reason, denaturation temperature of MC usually does not exceed 30 °C. Thus, to prevent the protein unfolding at room temperature (25 °C), it is necessary to keep the environmental temperature at 4–10 °C during the whole process.

With reference to single extraction steps, a study by Liu et al. evaluated the effect of NaOH concentration of the alkaline pre-treatment on the collagen yield [107]. In particular, a concentration from 0.05 to 0.1 M was suggested to effectively remove non-collagenous proteins without inducing protein structure loss. Moreover, it was demonstrated that NaOH concentration above 0.2 M caused significant protein structural modification and a yield decrement [107].

The acidic extraction is principally influenced by acid concentration and time. For example, the acetic acid concentration has been directly correlated to the yield: a gradual increase of extracted collagen occurs up to a concentration of 0.6 M, while a further increase of acid concentration induces a yield reduction due to concurrent collagen denaturation [71]. As regards the influence of the treatment time length, higher yields are observed up to 36 h of treatment, while a gradual decline is observed for longer times due to protein degradation by the acetic acid solvent [71].

Also in the case of the enzymatic extraction, the yield is influenced by enzyme concentration and time. Generally, an increase of the enzyme concentration corresponds to an increase in yield [108]. This aspect was confirmed by several studies by Yu et al., that revealed a significantly increase of extracted collagen from 66% to 80% when pepsin concentration varied from 800 to 1200 U/g. However, when pepsin concentration exceeded 1200 U/g [34] the increase was not relevant, thus suggesting an upper limit of effective enzyme concentration. Moreover, the extraction yield of PSC was significantly increased by longer treatment times [34,108].

The effect of pH on the solubility of collagen has been recently investigated by Tan et al. [109]. Fish collagens extracted by different methods have been found to solubilize in the pH range 1–4 [109], with both ASC and PSC showing the highest solubility at pH 2 [109]. However, individual collagens show maximal solubility at various pH values, depending on the marine species [110]. At a pH value higher than 5, that is close to the protein isoelectric point, solubility sharply decreases because of molecular precipitation [111].

As regards the efficacy of the salt precipitation phase, the used NaCl concentration significantly affects the yield of the process. In general, the solubility of collagen tends to decrease with increasing NaCl concentrations [77,110]. This is ascribed to the fact that high concentrations of NaCl (e.g. > 3% *w/v* [112]) increase the ionic strength of the solution, and thus lead to protein precipitation by improving the hydrophobic-hydrophobic interactions between protein chains [100,109]. Moreover, the optimal NaCl concentration to be used also depends on the type of soluble collagen to precipitate, since ASC tends to be less soluble than PSC (likely due to its higher crosslinking) [111].

Table 1 provides some quantitative information on the extraction yields of type I collagen (calculated with respect to the wet weight, dry weight or by mean of the Hyp content on dry weight) reported in the literature for different fish species and tissues (skin and scales). As discussed above, the different yields are dependent not only on the species and tissues, but also on the specific extraction parameters used.

**Table 1**
An overview of reported extraction yields (calculated on the dry weight, on the wet weight* basis or by mean of the hydroxyproline content with respect to the dry weight**) of type I collagen from different fish species and tissues (skin and scales).

| Fish tissue | Species | % Yield(s) | | Reference(s) |
|---|---|---|---|---|
| | | ASC | PSC | |
| Skin | Balloon fish | 4.0% | 19.5% | [85] |
| | Bamboo shark | 9.4% | 8.9% | [85]* |
| | Bigeye snapper | 11.0% | – | [112]* |
| | Bighead carp | 21.0% | 30.0% | [115] |
| | Black carp | 16.0% | 27.0% | [115] |
| | Bonylip Barb Fish | – | 6.2% | [92]* |
| | *Brama australis* | 1.5% | – | [116]* |
| | Brownstripe red snapper | 9.0% | 5.0% | [111]* |
| | Catfish | 16.8% | 28.0% | [85] |
| | Catla carp (Indian carp) | 63.0% | 69.0% | [91] |
| | Carp | 41.0% | – | [117] |
| | Channel catfish | 26.0% | 29.0% | [93] |
| | Deep-sea redfish | 48.0% | – | [118]** |
| | Giant croaker | 15.0% | 20.0% | [119] |
| | Globefish | 62.0% | 67.0% | [120]** |
| | Grass carp | 26.0% | 20.0% | [115] |
| | Loach | 22.0% | 27.0% | [85] |
| | Horse mackerel | 17.3% | 22.5% | [121]* |
| | Nile tilapia | 26.0–39.0% | 22.0% | [93,100,122] |
| | Ornate threadfin bream | 25.0% | – | [85,123] |
| | Paddlefish | 53.0% | 66.0% | [120]** |
| | Rohu carp | 46.0% | 65.0% | [91] |
| | Seabass | 16.0% | – | [124] |
| | Surf smelt | 24.0% | – | [125] |
| Scales | Carp | 1.4% | – | [117] |
| | Deep-sea redfish | 7.0% | – | [118]** |
| | Flying fish | 0.7% | – | [110]** |
| | Grey mullet | 0.4% | – | [110]** |
| | Horse mackerel | 0.6–1.5% | – | [110]** |
| | Japanese seabass | – | 41.0% | [126] |
| | Lizard fish | 0.7–0.8% | – | [110]** |
| | Nile tilapia | 3.0% | – | [100] |
| | Red sea bream | – | 38.0% | [126] |
| | Sardine | – | 51.0% | [126] |
| | Seabass | 0.4% | 1.0% | [81] |
| | Yellowback seabream | 0.9% | – | [110]** |

Therefore, Table 1 should be intended as an overview of literature findings, rather than a direct comparison among fish species. However, what is worth noting is that, in some cases, the achieved yield can be quite low, despite the extraction process being complex and time-consuming.

Therefore, various process optimizations or modifications to enhance the MC yield, while also trying to reduce the extraction time and improve the process scalability, have been recently proposed. For example, ultrasonic treatment combined with acidic method has been shown to increase the MC yield and reduce the processing time of fish skin (Japanese seabass), compared to conventional ASC extraction [113,114]. Ultrasonic treatment time and amplitude have been found to affect the yield and the rate of collagen extraction [113].

In a similar approach, an acid-based treatment coupled with physical disruption (via ultrasonic treatment and vigorous mechanical stirring) has been recently proposed as an effective method for isolation of jellyfish collagen [65]. In particular, ultrasonic treatment and vigorous stirring of acidic suspensions of jellyfish (*Acromitus hardenbergi*) tissue have been shown to allow for collagen yields (on a dry weight basis) seven and two times higher than acid-based and pepsin-based extraction, respectively (≈40% yield for the optimized process vs. ≈8% and ≈20% for ASC and PSC) [65].

With particular focus on the industrial scalability of MC extraction, it is worthwhile to mention a novel and green technology proposed for the isolation of collagen from *Chondrosia reniformis* [58,105]. The process is based on a single and rapid step (3 h), in which collagen is

**Table 2**
Amino acid composition of type I collagen (ASC) extracted from different fish species and tissues (skin and scales). The high content of glycine, proline and hydroxyproline suggests the presence of the typical repetition $(Gly\text{-}X\text{-}Y)_n$ and the triple helical structure.

| | | Skin | | | | | | Scales | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fish species | Loach | Tilapia | Black Carp | Cod | Starry triggerfish [135] | Paddlefish [120] | Seabass [81] | Black Carp [86] | Tilapia [100] |
| | Reference(s) | [85] | [98] | [86,117] | [117] | | | | | |
| Amino acids | Alanine | 114 | 122 | 119 | 107 | 144 | 115 | 133 | 118 | 137 |
| | Arginine | 53 | 54 | 53 | 54 | 50 | 42 | 52 | 50 | 41 |
| | Aspartic acid | 53 | 52 | 48 | 53 | 46 | 44 | 44 | 52 | 58 |
| | Cysteine | 0 | 7 | 0.2 | 0 | 0 | 2 | 0 | 0.4 | 2 |
| | Glycine | 316 | 319 | 314 | 342 | 322 | 343 | 327 | 308 | 276 |
| | Glutamic acid | 89 | 74 | 78 | 80 | 73 | 61 | 71 | 83 | 47 |
| | Histidine | 5 | 4 | 4 | 8 | 6 | 5 | 7 | 4 | 13 |
| | Hydroxylysine | 5 | 0 | 2 | 7 | 5 | 8 | 6 | 4 | 10 |
| | Hydroxyproline | 95 | 57 | 69 | 51 | 84 | 86 | 85 | 94 | 72 |
| | Isoleucine | 11 | 13 | 12 | 12 | 10 | 10 | 11 | 13 | 12 |
| | Leucine | 21 | 28 | 22 | 22 | 16 | 16 | 21 | 25 | 25 |
| | Lysine | 22 | 29 | 29 | 29 | 26 | 25 | 27 | 25 | 52 |
| | Methionine | 13 | 15 | 14 | 15 | 13 | 14 | 15 | 11 | 12 |
| | Phenylalanine | 14 | 16 | 14 | 12 | 14 | 15 | 14 | 16 | 18 |
| | Proline | 117 | 106 | 133 | 103 | 109 | 112 | 108 | 112 | 135 |
| | Serine | 37 | 37 | 37 | 59 | 35 | 47 | 28 | 32 | 20 |
| | Threonine | 0 | 28 | 25 | 23 | 25 | 25 | 24 | 27 | 27 |
| | Tryptophan | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Tyrosine | 2 | 5 | 0 | 4 | 4 | 10 | 5 | 4 | 6 |
| | Valine | 16 | 25 | 22 | 19 | 18 | 23 | 22 | 22 | 35 |
| | Imino acid | 212 | 163 | 202 | 154 | 193 | 198 | 193 | 206 | 207 |
| | TOT. | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |

extracted in water that is acidified and pressurized with carbon dioxide, without the use of organic solvents. Besides being faster and more easily scalable than conventional methods, this process has been shown to lead to a 30% improvement of the extraction yield of the acid soluble fraction (resulting in about 12% collagen per gram of wet tissue).

## 3. MC properties

### 3.1. Amino acid composition

Collagen derived from aquatic species presents only slight differences in amino acid composition with respect to collagen from mammals. In particular, MC has been found to show high percentages of homology with bovine and porcine collagen [34,98,127,128]. With specific reference to fish collagen, several studies demonstrated that the collagen of many fish species is characterized by the typical repetition $(Gly\text{-}X\text{-}Y)_n$, and thus a high glycine (30%) and hydroxyproline (8–10%) content [92] (Table 2). Furthermore, low levels of cysteine, tyrosine, histidine, hydroxylysine and methionine and high concentrations of alanine, proline, hydroxyproline and glutamic acid are generally observed. Interestingly, low levels of histidine are commonly considered as an index of low antigenicity, since histidines are precursor to histamines that are associated to allergic responses [129].

Similar findings on amino acid composition have been reported for collagen isolated from jellyfish, with glycine being the most abundant amino acid (22–32%), followed by glutamic acid (10–12%), alanine (8–10%), proline (7–8%) and hydroxyproline (4–7%) [43,47,65,130–132]. It is worth noting that these reported findings are not universal for all jellyfish species, since some species revealed to be characterized by lower levels of glycine (12–17%), alanine (3–7%) [68,133], and few or almost no hydroxylated residues [134].

The high glycine content of MC indicates the stability of the triple helix configuration, since glycine is responsible for the formation of the α helix, and consequently for collagen conformation.

However, low glycine levels have been also found in some marine sources, like several sponge species. For example, a glycine content of only 18.9% has been reported in *Chondrosia reniformis*, together with a proline content of 5.4% and a hydroxyproline content of 2.4% [61]. Noteworthy, in glass sponges like *H. sieboli*, the X and Y positions can be both occupied by 3-Hyp and 4-Hyp, an unusual double hydroxylation that plays an important role in mineralization [27,51].

The imino acids, i.e. proline and hydroxyproline, play an important role in maintaining the structural integrity of collagen, thanks to the presence of pyrrolidine rings which reduce the degree of freedom of the polypeptide chain, strengthening the triple helix and consequently affecting the thermal stability of the molecule [84,85]. Therefore, the denaturation temperature is found to increase with the imino acid content [91,136]. In this regard, the content of cysteine also contributes to the thermal stability of the collagen molecule, since cysteine forms disulfide bridges between α chains of the collagen triple helix [66].

As a final consideration, it should be underlined that marine tissues contain a high amount of glycoproteins, which are strongly associated with collagen, and are thus commonly found as impurities [126,137]. This is the main reason why a certain discrepancy in the amino acid composition is observed among studies regarding the extraction of collagen from the same animal species. Indeed, specific extraction methods can affect the glycoprotein residues, thus leading to compositional differences. In this regard, the extraction site should also be taken into account when comparing different studies, since tissue-specific structural and chemical differences are present within the same animal species. For example, by looking at the amino acid composition of ASC from different fish species and tissues (Table 2), it is possible to observe slight variations in the amino acid content between ASC obtained from skin and scales of the same species (e.g. tilapia and black carp). These variations are attributable to technical issues associated to the respective extraction process and the residual glycoprotein amount [51,58,61].

### 3.2. Thermal stability

The amino acid composition, which determines the collagen triple helix stability, is strongly influenced by the animal species and the natural habitat temperature. In general, one of the major drawbacks of MC compared to mammal collagen is the reduced denaturation temperature, which may be particularly relevant in clinical applications [126,138].

**Table 3**
Denaturation temperatures of type I collagen extracted from different fish species and tissues (skin and scales). The denaturation temperature of collagen extracted from cold-water species is lower than that of collagen from warm-water species.

| Extraction site | Fish species | Td (°C) | | Reference(s) |
|---|---|---|---|---|
| | | ASC | PSC | |
| Skin | Amur stungeon | 33 | – | [98] |
| | Alaska pollack | 17 | – | [136] |
| | Balloon fish | 29 | 30 | [136] |
| | Bigeye snapper | 29 | – | [112] |
| | Bighead carp | 36 | – | [86] |
| | Black carp | 36 | 36 | [86,115] |
| | Black drum | 34 | 36 | [136] |
| | Bonylip barb fish | – | 32 | [92] |
| | Brama australis | 24 | – | [116] |
| | Brown backed toadfish | – | 28 | [136] |
| | Brownstripe red snapper | 32 | 31 | [111] |
| | Catla carp | 31 | 35 | [91] |
| | Carp | 28 | – | [117] |
| | Channel catfish | 32–36 | 36 | [93,109] |
| | Cuttlefish | – | 27 | [142] |
| | Deep-sea redfish | 16 | – | [118,136] |
| | Flatfish | 27 | – | [98] |
| | Globefish | 27 | 27 | [120] |
| | Grass carp | 36–37 | 36 | [37,86,98] |
| | Hake | 10 | – | [143] |
| | *Misgurnus anguillicaudatus* | 36 | 33 | [85] |
| | Nile perch | 36 | – | [144] |
| | Nile tilapia | 32–37 | 32–35 | [98,122,127,128] |
| | Ocellate puffer | 28 | 28 | [145] |
| | Pacific saury | – | 24–25 | [136] |
| | Paddlefish | 30 | 28 | [120] |
| | Rohu carp | 35 | 35 | [91] |
| | Salmon | 30 | – | [139] |
| | Seabass | 33 | – | [124] |
| | Sheepshead | 34 | 34 | [136] |
| | Silver carp | 28–37 | – | [86,98,136] |
| | Surf smelt | 33 | – | [125] |
| | Tiger puffer | 28 | – | [136] |
| Scale | Bighead carp | 36 | 35 | [86,146] |
| | Black carp | 35 | – | [86] |
| | Carp | 28 | – | [117] |
| | Deep-sea redfish | 18 | – | [118,136] |
| | Grass carp | 35 | 35 | [37,86] |
| | Grey mullet | 27 | – | [110] |
| | Horse mackerel | 25 | – | [110] |
| | Lizard fish | 27 | – | [110] |
| | Nile tilapia | 33 | 32 | [89] |
| | Northern pike | 29 | 27 | [136] |
| | *Pagrus major* | – | 28–30 | [126,136] |
| | Sardine | – | 28 | [126] |
| | Seabass | 38 | 28–39 | [81,126] |
| | Silver carp | 34 | – | [86] |
| | Yellowback seabream | 28 | – | [110] |

For instance, cold-water fish species present a decreased imino acid content [118,136], thus their collagen shows a lower denaturation/melting temperature (15–20 °C) than that derived from warm-water species (30–35 °C) (Table 3). This clearly discourages the use of collagen from cold-water species in the biomedical field, while encouraging the use of collagen from warm-water species. Several studies reported denaturation temperatures around 30 °C in salmon [139], 32–36 °C in channel catfish [93,109], 36 °C in carp [86,115], and 32–37 °C in tilapia [98,122,127,128]. The slight changes encountered in the literature on the denaturation temperature of collagen derived from the same aquatic species (and tissues) may be due not only to the use of different extraction methods, but also to the use of different experimental techniques to investigate the collagen thermal stability (e.g. differential scanning calorimetry, dynamic mechanical analysis, circular dichroism) [140]. As a matter of fact, denaturation temperature of salmon collagen was found to be about 30 °C if deduced from dynamical mechanical analysis [139] and about 20 °C if recorded by differential scanning calorimetry [141]. However, what emerges from Table 3 is that collagens from carp and tilapia, by presenting a thermal resistance higher than other marine species, seem to be promising alternative sources of collagen for clinical use.

Also with regard to invertebrate MC, the species and the natural habitat temperature significantly affect the observed thermal stability. As for jellyfish, denaturation of collagen derived from *Rhizostoma pulmo* has been detected at about 29 °C [49], while collagen from *Catostylus mosaicus* has been shown to denature at about 32 °C [66]. Several marine sponge species also seem to possess quite similar melting temperatures, e.g. 24–25 °C for *Axinella cannabina* [38], 30–31 °C for *Chondrosia reniformis* [58] and 28–32 °C for *Suberites carnosus* [38].

Although a low denaturation temperature may suggest different uses of MC, other than biomedical or clinical ones, it is noteworthy to recall that suitable crosslinking treatments (e.g. carbodiimide-based) can be performed to enhance the collagen thermal stability. Crosslinked jellyfish collagen, for example, has been successfully employed for in vitro cell culture [43,68] and implanted in vivo on animal models [69], showing great promise for future use in humans.

### 3.3. Safety

As previously mentioned, compared to mammal collagen, MC possesses a lower threat of diseases transmissible to humans, and is thus generally considered as safe.

However, when considering fish collagen, it is worth recalling that fish and fish products can be a source of allergy/anaphylaxis. Fish allergy is a pathophysiological immune response to selected fish proteins, mediated by IgE-type antibodies. In particular, type I collagen has been identified as a fish allergen [147], with a recent study evidencing that 50% of Japanese patients with fish allergies show specific IgE against fish collagen [148]. Collagen from cartilaginous fish has been found to show lower allergenicity than collagen from bony fish [34,149]. Fish gelatin, which is often used in the food industry and in pharmaceuticals to replace mammalian gelatin, has been also involved in allergic reactions [127,128,149]. Although the incidence of fish allergy is variable (from 0% to 8%), depending on local food habits and fish consumption, it is estimated that fish and fish-based products can trigger immune reactions in < 1% of the general population [149,150].

With regard to jellyfish collagen, the toxicity of the species should be obviously considered before selecting and studying a given source for collagen extraction [69]. In this perspective, edible jellyfish types represent preferential MC sources [65]. Among marine sponges, it is worth citing that *Chondrosia reniformis* has been one of the first species to be explored for MC extraction, mainly due to its edibility [59,62,105,151,152].

In general, specific biocompatibility tests are needed to prove the safety of the extracted material. For the production of medical devices or pharmacological products, MC should be compliant with all the international/national regulations or pharmacopeias. For instance, medical devices to be commercialized in Europe should meet the essential requirements defined in the Annex I of the Council Directive 93/42/EEC (which is going to be replaced by the Medical Device Regulation (MDR) 2017/745 in May 2020) [122,153]. The manufacturing process of MC and/or the related device, including all aspects going from the raw materials to the delivery of the final product, should be fully validated to ensure reproducibility and safety for human use [27]. In this context, a number of research studies are currently directed to investigate the biocompatibility of MC and related devices, in accordance with the biocompatibility tests prescribed by the standard ISO 10993 [34,127,128,154]. Several devices based on Tilapia type I collagen, for example, have been shown to be highly biocompatible by in vivo and in vitro assays [82,155–157]. Similarly, encouraging *in vitro* and *in vivo* findings on collagen derived from jellyfish [43,49,68,69] and sea sponges [61,62,158] support their use as safe alternatives to

mammalian collagen, for the production of medical or pharmacological products.

For the food sector, where fish gelatin (rather than collagen) finds extensive application, the material must meet the requirements dictated by specific regulations, e.g. the Food Hygiene Regulation 853/2004 in Europe [150].

## 4. Applications

Being the main component of the ECM, collagen is intrinsically bioactive [2]. Widely known is, for example, the ability of collagen to improve wound healing [159] and reduce scar formation [159,160]. In addition to the topical application of collagen-based products (e.g. pomades) to enhance skin healing or skin appearance [95,161,162], ingestion of collagen-based supplements is an alternative route for administration, which is increasingly adopted in the cosmetic and nutraceutical sectors [163–165]. The formulation of several dietary supplements includes small collagen peptides or fragments, which are highly absorbed by the intestinal barrier, and thus have superior bioavailability compared to other collagen products.

With focus on MC, MC-based supplements have been found to lead to a faster and more complete healing of wounds [27,166], thanks to their anti-inflammatory action that inhibits the expression of key pro-inflammatory cytokines such as the tumor necrosis factor alpha (TNF-α), the interleukin 1 beta (IL-1β), the interleukin 8 (IL-8), and the inducible nitric oxide synthase (iNOS) [167]. MC peptides can also help the absorption of calcium and other minerals essential for bone strength, nails and hair [168–171], and can stimulate collagen synthesis in bones, joints and skin. When present in injectable gel formulations and/or in dietary supplements, MC contributes to reduce the risk of joint deterioration and inflammation [172,173]. Interestingly, recent studies also suggest the potential of ingested MC or MC fragments for weight management [174,175] and glycemic control [176–178]. Finally, MC represents a useful source of various antimicrobial peptides [179]. Among them, collagencin has been shown to inhibit the growth of a broad spectrum of bacteria, by interacting with both anionic (phosphatidylglycerol) and zwitterionic (phosphoethanolamine and phosphatidylcholine) lipids [180].

Based on the above-mentioned bioactive properties, MC use in health-related sectors is rapidly expanding, and involves multiple MC forms, including: a) the native collagen form, for specific biomedical applications (e.g. regenerative medicine); b) the gelatin form, for the encapsulation of drugs and nutrients; c) the peptide form, with peptides of low (0.1–1 kDa) [166] and high (1–10 kDa) [181,182] molecular weight (MW), for cosmetics, nutraceuticals and nutricosmetics, and dietary supplements. Some of the current uses of MC and its derivatives are discussed in more detail in the following.

### 4.1. Food and nutraceuticals

The food industry makes extensive use of gelatin from both marine species and mammals (Fig. 4). In particular, gelatin is prevalently used as a consistence enhancer and a food stabilizer. The latter role is due to its anti-microbial and anti-lipoxidation activity, which helps to preserve the taste and the shelf life of foods. Lipid oxidation develops undesirable toxic reaction products that are dangerous for human health [183]. Thus, the addition of gelatin, which inhibits the peroxidation, prevents foods from deterioration and protects the consumers from related diseases [164]. Gelatin can also serve as an outer protection film against dehydration, light, and oxygen [73,135]. The layer indeed acts as a barrier that controls the migration of oxygen, provides permeability to water vapor and prolongs the shelf life of foods.

As a nutraceutical/active ingredient, gelatin is further employed to enrich food protein content. In the larger context of food and beverages, it is worth noting that the production and consumption of functional drinks enriched with hydrolyzed collagen has considerably increased

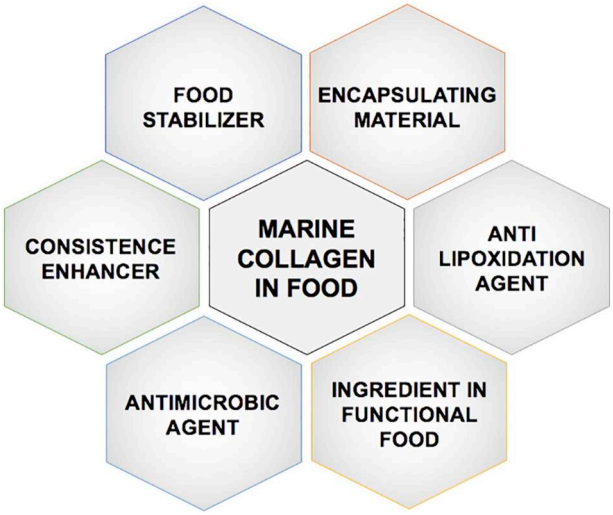


**Fig. 4.** Multiple functions of the seafood derived gelatin in the food industry. The food industry makes extensive use of gelatin from both marine species and mammals. Marine collagen, that is BSE-risk free, is widely used as food stabilizer, thanks to its anti-microbial and anti-lipoxidation activities that help in preserving food consistence, taste and shelf life. Collagen-based films are also used to encapsulate drugs, nutrients, heat-sensitive molecules and to protect aliments from dehydration, light, and oxygen. Moreover, marine collagen potential to improve health, increase longevity and reduce the risk of diseases supports it use as a nutraceutical ingredient.

over years [76], driven by the collagen potential to improve health and increase longevity [184–188]. Thanks to some evidences on the influence of specific MC peptides on bone formation, bone mineral density and osteoarthritis [171,172,189], Konig et al. investigated the effect of 12-month daily oral administration of 5 kDa peptides on post-menopausal women with low bone mineral density [170]. This clinical study demonstrated that the intake of MC peptides could increase bone mineral density and formation, while reducing degradation [170].

With regard to the development of capsule-based nutraceutical formulations, a great consumption of fish-derived gelatin is found in the capsule industry. Gelatin is indeed commonly employed for the encapsulation of heat-sensitive vitamins and other nutrients [190,191].

### 4.2. Cosmetics and nutricosmetics

For centuries, foods enriched in collagen have been favored by Asian people with the expectancy of an anti-aging effect on skin, with no documented scientific proofs [162]. Only in recent years, a small but growing number of evidences have pointed out the role of MC as enhancer of skin hydration and elasticity, minimizer of wrinkles and repairer of photo-damaged collagen and elastic fibers [61,161,162,192–199].

Human skin is composed of epidermis, dermis, and subcutaneous tissue [200]. In the dermis, collagen and elastin maintain the structure and the elasticity of skin [201]. Notably, type I collagen, which constitute the 80% of dermal collagen [162], plays a major role in maintaining the mechanical strength of skin. With age, new collagen production decreases and consequently dermis structure declines [200]. Common skin anti-aging formulations are based on collagen for its natural moisturizing, softening and glowing properties [76,139]. Compared to high MW collagen, cosmetic formulations use hydrolyzed collagen for its superior solubility at neutral pH, its easy dermis penetration and its water-binding properties [76].

In particular, MC showed to be effective in cosmetics due to its antioxidant properties. The oxidative stress has a key role in the aging process [202]. Normally, the endogenous antioxidant enzymes can scavenge reactive oxygen species (ROS) to protect skin from the

injuries. With age, the antioxidant capacity inevitably decreases, leading to oxidative stress and skin aging. MC gelatin and peptides significantly increase the activities of antioxidant enzymes and consequently ameliorated the oxidative stress of skin [162].

The efficacy of ingestible MC formulations is based on the high digestibility of peptides and the related almost immediate availability in the blood stream [162, 195]. Upon oral intake of collagen hydrolysates, small peptides derived from collagen (such as the tripeptide Gly-Pro-Hyp) can be detected in the human blood, with peak concentrations observed after 1–2 h [203,204]. Peptides then transfer from the blood vessels to the integumentary system, thus stimulating dermal fibroblasts to synthesize collagen, elastic fibers, and hyaluronic acid.

The effect of marine dietary supplements on human skin appearance has been explored in recent clinical trials [205]. Two randomized, double-blind, placebo-controlled studies on 30–60 aged people have showed that the administration of MC peptides in daily drinking formulations can help improving skin hydration, wrinkling, elasticity and density [192, 195]. The designation of marine peptides as an effective anti-aging ingredient opens a new way to MC in the cosmetic sector. Interestingly, one of the above-mentioned clinical studies also highlighted that MC can act not only on skin appearance, but also on hormonal levels. Indeed, MC peptides of about 2000–3000 Da positively have been found to influence the Plasma Growth Hormone (PGH) and the Insulin-Like Growth Factor-1 (IGF-1) [192].

Thus, all pre-clinical and clinical trials, albeit few, confirmed MC ability to maintain or improve skin conditions. The designation of marine peptides as an effective anti-aging ingredient opens a new way to collagen byproducts in the cosmetic sector.

### 4.3. Weight management and glycemic control

An emerging application of marine gelatin and peptides arises from the recent discovery of their implication in the regulation of the sense of satiety, fat metabolism and type 2 diabetes (T2D) [177,206–208]. Obesity is a global epidemic involving the accumulation of body fat. In 2016, the number of obese people in the world was estimated to be > 650 million, and this incidence is expected to rapidly increase [209]. Obesity not only generates social and aesthetic problems but most of all, increases the risk of associated comorbidities like T2D, cardiovascular diseases, obstructive sleep apnea, osteoarthritis and depression [210]. Among these, T2D is the most common comorbidity and the most widespread type of diabetes, in which excessive blood glucose levels are due to body insulin resistance and/or insufficient insulin production. It is estimated that 422 million adults lived with diabetes in 2014, and the prevalence is growing similarly to what observed for obesity [209]. For this reason, treatment options for T2D have greatly expanded in the last decades. Interestingly, based on in vitro and in vivo preclinical studies, as well as on recent clinical trials, dietary supplements including marine bioactive peptides seem to have the potentiality to prevent and treat at the same time obesity and T2D [206,211].

In obese patients without T2D, it is important to try to prevent the insurrection of the disease itself by limiting the nutritional intake of fat through a healthy diet. The standard prophylaxis procedure could be supported by the ingestion of collagen-based preparations that induce weight loss and reestablish lipid and blood sugar levels. In vitro studies on 3T3-L1 preadipocytes revealed that collagen peptides inhibits the adipogenic differentiation by reducing intracellular lipid droplets, the expression of a key adipogenic gene 2 (aP2), the levels of the α master adipogenic transcription factors (C/EBP-α) and of the γ-peroxisome proliferator-activated receptor (PPAR-γ) [212]. Further cell studies confirmed the inhibition of the adipogenic differentiation of mesenchymal stem cells by fish hydrolysates [213].

A number of recent works have also investigated the in vivo effects of marine hydrolysates on fat metabolism [163,212] (Table 4). Orally administered MC peptides were found to significantly reduce body fat and weight in high caloric diet fed mice [185,212,214,215]. Moreover, as demonstrated in vitro, collagen peptides drastically reduced the expression aP2, C/EBP-α and PPAR-γ in the epididymal adipose tissue of obese mice [206,212]. At the same time, the expression of the genes involved in the biosynthesis of unsaturated fatty acids, PPAR signaling pathway and fatty acid metabolism were up-regulated [174]. In particular, the peroxisome proliferator activated receptor-α (PPAR-α) was significantly expressed. This nuclear hormone receptor plays an important role in the modulation of insulin sensitivity, besides functioning as a lipid sensor that controls fatty acids burn [178]. Thus, its overexpression led to an increase of the fatty acid metabolism and then fats burn. Also in serum, levels of total cholesterol, triglyceride and Low-Density Lipoproteins (LDL) decreased while High-Density Lipoproteins (HDL) increased [174,211,212,214]. Furthermore, it has been discovered that MC peptides can inhibit angiotensin I-converting enzyme (ACE) [216], and therefore have the potential to reduce both hypertension and hyperlipidemia [217].

As regards T2D, insulin resistance is a primary pathophysiological factor. As a cascade of events the oxidative stress, the activity of the glucose transporter type 4 (GLUT-4) and the PPAR-α activity are all implicated in the pathogenesis of insulin resistance [177,206,218,219]. Orally administered MC peptides have been found to reduce oxidative stress, inflammation, and modulated GLUT-4 and PPAR-α expression in rat models of T2D [178]. PPAR-α expression in the liver of diabetic rats suggests that MC peptides can act on diabetes by enhancing insulin sensitivity. Almanza-Perez et al. have recently proposed that the regulatory effect of collagen on some factors related to storage and energy burning, such as PPAR-α, -γ, -δ, and uncoupling protein type 2 (UCP2), is due to the amino acid glycine [211,220]. The supplementation with collagen peptides effectively attenuated hepatic fat accumulation through the inhibition of fatty acid synthesis and the enhancement of the β-oxidation in fat mice liver [211].

Further studies in rats with induced T2D demonstrated that marine hydrolysates increased adiponectin and decreased leptin and resistin levels, thus suggesting that MC peptides can enhance insulin sensitivity through the regulation of adipocytokines release, a family of inflammatory mediators correlated with diabetes [178,214]. The blood glucose values in diabetic mice fed with MC peptides were also reported to be significantly reduced, with an evident hypoglycemic dose-dependent effect [163,215]. The ability of MC peptides to reduce blood sugar levels could be attributed to their antioxidant property, detectable by superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) activities: in diabetic mice fed with MC peptides, SOD and CAT activities were increased and MDA activity was decreased, so that to assume enzymatic levels close to normal levels of non-diabetic group [163,215]. This hypothesis was also confirmed by Zhu et al. who evaluated the oxidative stress on glutathione (GSH) and nitric oxide (NO) [178].

Although there are no regulatory limits on the maximum amount of MC peptides to be administered, it should be noted that an excessive peptide concentration might induce damage to internal organs. Experiments on mice indeed revealed that, while body weight was significantly reduced by the high-dose administration of collagen peptides (10%), internal organs such as liver, thymus, spleen and kidney markedly increased their volume, suggesting the hyperplasia of the primary immune and metabolic organs [162].

Overall, based on the encouraging preclinical results and on the absence of any adverse or toxic effects upon proper peptide dosage [174], clinical studies on MC dietary supplementation have been also performed (Table 5). The oral administration of fish collagen formulations is preferred, considering the high absorption of MC derivatives through the intestinal wall [162, 195, 203, 204]. Naticol® is an example of commercially available fish type I collagen peptide [185] that is currently undergoing a randomized clinical trial (NCT03872297) on overweighed participants to assess its effect on body weight and fat mass.

**Table 4**
Effect of MC on weight management and glycemia control: in vivo preclinical studies on animal models.

| Investigated MC and dosage | Animal model and administration route | Time length of administration | Parameters of interest | Outcomes | Ref. |
|---|---|---|---|---|---|
| Type I collagen peptides from Tilapia skin of 180–1000 Da<br>Dose: 0.85–1.70 g/kg/day | KM mice<br>Intragastric administration | 1 month | - Glucose levels<br>- Enzymatic assay on pancreatic extracts | - Free radicals scavenging activities<br>- Hypoglycemic effects<br>- Decrease of polydipsia Decrease of polyphagia | [163] |
| Type I Collagen oligopeptides of 130–3000 Da from skin salmon<br>Dose: 2.25–4.5-9.9 g/kg/day | Wistar rats<br>Intragastric administration | 1 month | - Glucose and insulin levels<br>- Oxidative stress<br>- Inflammation | - Decrease of glucose and insulin levels<br>- Increase of blood lipid levels<br>- Decrease of oxidative stress<br>- Decrease of inflammation<br>- Enhance insulin sensitivity<br>- Improve liver steatosis | [178] |
| Type I collagen peptides of 200–500 Da from tuna skin and scales<br>Dose: 0.3 g/kg/day | ICR mice<br>Intragastric administration | 2 months | - Body weight<br>- Food intake<br>- Adipose tissue analysis<br>- Plasma levels of total cholesterol, triglycerides, HDL and LDL | - Inhibition of lipid accumulation<br>- Decrease of body weight without a significant difference in food intake<br>- Decrease of total cholesterol, triglyceride and LDL<br>- Increase HDL | [212] |
| Type I collagen from fish 4000–600 kDa<br>Dose: improvement of diet protein content of 6% | BALB/cCrSlc mice<br>FD supplementation | 2 months | - Body weight<br>- Food and water intakes<br>- Plasma levels of total cholesterol, triglycerides, HDL and LDL | - No significant differences in body weight gain and liver lipid content<br>- Increase of liver lipid metabolism<br>- Decrease of total cholesterol | [174] |
| Type I (mainly) and III collagen peptides of about 2 kDa from warm sea fish skin (Naticol®)<br>Dose: 4 g/kg/day | C57Bl6/J mice<br>In drinking water | 5 months | - Glucose and insulin levels<br>- Metabolic and inflammatory parameters<br>- Body weight<br>- Body composition | - No alteration of food or water intake<br>- Decrease of body weight due to a decrease in fat mass<br>- Lower rise in basal plasma glucose levels<br>- Higher levels of triglycerides with lower amount of plasma cholesterol<br>- Slight but not significant decrease in plasma insulin | [185] |
| Type I collagen peptides of 1050 Da from skin skate<br>Dose: 0.1–0.2-0.3 g/kg/day | C57BL6/J mice<br>In drinking water | 2 months | - Body weight<br>- Adipose tissue weight and size<br>- Lipid levels in plasma and hepatic tissue<br>- Liver beta-oxidation, fatty acid synthesis, cholesterol metabolism | - Decrease of body weight and visceral adipose tissue weight<br>- Decrease of plasma and hepatic lipid levels<br>- Suppression of hepatic lipid accumulation<br>- Decrease of the lipid droplet size in the adipose tissue (dose dependent effect) | [211] |
| Type I collagen and other proteins from sardine by-products<br>Dose: 20 g/kg/day | Wistar rats<br>HFD supplementation | 28 days | - Body weight<br>- Plasma levels of total cholesterol, triglycerides, HDL and LDL | - Decrease of food intake<br>- Decrease of body weight<br>- Decrease of blood cholesterol, LDL, HDL | [214] |
| Type I collagen from fish scales<br>Dose: 1 g/kg/day | Wistar rats<br>Intragastric administration | 1 month | - Body weight<br>- Glucose and insulin levels<br>- Antioxidant activity<br>- Cytokines levels | - Decrease of body weight and body mass index<br>- Antioxidant effect<br>- Decrease proinflammatory cytokines<br>- Decrease glucose level | [215] |

**Table 5**
Use of marine collagen gelatin/peptides in clinical trials. Clinical trials demonstrated that the daily intake of marine peptides as dietary supplements or beverages (by increasing the protein content in the ratio protein/carbohydrates/fat, normal ratio 10:55:35) can induce weight loss, limit the food intake and reestablish lipids, insulin and blood sugar levels. Asterisk (*) indicates the ongoing Naticol study "Fish Collagen Peptide Food Supplement on Weight and Body Composition (NATICOL)", with a ClinicalTrials.gov identifier: NCT03872297.

| Food supplement and dosage | Participants (number, age and health conditions) | Time | Analyses | Outcome(s) | Ref. |
|---|---|---|---|---|---|
| Hydrolyzed gelatin<br>Dose: 20 g | 22<br>19–48 years<br>Obese | 3 h | Blood analysis | Increase of GLP-1 and insulin<br>Increase of sense of satiety | [175] |
| Gelatin<br>Dose: 25/55/20 | 24<br>23–27 years<br>Healthy | 3 d | Blood analysis<br>Appetite evaluation | Hunger suppression of gelatin 40% more compared to casein | [232] |
| Gelatin<br>Dose: 25/55/20 | 23<br>18–55 years<br>Healthy | 3 d | Blood analysis<br>Appetite evaluation<br>Energy expenditure | Positive energy balance<br>Hunger suppression 10%<br>Increase of GLP-1 | [221] |
| Oligopeptides 130–3000 Da<br>Dose: 13 g | 150<br>60–65 years<br>T2DM | 3 m | Blood analysis | Decrease of glucose, total triglycerides, total cholesterol, LDL and free-fatty acids<br>Increase of insulin sensitivity and HDL | [177] |
| Peptides<br>(MW not reported)<br>Dose: 10 g | 60<br>21–50 years<br>T2DM | 3 m | Blood analysis<br>Urine analysis | Decrease of FBG and HbA1c<br>Increase of insulin sensitivity and GLP-1 | [186] |
| Peptides (MW not reported)<br>Dose: 2 g | 90<br>20–60 years<br>Obese | 3 m | Blood analysis<br>Body fat analysis | Decrease of body fat and body fat mass<br>No improvement of lipid profile (in contrast with animal studies) | [226] |
| Peptides<br>(MW not reported)<br>Dose: - | 40<br>18–60 years<br>Obese | 3 m | Blood analysis<br>Body fat analysis | Results not reported | * |

Besides all the aforementioned influences of MC on fat acid metabolism, insulin resistance and oxidative stress, clinical trials on humans are also focused on the capability of gelatin and peptides to induce satiety, likely by swelling and retaining great amounts of water in the stomach [177,221]. In this regard, several studies reported that an increased protein intake increases satiety and favors weight loss [221–224]. Furthermore, clinical evidences show that MC peptides act on lipid metabolism by reducing total triglycerides, total cholesterol, LDH, free-fatty acids levels, along with fasting blood glucose and insulin [175,177,186,225]. Thus, it seems that marine peptides help in weight maintenance by also reducing the post-prandial blood sugar levels [206–208,226].

In this context, a possible explanation for the role of MC in the management of both weight and T2D is based on the down-regulation of the Dipeptidyl Peptidase IV (DPP-IV) [186], an enzyme present in the liver, kidney and small intestine and also as a soluble circulating form. This serine protease enzyme specifically acts on proline or alanine residues in the second position of the N-terminus of polypeptides [227,228]. Since the levels of the glucose-dependent insulinotropic polypeptide (GIP) and the glucagon-like peptide-1 (GLP-1) increase thanks to DPPH inhibitors, a therapy against the protease enzyme could be effective for the treatment of T2D [229,230]. MC peptides, that are rich in proline, have a DPP-IV inhibitory property. Therefore, by oral ingestion of collagen formulations, the inhibition of DPP-IV increases the level of GIP and GLP-1 hormones and subsequent the insulin level [231]. Devasia et al. conducted a clinical trial based on the intake of food supplements as DPP-IV inactivators, and observed reduced levels of blood glucose and glycosylated hemoglobin (HbA1c) coupled with an increased insulin sensitivity. This suggested the anti-diabetic properties of MC peptides by increasing the GLP-1 level [186].

### 4.4. Tissue engineering and wound healing

Tissue engineering is an interdisciplinary field of science, which deals with the regeneration or reconstruction of a variety of tissues or organs [233] by means of three elements: scaffold, cells and regulators. The scaffold is meant to mimic the ECM, i.e. a 3D network composed by collagen fibers and proteoglycans that provides structural support to cells besides biochemical and biomechanical signals important for morphogenesis, differentiation and homeostasis. First of all, the materials used for the scaffold synthesis should be biocompatible, i.e. they should not induce cytotoxic or immunological reactions, and should be bioactive. As such, among natural materials collagen is definitely considered as the most suitable one for scaffold manufacturing, as it is not only highly biocompatible but also promotes cellular migration, cell-matrix interactions and tissue regeneration.

In particular, several research studies have recently highlighted the suitability of MC as a biomaterial for tissue engineering and wound healing applications [76,83,86,88,128,138,157,160,169,234–243]. In these applications, collagen thermostability is a not negligible parameter, since the material/device should withstand the human physiological temperature (37 °C) for enough time to allow for tissue regeneration [138]. As aforementioned, collagen from aquatic sources generally has a denaturation temperature below 37 °C, although depending on the animal species and habitat (Table 3). Therefore, thermostability of MC-based scaffolds is often improved by crosslinking with chemical compounds, such as 1-ethyl-3-(3-dimethylaminoprophyl)-carbodiimide (EDC), genipin (GEN), tea polyphenol (TP), nordihydroguaiaretic acid (NDGA), diphenyl phosphoryl azide (DPPA) and glutaraldehyde (GTA), and/or with physical treatments (e.g. dehydrothermal crosslinking) [83,128,157,236–238]. Crosslinking is also useful to increase the mechanical strength and the stiffness of collagen-based devices, while delaying the degradation rate.

With the same aim of enhancing the material stability, few attempts have been also made by blending MC with other biomaterials, thus developing multicomponent scaffolds [236–240]. In some formulations, MC is specifically added to facilitate cell adhesion and proliferation, as well as to improve mechanical properties such as strength and elasticity, as found for chitosan-based scaffolds [240]. As an example, Cao et al. developed composite collagen/chitosan/chondroitin sulfate scaffolds for skin regeneration (with collagen derived from silver carp skin), further containing PLGA microparticles for localized delivery of the basic fibroblast growth factor (bFGF) [238]. A similar approach has been proposed by the same research group for the synthesis of collagen/

chitosan films added with biotinylated glycol chitosan nanoparticles for localized delivery of Doxorubicin [239]. Raftery et al. attempted to develop a multifunctional sponge-like device for bone regeneration, based on a salmon collagen/chitosan blend in alternative to bovine collagen/chitosan [236]. However, in this case the use of salmon-derived collagen did not allow reaching mechanical properties and degradation resistance suitable for use in orthopedic tissue engineering [236]. Recent efforts have been made to exploit marine extracts as bioinks for 3D-bioprinting [244–247]. Fish skin collagen [247], gelatin [245] and sea anemone-derived silk-like protein (aneroin) [246] were employed in blend with other biomaterials for the development of natural printable and biocompatible inks to be used for the construction of 3D scaffolds with eligible mechanical properties and biodegradability.

With focus on bone regeneration, it is worth noting that, among various kinds of bone grafts, marine xenografts have attracted much interest in the last years, based on the evidence that hydrolyzed MC, with MW ranging from 0.7 to 1.3 kDa, can induce the multidirectional differentiation of rat bone marrow mesenchymal stem cells and the osteogenic differentiation of human periodontal ligament cells [86,169]. Furthermore, in vivo tests on rabbits using decellularized fish scales as defect fillers revealed an increase of spongiosa and cortex formation, along with the presence of hypertrophic osteoblasts as a sign of good bone healing [88].

As regards wound healing, several studies showed that collagen from MC could rapidly and effectively accelerate wound healing and can thus be used as a promising dermal substitute to treat severe wounds [160]. For example, in vivo tests with collagen sponges, based either on ASC or PSC from tilapia skin, led to increased wound healing in a rat model, with enhanced fibroblast proliferation, collagen synthesis, re-epithelialization and dermal reconstitution [160]. Zhou et al. developed sponges and electrospun meshes based on tilapia skin collagen as potential wound dressings and reported favorable results both in vitro and in vivo [241,242]. A cuttlefish skin collagen-based gel formulation was also characterized and proposed for topical application: in a rat model, the gel led to a significant increase in the percentage of wound closure over a period of 12 days, when compared to the standard drug-treated animals [243]. Several studies also demonstrate the potential of jellyfish-derived collagen sponges for wound healing applications. In a study by Cheng et al. [43], EDC-crosslinked collagen sponges derived from *Rhopilema esculentum* demonstrated high hemostatic ability compared to control gauzes. In another study, both uncrosslinked and EDC-crosslinked collagen sponges derived from *Rhizostoma pulmo* were found to be well tolerated in vivo upon subcutaneous implantation in a rat model, similarly to control bovine collagen sponges [69].

## 5. MC market

Based on the multiple and extensive uses of collagen (and its derivatives) in the biomedical, cosmetic, food and pharmaceutical fields, it is quite difficult to provide precise estimates of the industrial collagen demand, as well as of the global market size, its segmentation and the respective growth rates. A few years ago, the industrial demand of collagen was reported to account for > 326,000 tons per year [58]. While in 2018 the global market size was estimated at USD 620.3 million, a rapid growth is expected in the next few years, due to the increasing uses of collagen in various industrial sectors. In particular, the global collagen market is expected to attain a value of USD 897.5 million by 2023 [248]. Among the different animal sources of collagen, bovine collagen currently dominates the market, accounting for a share of nearly 35% [249]. This is due to the abundance of bovine-based raw materials and the lower prices compared to marine and porcine sources [249].

Based on the current average selling prices of collagen-based products, the target cost of a collagen source should be < 100 USD/kg for food applications and in the range 5000–50,000 USD/kg for healthcare products, in order for it to be commercially competitive [27]. The reportedly higher cost of MC over bovine collagen may be related to the difficult scale-up of the extraction process, as discussed previously, as well as to the relatively low yield. According to a recent business report [250], the average industrial yield of MC is estimated at about 1–2% vs. 8–20% for bovine sources, with average prices of ≈44,000 USD/metric ton for MC and ≈33,000 USD/metric ton for bovine collagen.

Despite the higher MC cost, MC share is expected to witness the fastest growth in the global collagen market [249]. MC is indeed increasingly attracting the interest of researchers and industries, especially for applications in the healthcare and cosmetic sectors. Here, MC seems superior to mammalian collagen owing to its higher absorption rate and bioavailability [22,150]. Therefore, the possibility to obtain high added value products, with improved properties for healthcare and wellbeing, may justify the use of expensive MC over other collagen sources. Conversely, for low-value products, e.g. food, bovine source holds the cost leadership, thus being much more advantageous. This is quite in line with the market report of the Trash2Cash project in 2011, which reported a small request of fish derivatives (gelatin and peptides, mostly used in the food sector) among the entire gelatin market (2000–3000 tons per year with a cost 11–17 USD/kg) [251].

Overall, the increasing demand for healthcare and nutraceutical supplements is currently driving the MC market, which was valued at USD 581.3 million in 2017 and is forecasted to reach USD 897.5 million by 2023 [248,252]. The healthcare sector was the largest application segment in 2018 and is expected to retain its dominant position accounting for a 48% share of the market volume in 2025 [249]. The use of collagen in medical devices and drug delivery systems is expanding owing to the increased trend towards minimally invasive technologies and its effectiveness in wound healing [160,166]. Several researches promote the use of MC for bone regeneration, due to its assessed ability to encourage bone development [88,171,213,253–256]. Moreover, MC increasing consumption as a dietary supplement is carried on by its overall benefits such as bone growth, weight loss promoter and above all anti-aging activity [170,189,257,258].

The second profitable area of interest is that of cosmetics, a sector for which the increase of lifetime and of aging factors, such as stress and pollution, boosted the search for effective anti-oxidant and anti-aging products [139,198]. Thus, anti-aging formulations are one of the key market drivers for MC [248].

Among the different marine sources, it is worth noting that fish collagen (FC) holds the lion's share in the commercial MC exploitation [248]. This is likely ascribable to the large availability of low cost discards of the fish processing industry and the resulting environmental pollution, which have fueled the FC research in the last two decades. For given marine sources (e.g. some invertebrate species), the sustainability of the source, in terms of supply costs and sufficient and continuous quantity, may be one of the limiting factors for achieving an effective industrial exploitation [27]. Yet, several companies (e.g. Jellagen Ltd., https://www.jellagen.co.uk; Jellyfish Research Laboratories Inc., https://www.jfish-lab.com/home-en; Certified Nutraceuticals Inc., https://www.certifiednutra.com) are currently performing significant investments on marine sources different from fish for collagen extraction, as the biocompatible and bioactive properties of the isolated collagens hold promise for advanced clinical and cosmetic applications, where higher costs of source supply and collagen production are still acceptable.

Some of the global leaders in the production of FC are listed in Table 6, along with their main interest areas. It can be observed that most of the target sectors currently involve the use of FC derivatives (i.e. gelatin and peptides), rather than native collagen. However, the promising results of recent studies, based on the use of both FC and marine invertebrate collagen [249], suggest that the market of native collagen is also going to increase in the future, to allow for the development of novel medical devices (especially for wound healing) [249].

**Table 6**
The most important world suppliers of fish collagen and its derivatives. MC companies are mainly based in Asia and America.

| Company | Fish tissue | Products | Interest areas | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Europe<br>America<br>Asia | Skin scale | Collagen<br>Gelatin<br>Peptides | Cosmetic | Nutricosmetic | Pharmaceutical | Food | Nutraceutical | Weight management | Others |
| Italgelatine (Italy) www.italgelatine.com | – | Peptides | x | x | x | x | | | |
| Lapi gelatine (Italy) www.lapigelatinee.com | Skin | Gelatin<br>Peptides | x | x | | x | x | | |
| COLLAswiss (Switzerland) www.collaswiss.com | – | Peptides | x | | | | x | x | |
| Weischardt group (France) www.weichards.com | Skin | Gelatin<br>Peptides | x | x | | x | x | x | |
| Copalis (France) www.copalis.fr | by-products | Collagen<br>Peptides | x | x | | | | x | Pet food |
| Seagarden (Norway) https://seagarden-norway.com/ | Skin | Peptides | x | | | | x | | |
| BHN Beauty Health Nutrition (Japan) http://bhn.co.jp/ | Skin and scales | Gelatin<br>Peptides | x | | x | x | | | |
| Nitta Gelatine Inc. (Japan) www.nitta-gelatine.com | Scales | Collagen<br>Gelatin<br>Peptides | x | x | x | x | | x | Photosensitive material<br>Automobile safety research |
| Jellice (Japan) http://jellice.com.tw | Skin | Gelatin | | | x | x | | X | |
| Taiaitai (China) http://taiaitai.company.weiku.com/ | Skin | Peptides | x | x | | | | | |
| Geltech Co Lt (Korea) www.geltech.co.kr | Scales | Gelatin | | | x | | | | |
| ANS Ingredients (USA) https://ansingredients.com/ | Skin | Collagen | x | | | | | x | |
| Neocell (USA) www.neocell.com | By-products | Collagen<br>Peptides | x | | | | | x | |
| Nippi (Canada) http://nippicollagen.com/ | Skin and scale | Peptides | | | | x | | x | |
| withinUs (Canada) https://withinus.ca | Scales | Peptides | x | | | | x | | |
| Norland Products (USA) www.norlandproducts.com | Skin | Collagen<br>Gelatin<br>Peptides | x | x | | x | | | Photoengraving glue<br>High tack glue |

Nevertheless, this future step will require demonstrating the reproducibility and safety of the medical products, and the consistency of native MC production.

## 6. Conclusion and perspectives

Collagen is the most abundant structural protein of the human body. Due to its bioactivity, biocompatibility and biodegradability, collagen derived from animal tissues is widely employed in several applicative areas such as medical care, cosmetics, pharmaceutics and food. In this scenario, MC has recently emerged as a versatile and sustainable resource alternative to mammal collagen. MC, particularly fish collagen, indeed shows a lower threat of transmissible diseases to humans, compared to bovine and porcine collagens, and is also free from ethical/religious concerns. Moreover, the utilization of the discards of the fish processing industry as a collagen source provides the double advantage of reducing pollution while valorizing the discards.

Numerous in vitro and in vivo studies suggest that MC from various aquatic species and processed in multiple shapes (e.g. films, sponges, beads, ointments) could be a valid bio-resource for healthcare applications. MC, used in the native, denatured or in peptide form, shows the ability to improve cell proliferation, renewal and wound healing, reduce inflammation, help the absorption of calcium and other minerals, inhibit the growth of bacteria, act as an antioxidant and help in the management of body weight and type 2 diabetes. While it is reasonable to foresee an increased MC demand in sectors such as cosmetics and nutraceuticals, where gelatin and peptides are mostly used, the established use of native MC in the manufacture of medical devices and pharmaceutical products appears much more challenging. This is mostly due to the fact that a high control of the batch-to-batch

consistency is required, in order to improve the reproducibility and the safety of the final products. Moreover, further knowledge is needed with regard to the immunological response to MC, as well as its bioactivity and the underlying mechanisms of action, which are not well known yet.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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