

Physical, biochemical, densitometric and spectroscopic techniques for characterization collagen from alternative sources: A review based on the sustainable valorization of aquatic by-products

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ABSTRACT

Collagenous biopolymers can be analyzed using physic-chemical, densitometric, and spectroscopic analysis to obtain their main characteristics, aiming at their biotechnological manipulation. Collagen extracted from fishery resources is a product of high added value, with potential use in food, biopharmaceutical and cosmetic industries. Some factors make the use of the polymer from fishery resources promising, such as: high availability; greater ontogenetic distance between fish and humans; absence of sociocultural barriers; and absence of toxicity. The collagen extraction method (acid-soluble, pepsin-soluble, electrodialysis, ultrasound, isoelectric precipitation) will directly influence its properties. Thus, this work aims to provide an overview of the extraction methods, characterization techniques (solubility, zeta potential, viscosity, thermogravimetry, differential scanning calorimetry, SDS-PAGE, densitometry, gel strength, centesimal composition, color, aminogram, hydroxyproline determination, X-ray diffraction, circular dichroism, ultraviolet, Raman, and FTIR spectroscopy), and potential analysis with a focus on aquaculture and fisheries sources, through a compilation of scientific information that can be useful to guide aquatic biotechnology professionals, considered that its properties are similar to collagen extracted from mammals.

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1. Introduction

Polymers are widely distributed in nature, built by repeated units of monomers (low molecular mass molecules) connected by covalent bonds, forming macromolecules (high molecular mass molecules). When a polymer comes from living organisms, it is called "Biopolymer", and when it comes from the aquatic freshwater and/or marine environment, it is called "Aquatic Biopolymer"

[1]. Aquatic biopolymers are numerous and diversified, desired by-products by the food, pharmaceutical, cosmetic and textile industry, and others [2]. Among the by-products that can be extracted from the aquatic ecosystem, there are chitin, alginates, fucoidan, carrageenans, agar, ulvans, laminarins, aquatic plants and algae proteins, collagen, enzymes, starch, cellulose, polyesters, and others aquatic biopolymers (phlorotannins, bioluminescent proteins, biofluorescent proteins, xyloglucan, pectin, lignin, peptides from frog skin, others) [1].

New research has emerged on collagen-based biopolymers or collagen derived-associated products, which has increased the interest from the industry and guaranteed investments for this sector. The wide variety of aquatic organisms also signals the possibility of diverse characteristics for each collagen type. Advantageously,

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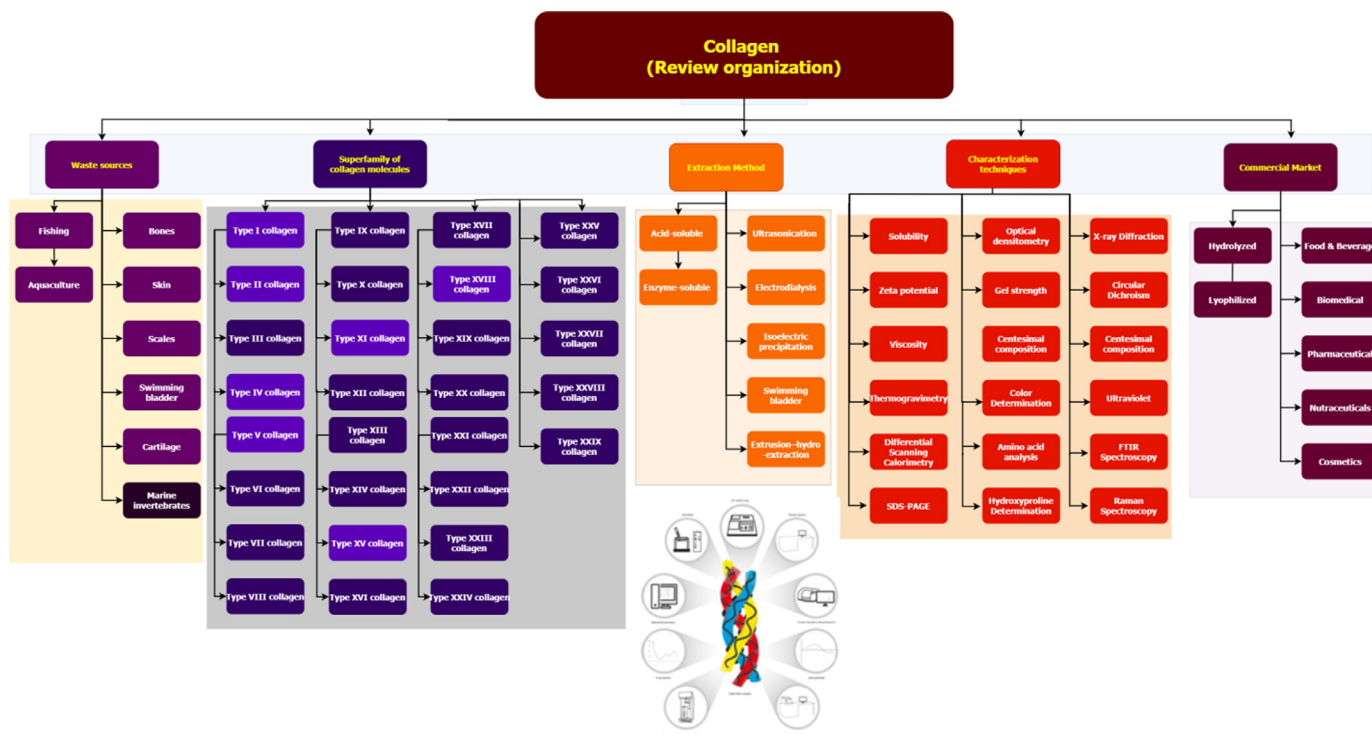


Fig. 1. Structural review organization: sources of collagen superfamily, extraction methods, characterization techniques (physicochemical, densitometric, and spectroscopic), and commercial collagen market. Image prepared in Flowchart Maker and Online Diagram Software (app.diagrams.net).

the collagens extracted from the aquatic environment are free of sanitary and sociocultural restrictions when compared to the collagen from mammals (possibility of transmission of bovine spongiform encephalopathy-BSE, transmissible spongiform-TSE, foot-and-mouth disease -FMD, in addition to allergic reactions) [3–10].

The use of aquatic biopolymers also contributes to the environment, economy and protein market: i) utilizing fishery resources (carcasses, bones, skin, scales, fins, cartilage, swimming bladder and other internal viscera of fish such as stomach, intestines, liver; crustacean shells; mollusk shells, among others), which are almost always discarded inappropriately, as a way of adding value to fishery and aquaculture by-products; ii) the diversity of by-products also translates into the chemical diversity of compounds, which favors multiple biotechnological applications; iii) aquatic polymers are possible sources for the replacement of synthetic polymers, which contributes to the reduction of environmental impact [1].

Several researchers resort to physical and biochemical techniques to determine or qualify the properties of the collagenous molecule, and spectroscopic techniques to collect structural information, which can be used separately or combined, in order to identify the profile of the collagen obtained. Currently, optical densitometry has been used as a quick method of physical comparison of isolated collagen with that of others already available in the literature [5]. The choice for the most appropriate method varies according to the main goal of the extraction, as not all techniques are always available to researchers. In that sense, some points must be considered: form of extraction, availability of equipment for characterization, cost of reagents and other chemical products used, the time to gather results and the degree of reliability [5,11–25]. Thus, this study aimed to provide an overview of collagen, from physical, biochemical, densitometric and spectroscopic approaches used for its identification and characterization, focusing on the residual sources of fisheries and aquaculture. The review structure can be seen in Fig. 1.

2. Collagen sources

Various collagen sources have been registered, including vertebrates and invertebrates (Fig. 2). Some sources are more frequently used, such as: human tendons and placenta [26]; feet, skin, and sternal cartilage from domestic birds [27–29], for instance, chickens (broiler and laying hens), turkeys, quails, ducks and geese; bovine skin, tendon and bones [30], buffalos [31], lamb [32], equine [33], porcine [34], ovine [35,36], and rabbits [37]. Marine mammal species are also sources of this biopolymer, such as whales, seals, sea otters and polar bears [4].

Collagen from marine sources is obtained from a variety of by-products, with similar biochemical and biophysical properties to those of porcine and bovine collagen [38]. Thus, from freshwater and/or marine environments, teleost and cartilaginous fish and/or marine invertebrates are promising resources.

The species of marine teleost fish that have already been investigated as sources of collagen are: *Rachycentron canadum* (Cobia) [39], *Labeo rohita* (Rohu), *Catla catla* (Catla) [40], *Esox lucius* (Northern pike) [41], *Sciaenops ocellatus* (Red drum fish) [42], *Gadus morhua* (Atlantic codfish), *Salmo salar* (Atlantic Salmon) [43], *Cyclopterus lumpus* (Lumpfish) [44], *Sardinella fimbriata* (Sardinella) [45], *Coryphaena hippurus* (Mahi mahi) [22], *Takifugu flavidus* (Yellowbelly pufferfish) [46], *Thunnus obesus* (Bigeye Tuna) [47], and *Scomber japonicus* (Mackerel) [24]; freshwater teleost fish, such as *Cyprinus carpio* (Carp) [48,49], *Oreochromis niloticus* (Tilapia) [7,50,51], and *Cichla ocellaris* (Peacock bass) [5]; to a lesser extent, aquatic reptiles, such as the soft-shelled turtle [52].

By-products of cartilaginous fish are also options for collagen extraction. Have already been investigated: *Chiloscyllium punctatum* (Brownbanded bamboo shark) [53], *Carcharhinus limbatus* (Blacktip shark) [54], *Carcharhinus albimarginatus* (Silvertip Shark) [55], *Rhincodon typus* (Whale shark) [11], *Prionace glauca* (Blue shark), *Scyliorhinus canicula* (Small-spotted catshark) [56], *Pangasius pan-*



Fig. 2. Main collagen sources in nature (focusing on fisheries and aquaculture resources). Image prepared in Adobe Illustrator Software.

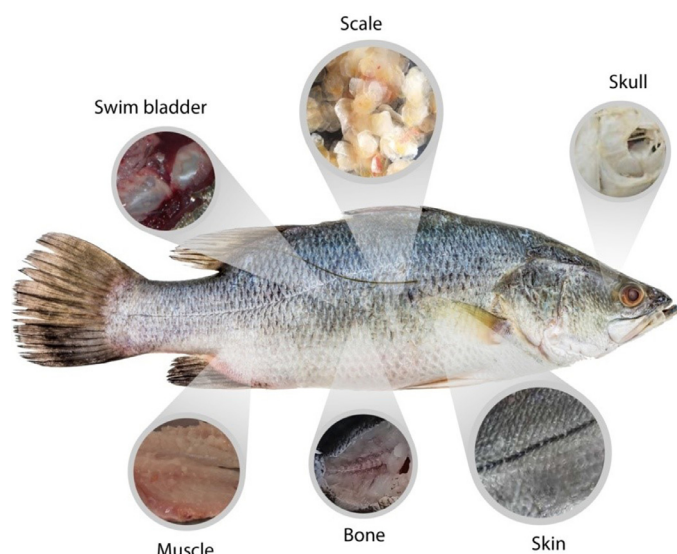


Fig. 3. By-products of teleost fish as potential sources of collagen: skin, scales, bones, skull, swimming bladder and remnants of post-evisceration muscles. Biological model: *Centropomus undecimalis* (Common snook). Image prepared in Adobe Illustrator Software.

gasius (Shark catfish) [57], and *Mustelus mustelus* (Smooth-hound) [58].

The most used by-products for the extraction of collagen from teleost fish are: (Fig. 3): carcass, bones [24,59–62]; spines, skulls [63], fins [64], skin [5,24,50,60], scales [48,60,65–67], remains of filleting muscles [59], and swimming bladder [68–71]. The marine teleost fish notochord has also been used as a promising source of collagen [68].

Marine invertebrates that are sources of collagen: Sea anemones [4,33,72], Corals [73], Sponge [74–76], Starfish [77], Octopus [78,79], Cuttlefish [80], Sea urchins [77,81], Sea cucumber [25,77,82], Jellyfish [83–86], Squid [86,87], Mussels [88], and Shell [89]. Some specific parts of marine invertebrates receive special attention because they have already been successfully used in collagen obtention, such as the starfish wall [90], cuttlefish skin [80], squid mantle, muscles, and skins [18,19,87], jellyfish filaments [85], and the sea cucumber body wall [25].

Collagenous biopolymers extracted from teleost fish, cartilaginous fish and marine invertebrates have physicochemical and spectroscopic characteristics close to those from mammals with some advantages: 1) high availability of fishing and aquaculture by-products, mainly from fish skin and scales; 2) greater ontogenetic distance between fish and humans (low risk of disease transmission when compared to mammal collagen); 3) absence of cultural and religious barriers; 4) easier extraction processes (many times supported by new technology, such as sonication); 5) high versatility; 6) bioresorbability; 7) absent (fish) or almost insignificant (marine invertebrates) toxicity; 8) minimal inflammatory response; 9) low melting point; 10) low viscosity; 11) high glycine and alanine content, reasonable arginine, and glutamic acid content; 12) readiness of isolation, purification and characterization; 13) good homeostatic properties; and, 14) metabolic compatibility [72,80,91–96].

3. Collagen superfamily

Collagen belongs to a superfamily of structural and protective proteins in the extracellular matrix (EMC), both in the vertebrate and invertebrate taxa. In vertebrates, collagen can account to up to 30% of the total protein content [72,97–102]. This biopolymer structure is formed by three polypeptide chains interconnected in a triple helix, linked by hydrogen bonds [103,104]. Collagen fea-

tures physicochemical, densitometric and spectroscopic attributes characterized according to the source, differentiated by the intrapolypeptide and interpolypeptide molecular arrangement, stability, elasticity and immunophysiological properties [5,35,57,100,105–108].

The general structure of the collagen molecule is characterized by the repetition of “Glycine-X-Y” domains, where “X” and “Y” are occupied by different amino acids that vary throughout the triple helix, though “X” is frequently proline (Pro) and “Y” is hydroxyproline (Hyp). This pattern leads to the formation of the triple helix of 3 polypeptide chains found in all members of the collagen family [4,97,103,109–112].

So far, 29 members of the collagen family have been reported, identified from I to XXIX [72,80,110,113–115]. The different types can be grouped into categories, according to their structure, which are, exemplifying: i) Fibril-forming collagens (Types: I, II, III, V, XI, XXIV, and XXVII); ii) Basal Membrane Collagen (Types: IV, VII, and XXVIII); iii) Short-Chain Collagens (Types: VI, VIII, and X); and, iv) Collagens with multiple interruptions (FACITs) (Types: IX, XII, XIV, XVI, and XIX to XXII) [10,97,101,110,114].

The specific type of collagen is identified by physicochemical (solubility, electrophoresis, others) and spectroscopic (FTIR, DC, Raman, others) studies of the extracted material. The presence of two alpha and one beta bands signals for Type I, which can be confirmed through spectroscopic tests to verify the structure of the collagenous material. Generally, the Type I production process in the organism can be divided in the following stages: i) formation of procollagen (collagen precursor); ii) formation of tropocollagen; and, iii) formation of collagen fibrils (fibrils are formed by the assembly of tropocollagen). Concisely, collagen biosynthesis begins with the transcription of collagen genes and formation of mRNA that will be translated into a chain of amino acids (procollagen) [103,110]. Among the post-translational modification processes [116], collagen undergoes lysine and proline residue hydroxylation, which influences the assembly of the 3 chains that form the triple helix and final molecule stability. After this, the molecules are packaged in the Golgi apparatus and secreted into the extracellular matrix, where they will be processed according to the collagen type. At this stage, specific enzymes responsible for the cleavage of the C-terminal and N-terminal portions of procollagen act, and this cleavage has impact on the final properties and arrangement of collagen [103,110].

Type I collagen is abundant in mammals, it is a heterotrimer with chains $[\alpha 1(I)]_2\alpha 2(I)$, with molecular assembly formed by monomers staggered by 67 nm, and fibers supramolecular structure with bands of 67 nm large diameter [114], found mainly in the skin, bones [116], tendons [97,114,117], and by-products from fisheries and aquaculture [5,24,43,60,65,66], in marine invertebrates such as *Apostichopus japonicus* (sea cucumber) [118]. Type II collagen is found in cartilage [119], vitreous, cartilaginous zones of tendon, intervertebral disk, with chains $[\alpha 1(II)]_3$ [97,114], molecular assembly formed by monomers staggered by 67 nm, and fibers supramolecular structure with 67 nm banded fibrils [114], abundant in cartilaginous fish [11,54], having already been extracted from marine invertebrates like jellyfish [120].

Type IV collagen with chains $[\alpha 1(IV)]_2\alpha 2(IV)$ is located in the basal membrane [97,114,121], molecular assembly formed by association of 4N- and 2C-termini, and supramolecular structure formed by nonfibrillar meshwork [114], forming the extracellular matrix, identified in marine invertebrates such as gastropod mollusks [122], and marine sponge [99,123]; while Type V collagen with chains $[\alpha 1(V)]_2\alpha 2(V)$, molecular assembly formed by monomers staggered by 67 nm, and supramolecular structure with 9 nm diameter banded fibrils [114], is mainly found in placental/embryonic tissue, dermis, bone, interstitial matrix of muscles, lungs [124], cornea, cell surfaces [97,114], and is found together

with Type I collagen, as reported to teleost fish and marine invertebrates [19,72,113,125].

Type XI (chains $[\alpha 1(XI)\alpha 2(XI)\alpha 3(XI)]$), with their fibrils identical to Type V collagen [114,125], distributed in articular cartilage, skeletal muscle, placenta, lung, tendons, testis, trachea [126]; Type XV (chains $[\alpha 1(XV)]_3$) and type XVIII (chains $[\alpha 1(XVIII)]_3$), both located in areas of the basement membrane [127,128], collagen can also be found in other invertebrate organisms [129]. The other types of collagen are found in low amounts and in specific tissues [115].

4. Collagen extraction methods

Collagen polymers from fishery and aquaculture sub products, especially when extracted from fish skin, need to undergo previous treatment such as washes using water and sodium chloride to remove impurities and fats, as well as grinding the skin to increase its contact surface with the liquid phase (squares of $\sim 1.5 \times 1.5$ cm) [5,115,130,131]. Then, the material is immersed in alkaline solutions for the removal of impurities and non-collagenous proteins [132], using sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), calcium hydroxide ($Ca(OH)_2$), or a combination of these, to then use butyl alcohol (10%) to remove oily parts [5,25,49,133–137].

After pre-treatment, extraction steps based on the solubility of the collagenous molecule follow. The most applied are: i) saline treatment for extraction by precipitation using sodium chloride (NaCl) [138,139], and/or guanidine hydrochloride ($CH_5N_3 \cdot HCl$) [89], having among the disadvantages of this technique, a low yield in the extractions; ii) acid treatment for the extractions using: acetic acid (CH_3COOH) [140], lactic acid ($C_3H_6O_3$), citric acid ($C_6H_8O_7$) [141], hydrochloric acid (HCl) [23,142], formic acid (CH_2O_2), sulfuric acid (H_2SO_4), or tartaric acid ($C_4H_6O_6$) [23], for example; and iii) enzymatic treatment using commercial enzymes (and/or purified enzymes), such as pepsin [4,5,138,143], papain [52,144,145], and/or collagenase [109,146]. For this type of treatment, the extraction takes place in a medium containing organic acid (CH_3COOH is the most used) with the addition of an enzyme (pepsin, for example). The use of inorganic acids, such as HCl and H_2SO_4 , has been reported to be less efficient in the extraction when compared to organic acids [23].

Enzymatic hydrolysis tends to remove the non-helical extremities, which increases the solubility of collagen, becoming the preferred method in the extraction of collagen from skin, scales and swimming bladder residues from teleost fish species. A disadvantage of this procedure is the possibility of irreversible denaturation of the collagen structure by enzymatic digestion [97], which can be identified by Fourier-transform infrared spectroscopy (FTIR). Fig. 4 illustrates the most employed stages of extraction for the obtention of collagen from freshwater and marine teleost fish skin.

Acid-soluble (ASC) and pepsin-soluble (PSC) treatment are the most used due to the high yield obtained from the extractions (Table 1), and can be applied separately, or combined, with the goal of optimizing the final yield [5,32,45,60,87,147–150].

The advancement of aquaculture biotechnology has contributed to the increase of yield of collagen from freshwater/marine species. New approaches are being brought forward to optimize the stages of collagen extraction, among them are: i) use of ultrasonication processor device, through the improvement of mass transfer by opening the collagen fibrils, facilitating acid and/or enzymatic hydrolysis and, consequently, increasing the extraction yield, making this method more efficient than the conventional one. The main variables are frequency (kHz), exposure time and temperature, all of which can be controlled according to the type of ultrasound equipment used [27,30,131,132,151,152]; ii) use of electro dialysis, a simple, viable and inexpensive technique that can be employed to substitute conventional dialysis, providing an increase in the ex-

traction yield and more agility in the process [46]; iii) use of iso-electric precipitation, a common technique in the separation of protein biomolecules and which can be introduced in the isolation of collagen process from marine sources, having already been used successfully in extractions of collagen from marine fish [47]; iv) Extrusion–hydro-extraction (EHE) process, a technique that has already been employed in the food industry for feed and food production, as well as in the extraction of collagen from sub products of *Oreochromis* sp. (Tilapia), facilitating the extraction of collagen by hot water treatment and generating minimal material waste [153].

The introduction of new techniques during the collagen extraction stages mainly aims to reduce the collagen processing time, reduce the consumption of energy and the number of chemical reagents used by the conventional methods [109,154]. Table 1 shows a comparison of the techniques used for collagen extraction (conventional and new approaches), illustrating the advantages of the introduction of new technology in the freshwater/marine collagen extraction phases.

The yield of extraction processes of collagen obtained from animal industry by-products is dependent on the extraction source, sex, age and body weight of the animals as well as the state of the by-products generated by the processing, in addition to the type of technique used [5,132]. All stages of extraction are performed under low temperatures (generally $4^\circ C$) as a way of preventing the denaturation of collagenous protein. The collagen extracted from teleost fish, cartilaginous fish and from marine invertebrate species displays a yield that varies between 0.05 to 94.4%, as illustrated in Fig. 5. From fish processing by-products, skin traditionally has been reported as a beneficial option for collagen extraction, with yield values superior to those obtained from terrestrial and marine mammals and from marine invertebrates. The following equation is used to calculate the yield of the collagen extracted: Yield (%) = (weight of lyophilized collagen (g) / weight of initially used dry tissue (g)) \times 100. This way, the yield is calculated based on the dry weight [5,155].

5. Collagen physical and biochemical characterization

5.1. Collagen solubility (pH and NaCl)

Collagen solubility is investigated in various pH levels (1–12) [5], and in various salt concentrations (NaCl), in acid medium, usually testing concentrations of 3 to 6 mg/mL [8,39,156]. The solubility of collagen is defined as the weight of its acetic acid-soluble fraction, expressed as the total percentage of collagen used in the essay [157]. The solubility range of collagen depends on the type of extraction employed. Collagen from skin, scales, swimming bladder, cartilage and bones fish usually are soluble within a range of pH 1–6, 1–5, 1–4, 1–5 and 1–4, respectively; while collagen extracted from marine invertebrates (sea cucumbers and squids) are commonly soluble in the pH range of 1–5. Collagens extracted from other non-aquatic sources (land mammals and birds) can be soluble in the pH range of 1–5, as shown in Table 1. The effect of pH is calculated according to the following equation: Solubility (%) = (concentration of protein (mg/mL) in supernatant / Concentration of protein (mg/mL) in sample (highest solubility)) \times 100.

For the purpose of using collagen as a functional ingredient in the formulation of food industry products, the most appropriate solubility is within the pH range of 2 to 4, while the addition of NaCl above 20 mg/g sharply reduces this parameter, as well as its functional characteristics [157]. The effects of NaCl concentrations are calculated according to the following equation: Solubility (%) = (concentration of protein (mg/mL) in supernatant / concentration of protein (mg /mL) in “control” sample (without NaCl)) \times 100. The solubility of collagen is also influenced by the structure

Table 1
Physico-chemical comparison of different types of collagen obtained from fishery and aquaculture by-products.

Collagen source	Name	Class	Method	Tissue	Yield (%)	Solubility		Zeta potential	Type	Ref.
						pH	NaCl			
<i>Scomber japonicus</i>	Mackerel	Teleost Fish	PSC	Bone	1.75	–	–	–	Type I	[24]
<i>Scomber japonicus</i>	Mackerel	Teleost Fish	PSC	Skin	8.10	–	–	–	Type I	[24]
<i>Holothuria cinerascens</i>	Sea cucumber	Marine Invertebrates	ASC	Body wall	72.2	1–3	0–2%	–	Type I	[25]
<i>Thunnus obesus</i>	Bigeye tuna	Teleost Fish	ASC	Skin	13.5	2–6	–	6.1	Type I	[60]
<i>Thunnus obesus</i>	Bigeye tuna	Teleost Fish	PSC	Skin	16.7	2–5	–	6.4	Type I	[60]
<i>Thunnus obesus</i>	Bigeye tuna	Teleost Fish	PSC	Scale	4.6	2–5	–	5.4	Type I	[60]
<i>Thunnus obesus</i>	Bigeye tuna	Teleost Fish	PSC	Bone	2.6	2–5	–	5.5	Type I	[60]
<i>Nibeia japonica</i>	Giant croaker	Teleost Fish	ASC	Swim bladders	11.33	1–4	0–2%	–	Type I	[69]
<i>Nibeia japonica</i>	Giant croaker	Teleost Fish	PSC	Swim bladders	15.35	1–4	0–2%	–	Type I	[69]
<i>Takifugu flavidus</i>	Pufferfish	Teleost Fish	SB ¹	Skin	67.3	1–3	0–3%	–	Type I	[46]
<i>Cyprinus carpio</i>	Common carp	Teleost Fish	ASC	Scale	13.6	–	–	–	Type I	[49]
<i>Rhopilema esculentum</i>	Jellyfish	Marine Invertebrates	PSC	Filaments	4.31	–	–	–	Type I	[85]
<i>Sardinella fimbriata</i>	Sardinella	Teleost Fish	ASC	Fringescale	7.48	1–6	–	6.0	–	[45]
<i>Sardinella fimbriata</i>	Sardinella	Teleost Fish	PSC	Fringescale	0.94	7–10	–	7.0	–	[45]
<i>Thunnus obesus</i>	Bigeye Tuna	Teleost Fish	PSC-SO ²	Skin	14.14	–	–	–	Type I	[47]
<i>Thunnus obesus</i>	Bigeye Tuna	Teleost Fish	PSC-IP ²	Skin	17.17	–	–	–	Type I	[47]
–	Hybrid sturgeon	Teleost Fish	ASC	Skin	5.73	–	–	6.56	Type I	[147]
–	Hybrid sturgeon	Teleost Fish	PSC	Skin	10.26	–	–	5.36	Type I	[147]
<i>Cichla ocellaris</i>	Peacock bass	Teleost Fish	PSC	Skin	2.9	2–6	0–3%	–	Type I	[5]
<i>Oreochromis niloticus</i>	Nile Tilapia	Teleost Fish	ASC	Skin	19.07	–	–	–	Type I	[7]
<i>Oreochromis niloticus</i>	Nile Tilapia	Teleost Fish	PSC	Skin	19.61	–	–	–	Type I	[7]
<i>Coelomactra antiquata</i>	Live surf clam shells	Marine Invertebrates	GSC ³	Body	0.59	–	–	–	–	[89]
<i>Coelomactra antiquata</i>	Live surf clam shells	Marine Invertebrates	PSC	Body	3.78	–	–	–	–	[89]
<i>Acipenser schrenckii</i>	Amur sturgeon	Teleost Fish	PSC	Skin	13.4	–	–	–	Type I	[68]
<i>Acipenser schrenckii</i>	Amur sturgeon	Teleost Fish	PSC	Swim bladder	16.5	–	–	–	Type I	[68]
<i>Acipenser schrenckii</i>	Amur sturgeon	Teleost Fish	PSC	Notochord	1.7	–	–	–	Type II	[68]
<i>Probarbus jullieni</i>	Golden carp	Teleost Fish	ASC	Skin	51.90	–	–	6.11	Type I	[151]
<i>Probarbus jullieni</i>	Golden carp	Teleost Fish	UASC ⁴	Skin	81.53	–	–	6.02	Type I	[151]
<i>Probarbus jullieni</i>	Golden carp	Teleost Fish	PSC	Skin	79.27	–	–	6.21	Type I	[151]
<i>Probarbus jullieni</i>	Golden carp	Teleost Fish	UPSC ⁴	Skin	94.88	–	–	6.56	Type I	[151]
<i>Sole fish skin waste</i>	Fish market	Teleost Fish	OVAT ⁵	Skin	1.93	–	–	–	Type I	[136]
<i>Pangasius sp.</i>	Silver catfish	Teleost Fish	ASC	Skin	4.27	4	–	–	–	[137]
<i>Pangasius sp.</i>	Silver catfish	Teleost Fish	PSC	Skin	2.27	1	–	–	–	[137]
<i>Lates calcarifer</i>	Barramundi	Teleost Fish	PSC	Skin	47.3	–	–	–	Type I	[50]
<i>Oreochromis niloticus</i>	Tilapia	Teleost Fish	PSC	Skin	52.6	–	–	–	Type I	[50]
<i>Mustelus mustelus</i>	Smooth-hound	Cartilaginous fish	ASC	Skin	23.07	–	–	–	Type I	[58]
<i>Mustelus mustelus</i>	Smooth-hound	Cartilaginous fish	PSC	Skin	35.27	–	–	–	Type I	[58]
<i>Ictalurus punctatus</i>	Channel catfish	Teleost Fish	ASC	Skin	–	1–2	0–2%	5.34	Type I	[142]
<i>Ictalurus punctatus</i>	Channel catfish	Teleost Fish	HSC ⁶	Skin	–	1–2	0–2%	5.73	Type I	[142]
<i>Ictalurus punctatus</i>	Channel catfish	Teleost Fish	PHSC ⁶	Skin	–	1–2	0–2%	5.42	Type I	[142]
<i>Misgurnus anguillicaudatus</i>	Loaches	Teleost Fish	ASC	Skin	22.42	–	–	6.42	Type I	[130]
<i>Misgurnus anguillicaudatus</i>	Loaches	Teleost Fish	PSC	Skin	27.32	–	–	6.51	Type I	[130]
<i>Miichthys miiuy</i>	Miiuy Croaker	Teleost Fish	ASC	Swim Bladders	1.33	1–4	0–2%	6.74	Type I	[70]
<i>Miichthys miiuy</i>	Miiuy Croaker	Teleost Fish	PSC	Swim Bladders	8.37	1–4	0–2%	6.85	Type I	[70]
<i>Nibeia japonica</i>	Giant Croaker	Teleost Fish	PSC	Skin	84.85	1–4	0–2%	–	Type I	[21]
<i>Probarbus jullieni</i>	Golden carp	Teleost Fish	ASC	Scale	0.42	1–3	0–	6.04	Type I	[65]
<i>Probarbus jullieni</i>	Golden carp	Teleost Fish	PSC	Scale	1.16	1–3	0–	6.22	Type I	[65]
<i>Catla catla</i>	Catla	Teleost Fish	ASC	Scale	1.72	3–6	0–	–	Type I	[12]
<i>Labeo rohita</i>	Rohu	Teleost Fish	ASC	Scale	2.7	3–6	0–	–	Type I	[12]
<i>Cyprinus carpio</i>	Carp	Teleost Fish	ASC	Scale	9.79	–	–	–	Type I	[48]
<i>Oreochromis niloticus</i>	Tilapia	Teleost Fish	ASC	Skin	27.2	1–3	0–3%	6.42	Type I	[51]
<i>Oreochromis niloticus</i>	Tilapia	Teleost Fish	ASC	Scale	3.2	1–3	0–2%	6.82	Type I	[51]
<i>Sciaenops ocellatus</i>	Red drum fish	Teleost Fish	PSC	Scale	4.32	1–3	0–6%	–	Type I	[42]
<i>Loligo vulgaris</i>	Squid	Marine Invertebrates	ASC	Mantle	5.1	1–4	0–	–	Type I and V	[19]
<i>Loligo vulgaris</i>	Squid	Marine Invertebrates	PSC	Mantle	24.2	1–4	0–	–	Type I and V	[19]
Commercial dry product	Dried squid	Marine Invertebrates	ASC	–	–	1–6	0–4%	–	Type I	[86]
Commercial dry product	Dried squid	Marine Invertebrates	PSC	–	–	1–5	0–4%	–	Type I	[86]
Commercial dry product	Dried jellyfish	Marine Invertebrates	ASC	–	–	1–5	0–2%	–	Type I	[86]
Commercial dry product	Dried jellyfish	Marine Invertebrates	PSC	–	–	1–5	0–2%	–	Type I	[86]
<i>Labeo rohita</i>	Rohu	Teleost Fish	ASC	Skin	64.2	–	–	5.9	Type I	[148]
<i>Labeo rohita</i>	Rohu	Teleost Fish	PSC	Skin	6.8	–	–	5.3	Type I	[148]
<i>Catla catla</i>	Catla	Teleost Fish	ASC	Skin	63.40	–	0–	–	Type I	[40]
<i>Catla catla</i>	Catla	Teleost Fish	PSC	Skin	69.53	–	0–	–	Type I	[40]

(continued on next page)

Table 1 (continued)

Collagen source	Name	Class	Method	Tissue	Yield (%)	Solubility		Zeta potential	Type	Ref.
						pH	NaCl			
<i>Labeo rohita</i>	Rohu	Teleost Fish	ASC	Skin	46.13	–	0– 0.4 mol.L ⁻¹	–	Type I	[40]
<i>Labeo rohita</i>	Rohu	Teleost Fish	PSC	Skin	64.94	–	0– 0.4 mol.L ⁻¹	–	Type I	[40]
<i>Doryteuthis singhalensis</i>	Squid	Marine Invertebrates	ASC	Outer skin	56.80	–	–	–	Type I	[18]
<i>Stichopus monotuberculatus</i>	Sea Cucumber	Marine Invertebrates	PSC	Body Wall	61.93	2–4	3–5%	–	Type I	[82]
<i>Chrysaora</i> sp.	Ribbon jellyfish	Marine Invertebrates	PSC	Umbrella	9–19.0	–	–	6.64	Type II	[120]
<i>Katsuwonus pelamis</i>	Skipjack tuna	Teleost Fish	ASC	Spine	2.47	1–5	0–2%	–	Type I	[63]
<i>Katsuwonus pelamis</i>	Skipjack tuna	Teleost Fish	PSC	Spine	5.62	1–5	0–2%	–	Type I	[63]
<i>Katsuwonus pelamis</i>	Skipjack tuna	Teleost Fish	ASC	Skull	3.57	1–5	0–2%	–	Type I	[63]
<i>Katsuwonus pelamis</i>	Skipjack tuna	Teleost Fish	PSC	Skull	6.71	1–5	0–2%	–	Type I	[63]
<i>Carcharhinus albimarginatus</i>	Silvertip Shark	Cartilaginous fish	ASC	Cartilage	–	5–6	1%	–	Type II	[55]
<i>Carcharhinus albimarginatus</i>	Silvertip Shark	Cartilaginous fish	PSC	Cartilage	–	5–6	1%	–	Type II	[55]
<i>Thunnus albacores</i>	Yellowfin tuna	Teleost Fish	ASC	Swim bladders	1.07	1–6	–	6.05	Type I	[71]
<i>Thunnus albacares</i>	Yellowfin tuna	Teleost Fish	PSC	Swim bladders	12.10	1–6	–	5.93	Type I	[71]
<i>Pelodiscus sinensis</i>	Soft-shelled turtle	Marine reptile		Lung	79.29	–	–	–	Type I	[52]
			PSC+Papain							
<i>Saurida</i> spp.	Lizard fish (Japan)	Teleost Fish	ASC	Scale	0.79	1–5	0.2–0.4M	–	Type I	[67]
<i>Saurida</i> spp.	Lizard fish (Vietnam)	Teleost Fish	ASC	Scale	0.69	1–5	0.2–0.4 M	–	Type I	[67]
<i>Trachurus japonicus</i> (Japan)	Horse mackerel	Teleost Fish	ASC	Scale	1.51	1–5	0.2–0.4 M	–	Type I	[67]
<i>Trachurus japonicus</i>	Horse mackerel (Vietnam)	Teleost Fish	ASC	Scale	0.64	1–5	0.2–0.4 M	–	Type I	[67]
<i>mulg cephalis</i>	gray mullet	Teleost Fish	ASC	Scale	0.43	1–5	0.2–0.4 M	–	Type I	[67]
<i>Cypselurus melanurus</i>	Flying fish	Teleost Fish	ASC	Scale	0.72	1–5	0.2–0.4 M	–	Type I	[67]
<i>Dentex tumifrons</i>	Yellowback seabream	Teleost Fish	ASC	Scale	0.90	1–5	0.2–0.4 M	–	Type I	[67]
<i>Acipenser schrenckii</i>	Amur sturgeon	Teleost Fish	PSC-I	Skin	92.40	–	–	–	Type I	[113]
<i>Acipenser schrenckii</i>	Amur sturgeon	Teleost Fish	PSC-V	Skin	2.16	–	–	–	Type V	[113]
<i>Acipenser schrenckii</i>	Amur sturgeon	Teleost Fish	SSC ⁷	Skin	4.55	–	–	–	Type I	[200]
<i>Acipenser schrenckii</i>	Amur sturgeon	Teleost Fish	PSC	Skin	52.80	–	–	–	Type I	[200]
<i>Lates calcarifer</i>	Barramundi	Teleost Fish	ASC	Skin	8.12	2–5	0–2%	–	Type I	[13]
<i>Lates calcarifer</i>	Barramundi	Teleost Fish	PSC	Skin	43.63	2–5	0–2%	–	Type I	[13]
<i>Lates calcarifer</i>	Barramundi	Teleost Fish	PaSC ⁸	Skin	43.91	2–5	0–2%	–	Type I	[13]
Hybrid <i>Clarias</i> sp.	Malaysian catfish	Teleost Fish	ASC	Skin	18.11	1–5	0–4%	–	Type I	[14]
Hybrid <i>Clarias</i> sp.	Malaysian catfish	Teleost Fish	PSC	Skin	26.69	1–5	0–4%	–	Type I	[14]
<i>Scomberomorus niphonius</i>	Spanish mackerel	Teleost Fish	ASC	Skin	13.68	1–4	0–2%	–	Type I	[15]
<i>Scomberomorus niphonius</i>	Spanish mackerel	Teleost Fish	PSC	Skin	3.49	1–4	0–2%	–	Type I	[15]
<i>Scomberomorus niphonius</i>	Spanish mackerel	Teleost Fish	ASC	Bones	12.54	1–4	0–2%	–	Type I	[15]
<i>Scomberomorus niphonius</i>	Spanish mackerel	Teleost Fish	PSC	Bones	14.27	1–4	0–2%	–	Type I	[15]
<i>Acanthaster planci</i>	Crown-of-thorns Starfish	Marine Invertebrates	PSC	Body wall	2.29	–	–	–	Type I	[90]
<i>Lates calcarifer</i>	Seabass	Teleost Fish	ASC	Skin	15.8	–	–	6.46	Type I	[149]
<i>Lates calcarifer</i>	Seabass	Teleost Fish	ASC	Swim bladder	28.5	–	–	6.64	Type I	[149]
<i>Evenchelys macrura</i>	Marine eel-fish	Teleost Fish	ASC	Skin	80.0	1–4	0–2%	–	Type I	[8]
<i>Evenchelys macrura</i>	Marine eel-fish	Teleost Fish	PSC	Skin	7.10	1–4	0–4%	–	Type I	[8]
<i>Rachycentron canadum</i>	Cobia	Teleost Fish	ASC	Skin	35.5	1–3	0–2%	–	Type I	[39]
<i>Rachycentron canadum</i>	Cobia	Teleost Fish	PSC	Skin	12.3	1–4	0–2%	–	Type I	[39]
<i>Diodon holocanthus</i>	Balloon fish	Teleost Fish	ASC	Skin	4.0	1–5	0–1%	–	Type I	[9]
<i>Diodon holocanthus</i>	Balloon fish	Teleost Fish	PSC	Skin	19.5	1–5	0–2%	–	Type I	[9]
<i>Nemipterus hexodon</i>	Ornate threadfin bream	Teleost Fish	PSC	Skin	24.9	–	–	6.40	Type I	[112]
<i>Aluterus monoceros</i>	Unicorn leatherjacket	Teleost Fish	APSC ⁹	Skin	8.48	1–6	0–2%	–	Type I	[143]
<i>Aluterus monoceros</i>	Unicorn leatherjacket	Teleost Fish	YPSC ⁹	Skin	8.40	1–6	0–2%	–	Type I	[143]
<i>Aluterus monoceros</i>	Unicorn leatherjacket	Teleost Fish	PPSC ⁹	Skin	7.56	1–6	0–2%	–	Type I	[143]
<i>Chiloscyllium punctatum</i>	Brownbanded bamboo	Cartilaginous fish	ASC	Skin	9.38	–	–	6.21	Type I	[53]
<i>Chiloscyllium punctatum</i>	Brownbanded bamboo	Cartilaginous fish	PSC	Skin	8.86	–	–	6.56	Type I	[53]
<i>Chiloscyllium punctatum</i>	Brownbanded bamboo	Cartilaginous fish	ASC	Cartilage	1.27	–	–	6.53	Type I and II	[54]
<i>Chiloscyllium punctatum</i>	Brownbanded bamboo	Cartilaginous fish	PSC	Cartilage	9.59	–	–	7.03	Type I and II	[54]
<i>Carcharhinus limbatus</i>	Blacktip shark	Cartilaginous fish	ASC	Cartilage	1.04	–	–	6.96	Type I and II	[54]
<i>Carcharhinus limbatus</i>	Blacktip shark	Cartilaginous fish	PSC	Cartilage	10.30	–	–	7.26	Type I and II	[54]
<i>Sebastes mentella</i>	Deep-sea redfish	Teleost Fish	ASC	Skin	47.5	–	–	–	Type I	[62]
<i>Sebastes mentella</i>	Deep-sea redfish	Teleost Fish	ASC	Bones	10.3	–	–	–	Type I	[62]

(continued on next page)

Table 1 (continued)

Collagen source	Name	Class	Method	Tissue	Yield (%)	Solubility		Zeta potential	Type	Ref.
						pH	NaCl			
Other sources of collagen										
-	Chicken	Birds	UPSCII ¹⁰	Sternal cartilage	87.17	-	-	5.64	Type II	[27]
Fibrillar type I collagen	Equine	Mammals	-	Tendon	-	-	-	5.0	Type I	[33]
Commercial type I collagen	Equine	Mammals	-	Tendon	-	-	-	5.5	Type I	[33]
-	Sheep	Mammals	ASC	By-products*	12.5	2-5	-	-	Type I	[32]
-	Lamb	Mammals	ASC	By-products*	18.0	2-5	-	-	Type I	[32]
-	Chicken	Birds	PSC	Fat lungs	-	1-4	0-2%	-	Type II	[152]
-	Chicken	Birds	UPSC ¹¹	Fat lungs	31.25	1-4	0-2%	-	Type II	[152]
<i>Ujumuqin sheep</i>	Ovine	Mammals	ASC	Bone	-	-	-	4.65	Type I	[36]
<i>Ujumuqin sheep</i>	Ovine	Mammals	PSC	Bone	-	-	-	5.76	Type I	[36]
-	Chicken	Birds	PSC	Skin	10-12.0	-	-	-	Type I	[28]
<i>Coturnix japonica</i>	Japanese quail	Birds	ASC	Feet	-	-	-	5.53	Type I	[29]
<i>Coturnix japonica</i>	Japanese quail	Birds	PSC	Feet	-	-	-	5.61	Type I	[29]
<i>Bubalus bubalis</i>	Water buffalo	Mammals	ASC	Skin	1.8	-	-	-	Type I	[31]
-	Rabbit	Mammals	PSC	Skin	71.0	-	-	-	Type I	[37]
<i>Dromaius novaehollandiae</i>	Emu	Birds	PSC	Skin	27.3	-	-	-	Type I	[16]

ASC- Extraction acid-solubilised collagen, and/or Extraction pepsin-solubilised collagen (PSC).

¹ Sodium bicarbonate and electro dialysis (SB)

² Extracted by salting-out (PSC-SO), and isoelectric precipitation (PSC-IP) methods

³ Extraction of guanidine hydrochloride soluble collagen (GSC).

⁴ Acid-soluble ultrasound-assisted method (UASC) and pepsin-soluble ultrasound-assisted method (UPSC).

⁵ The effect of acetic acid, NaCl, solid/solvent ratio and time on the extraction of collagen were studied by one variable at a time (OVAT) method.

⁶ Extraction of collagen with homogenization-aided (HSC) method, extraction of collagen with pepsin and homogenization aided (PHSC) method.

⁷ Extraction process using sodium chloride.

⁸ PaSC: Papain-soluble collagen.

⁹ PSC extracted with the aid of albacore tuna pepsin (APSC), yellowfin tuna pepsin (YPSC) and porcine pepsin (PPSC), respectively.

¹⁰ Extraction using pepsin soluble and ultrasound treatment time 36 min (UPSCII36).

¹¹ Pepsin-soluble collagen by ultrasound pre-treatment (UPSC). By-products

* Bone, cartilage, carcass trimmings and meat.

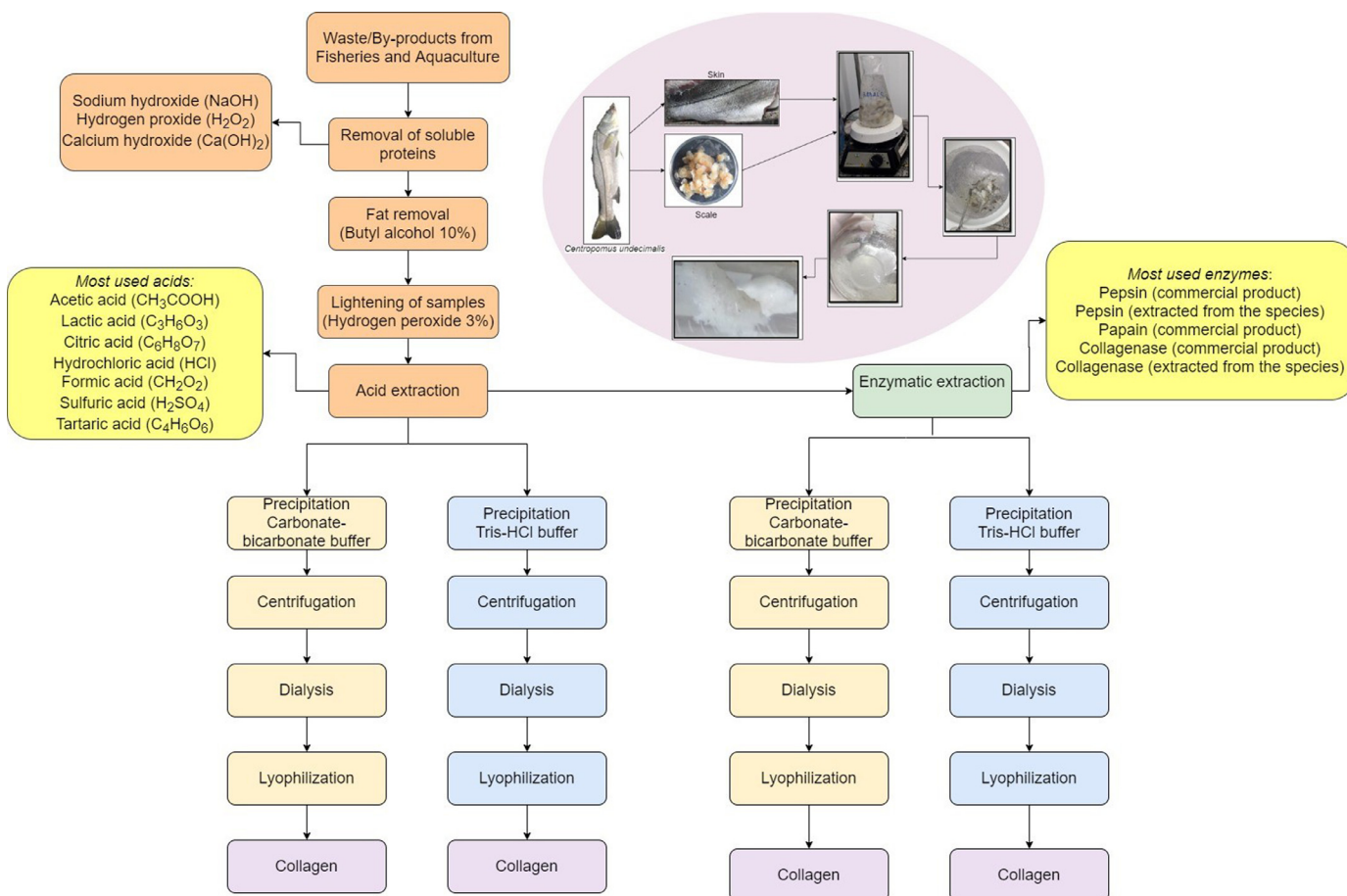


Fig. 4. Extraction of collagen from fish skin (acid and enzymatic treatment). Image prepared in Flowchart Maker and Online Diagram Software (app.diagrams.net).

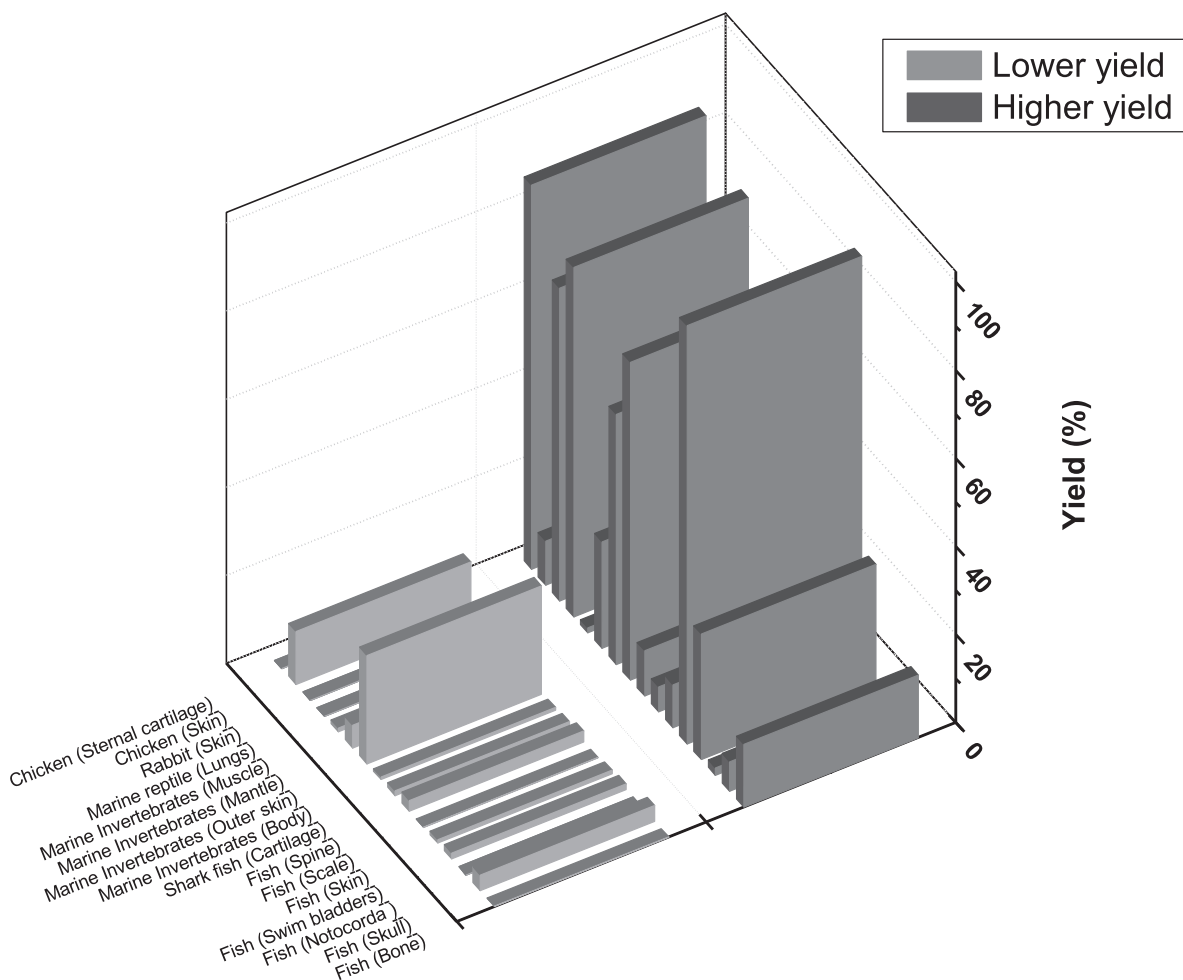


Fig. 5. Yield variation between different types of collagen sources, focusing on the extracted from the fishing and aquaculture by-products compared to the production of animal waste. Image prepared in the OriginLab® 8.0 Software.

and amino acid composition, mainly when exposed to high concentrations of NaCl [143]. Solubility is a determining factor when this protein is applied as a source in the production of moisturizing cosmetics, as this industrial segment uses hydrolyzed substances for cosmetic and medical cream formulations [25].

5.2. Zeta potential

Zeta potential (ζ) or electrokinetic potential in colloidal dispersion represents the electrical potential in the double layer around the dispersed particles or the potential difference between the medium (solvent solution) and the solvent molecules (or dissolved salt) adsorbed external to the double layer [158]. It quantifies the electrostatic repulsion or attraction between particles, representing an important parameter for the stability of the colloidal suspension, demonstrating the conditions that support its dispersion, aggregation and flocculation. Such information reduces stability testing time. The Zeta Potential equivalent to zero represents the isoelectric point (pI) of collagen [53,149,159]. The isoelectric point of collagen extracted from fishery and aquaculture by-products is described in the pH range of 4.71–7.26 [54,148], as illustrated in Table 1. ASC and PSC zeta potentials of collagen extracted from skin by-products of *Cylopterus lumpus* (Lumpfish) [44], and *Oreochromis niloticus* (Nile Tilapia) [160] were shown in pH 5.40 and 5.75 and pH 5.33 and 4.71, respectively. On its isoelectric point, collagen presents a more hydrophobic, compacted and less stable

structure due to the absence of repulsive forces between particles, this way, its chains lose the interaction with the solvent solution and precipitate, which harms the quality of the final product. The variations of isoelectric points between collagen types are attributed to differences in amino acid composition in the organism/source tissue and to the distribution of amino acid residues, mainly in surface domains [53,60,149,159].

5.3. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The characterization of isoelectric profile is performed with SDS detergent [161], which adds negative charges to the structure of proteins, facilitating their migration in the gel and increasing the resolution/separation of protein bands. In collagen characterization, the samples are mixed with a reducing agent such as β -mercaptoethanol, to break the disulfide bonds in the protein structure and separate the subunits. The mixture is applied to a large mesh gel (varying from 5% to 8%) along with molecular-weight markers and collagen pattern (usually bovine tendon collagen). The choice for a large mesh is due not only to the high molecular weight of the collagen chain subunits ($\alpha 1$ and $\alpha 2$) and its β dimers and γ trimer (120 – 200 kDa), but also to the presence of non-hydrolyzed called HMC (*high molecular weight cross-linked component*) and VMC (*very high molecular weight cross-linked component*). The gel stains used are the most common such as Coomassie blue or silver nitrate [5,29,39,112,162].

5.4. Optical gel densitometry

The optical densitometry of bands in SDS – PAGE electrophoresis wells can be assessed using the National Institute of Health (NIH) software ImageJ, available at: <https://imagej.nih.gov/ij/> and the ImageLab (Bio-RadLaboratories) software. The bands form peaks that are quantified as the areas under the peaks (area under the peaks - AUP) and as pixel integrated density (integrated density - ID, which represents the sum of pixel intensity values) in the Pixel Intensity graphs versus Distance ran in the gel [5,112,163]. For statistical analysis, the AUP values are multiplied by 10^{-4} (for band analysis).

5.5. Gel strength

Gel strength analysis measures the capacity of a colloidal dispersion of organizing itself into the correct construction of the polymer gel network, as well as resisting fragmentation of its protein structure [153,164], being important for the collagen quality and classified into 3 levels: low (<150 g), medium (150–220 g) and high (220–300 g) [142]. The determination is performed using a texturometer device with cylindrical probe (usually made of teflon) to penetrate the gel. The force is expressed as the maximum force (g) required for the probe to penetrate 4 mm into the sample under a 10 °C temperature [153,164]. This analysis investigates modifications in the collagen gel resistance due to composition, extraction method and solubilization variations, which contributes to or harms the rearrangement of the chains to their original form [153]. The variation in the amino acid composition and the size of protein chains of collagen from different species and tissues is possibly one of the reasons for the discrepancy in gel strength between the different types of collagen extracted from aquatic organisms. In temperatures lower than 10 °C, some short chain peptides in low viscosity collagenous solutions tend to strengthen the gel [142].

5.6. Amino acids analysis

In this analysis, the samples undergo acid hydrolysis (usually HCl or methanesulfonic acid), and are concentrated to be applied in amino acid analyzing devices using amino acid patterns for detection [29,39,100,112,165,166]. The main characteristic of the collagen primary structure is the presence of glycine and proline and hydroxyproline, forming tripeptide units such as glycine-X-proline or glycine-X- hydroxyproline, in which X can be any of the standard 20 amino acids [167]. The collagen molecules extracted from fishery resources are composed of amino acid concentrations varying according to the source of this biopolymer. In collagen isolated from fish skin, glycine (33.8%), alanine (12.2%), proline (11.3%), and imino acids (18.5%) are the most frequent [22], as illustrated in Table 2, which is a comparison of the amino acid composition of collagen from by-products (skin, scales, swimming bladder, cranium, tendons, among other) of aquatic and land animals processing.

5.7. Hydroxyproline determination

Hydroxyproline (Hyp) is a post-transductional imino acid of collagen molecules [168], and its content is quantified by means of a calorimetric technique [153,169–171]. For this type of essay, a calibration standard curve is performed (0.125 to 5 µg Hydroxyproline). In essays, an oxidizing solution (composed by 0.28 g chloramine T, 2 mL n-propanol and 16 mL citrate buffer) is used with a revealing solution, Erlich reagent (composed by 3.8 g p-Dimethylaminobenzaldehyde, 14 mL n-propanol and 5.9 mL 70% perchloric acid) is commonly employed. Initially, the oxidizing solution is added to the sample fractions. Hydroxyproline is formed

by oxidation of the carbon in the gamma position in the pyrrolidine proline ring through the action of prolyl-hydroxylase with ascorbic acid (vitamin C) as a cofactor. After oxidation, Erlich solution is added, forming the color red. The reading is done in a spectrophotometer, using a 550 nm wave length [169]. Hydroxyproline dosage is a commonly used technique in food segments, mainly in the assessment of products based on collagen extracted from skin and bones of fish, such as the production of gelatin and other products [172,173].

5.8. Centesimal composition of extracted material

The centesimal composition is determined according to Association of Official Analytical Chemists (AOAC), and online edition (<http://www.eoma.aoac.org/>), where they can be identified: i) Humidity, using gravimetric method by difference in weight before and after heating until constant weight is reached; ii) Protein, by the Kjeldahl crude protein method. The sample is digested with a mixture of copper sulfate e potassium sulfate. After adding sulfuric acid, the sample undergoes distillation and titration with hydrochloric acid. Results expressed in nitrogen percentage are converted in crude protein; iii) Lipids, determination occurs in the ether extract by the Soxhlet method, based on the difference in weight of the material before and after being subjected to extraction; iv) Ashes, determination of mineral residue or ashes by submitting samples to 550 °C in a muffle furnace with consequent destruction of organic matter; and, v) Carbohydrates, determination by the difference of the values found for humidity, proteins, lipids and ashes for a given amount of sample [174,175].

The presence of high levels of lipids and ash in the extracted collagen signals inefficient treatment (including demineralization stage) during the extraction process [176].

However, the centesimal composition will vary according to diets and culture systems, the source of collagen, between different species and with the stage of development of the specimens under study. The environment (marine or freshwater) is another factor of variability. This technique is not widely used to characterize and constitutes an option for the food industry, for example.

5.9. Color determination

Use of the CIE Lab system using the L*a*b color space scale [58,177], after calorimetric reading conversion. The L*a*b space is widely used for consistently correlating the numerical values of the scale with visual perception. The system is based in the concept of opposite or complementary colors that cannot occur at the same time (red-green and yellow-blue). The “L” represents the luminosity of black (0–50) to white (51–100). The “a” represents the scale from red (+a) to green (-a) and the “b” represents the variation from yellow (+b) to blue (-b). The three values are necessary to completely describe the color of an object/sample. The divergence of a sample to a predefined standard is represented by the differences in the three dimensions L, a and b (ΔL , Δa e Δb). The total difference in color is represented by $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$. Another parameter of sample differentiation is the saturation or color purity (chroma - C) which is given by $C = [(a)^2 + (b)^2]^{1/2}$. The color of collagen depends on the source and form of extraction [40]. When the ‘L’ value is high, it indicated a whiter sample, when ‘L’ is low; there probably is a low removal of pigments during the extraction steps, making the sample darker [13]. The color itself does not influence the functional properties of lyophilized collagen, but according to commercial data, lighter color foods are the preference of consumers, for this reason, lighter collagens are easily introduced in the food menu [40,178].

Table 2
Comparison of amino acid composition of collagen extracted from different sources, focusing on the fishing and aquaculture.

Tissue source	Fish ¹ Skin	Fish ² Scale	Fish ³ Spine	Fish ⁴ Skull	Fish ⁵ Bone	Fish ⁶ Swimming Bladder	Fish ⁷ Cartilage	Starfish ⁸ Body wall	Sea cucumber ⁹ Body wall	Jellyfish ¹⁰ Umbrella	Shell ¹¹ Body	Squid ¹² Skin	Squid ¹³ Muscle	Pig ¹⁴ Skin	Bovine ¹⁵ Tendon	Buffalo ¹⁶ Skin	Chicken ¹⁷ Feet	Ovine ¹⁸ Bone
Extraction Type Collagen	PSC Type I	ASC Type I	ASC Type I	PSC Type I	PSC Type I	ASC Type I	PSC Type II	PSC Type I	PSC Type I	PSC Type II	GSC Type I	PSC Type I	ASC Type I	PSC Type I	UPSC Type I	ASC Type I	SSC Type I	PSC Type I
Amino acid composition (Residues per 1000 total amino acid residues)																		
Alanine (Ala)	122.0	141.0	126.3	110.8	91.63	98.2	118.0	105.0	11.2	87.0	70.0	90.0	96.0	9.7	116.0	112.0	107.0	108.99
Arginine (Arg)	55.0	46.0	48.3	50.2	79.28	46.9	67.0	94.0	49.9	58.0	52.0	33.0	39.0	45.4	48.0	51.0	51.0	50.67
Aspartic acid (Asp)	45.0	63.0	46.8	43.7	44.48	43.9	39.0	78.0	47.0	76.0	81.0	97.0	71.0	28.1	42.0	43.0	42.0	47.35
Cysteine (Cys)	0.0	-	0.0	0.0	2.45	0.0	7.9	-	0.7	-	1.0	7.0	5.0	0.8	2.0	0.0	-	-
Glutamic acid (Glu)	80.0	87.0	66.7	77.4	101.17	46.6	67.0	106.0	85.8	101.0	118.0	87.0	82.0	57.4	76.0	76.0	99.0	84.08
Glycine (Gly)	338.0	429.0	339.1	330.2	215.45	322.4	336.0	232.0	31.2	320.0	244.0	278.0	315.0	31.3	336.0	332.0	239.0	316.69
Histidine (His)	5.0	0.0	5.3	3.9	9.45	9.0	5.0	-	-	-	11.0	5.0	5.0	-	3.0	6.0	15.0	4.94
Hydroxylysine (Hyl)	3.0	-	4.9	5.1	10.36	-	-	-	-	-	-	15.0	20.0	-	8.0	5.0	-	6.24
Hydroxyproline (Hyp)	72.0	52.0	73.8	69.8	87.22	73.4	42.0	111.0	-	70.0	65.0	37.0	60.0	-	99.0	97.0	54.0	101.42
Isoleucine (Ile)	9.0	8.0	12.7	24.8	14.24	8.4	21.0	19.0	7.2	23.0	26.0	18.0	14.0	9.2	9.0	12.0	34.0	11.35
Leucine (Leu)	20.0	20.0	26.0	30.9	27.86	16.2	56.0	16.0	17.1	31.0	42.0	44.0	33.0	21.4	21.0	19.0	60.0	26.17
Lysine (Lys)	27.0	6.0	29.5	27.9	39.34	23.4	20.0	18.0	8.6	17.0	35.0	14.0	16.0	41.6	25.0	25.0	58.0	27.92
Methionine (Met)	13.0	12.0	14.5	4.7	19.12	9.0	40.0	-	6.4	16.0	14.0	23.0	22.0	6.1	5.0	4.0	10.0	5.61
Phenylalanine (Phe)	12.0	10.0	14.3	20.9	20.19	9.1	17.1	4.0	10.4	14.0	17.0	29.0	22.0	14.9	2.0	9.0	25.0	13.10
Proline (Pro)	113.0	46.0	104.4	100.3	140.28	107.7	80.0	108.0	10.4	79.0	85.0	54.0	59.0	12.9	125.0	128.0	90.0	119.50
Serine (Ser)	40.0	33.0	33.3	36.8	38.36	25.4	35.4	40.0	28.8	44.0	55.0	83.0	51.0	29.4	37.0	31.0	40.0	30.24
Threonine (Thr)	25.0	30.0	25.2	27.8	33.40	16.7	-	340	31.2	34.0	35.0	29.0	20.0	14.7	20.0	19.0	37.0	20.10
Tyrosine (Tyr)	2.0	1.0	2.9	1.9	6.43	2.0	2.9	7.0	5.1	10.0	17.0	11.0	4.0	2.1	2.0	4.0	-	2.74
Tryptophan (Trp)	113.0	-	-	-	-	-	20.0	-	-	-	-	-	-	-	-	2.0	-	-
Valine (Val)	19.0	16.0	26.0	32.9	24.24	13.5	26.0	28.0	21.0	22.0	32.0	30.0	26.0	21.1	22.0	23.0	39.0	22.88
Imino acids*	185.0	98.0	178.2	170.1	226.85	181.1	122.0	209.0	-	149.0	150.0	91.0	119.0	-	224.0	225.0	144.0	220.90

¹ *Coryphaena hippurus* (Mahi mahi) [22].

² *Ctenopharyngodon idellus* (Grass Carp) [95].

³ *Katsuwonus pelamis* (skipjack tuna) [63].

⁴ *Katsuwonus pelamis* (skipjack tuna) [63].

⁵ *Thunnus obesus* (Bigeye tuna) [60].

⁶ *Nibea japonica* (giant croaker) [69].

⁷ *Prionace glauca* (Blue shark) [17].

⁸ *Acanthaster planci* (crown-of-thorns Starfish) [90].

⁹ *Holothuria cinerascens* (Sea cucumber) [25].

¹⁰ *Chrysaora* sp. (Ribbon jellyfish) [120].

¹¹ *Coelomactra antiquata* (Surf Clam Shell) [89].

¹² *Kondakovia longimana* (Antarctic squid) [87].

¹³ *Illex argentinus* (Sub-Antarctic squid) [87].

¹⁴ Porcine skin was purchased from the market [25].

¹⁵ Collagen-rich cattle short tendons (musculus extensor communis, musculus flexor digitorum, musculus digitorum profundis) [30].

¹⁶ *Bubalus bubalis* (Water buffalo) [31].

¹⁷ Sodium chloride-soluble collagen (SSC), Frozen chicken feet [138].

¹⁸ Ovine bones (Ujumuqin sheep) [36].*The iminoacid (proline + hydroxyproline).

5.10. Denaturation temperature (T_d)

High viscosity is an important physicochemical characteristic of collagen and viscometry, although it does not have enough sensitivity to detect the pre-transitional thermal stage in the viscosity curve, it is perfectly adequate for monitoring the main transitioning (inflection point of the curve) associated with the denaturation of collagen. Only one irreversible step is observed in viscosity data, during temperature variation until denaturation (in ASC and PSC), and it is associated with the transition stage relative to the disorder of the triple helix. Denaturation temperature (T_d) corresponds to the temperature in which the relative viscosity assumes the value of 0,5 [29,155,179–181]. High quantities of cross-links fragments such as HMC and VMC in the sample contribute to increase thermostability and T_d . Besides that, T_d values have the characteristic of being significantly lower than the physiological temperature of the collagen source organism [29,49,182]. The amount of imino acids (proline and hydroxyproline) interfere in the molecule stability, as higher proline hydroxylation improves collagenous protein thermal stability [69]. The study of denaturation (T_d) is of industrial importance, mainly in the production of biomaterials, as it influences biochemical, biophysical and biological properties in the collagen molecule [115]. Table 3 shows the denaturation temperature for collagen extracted from fisheries and aquaculture residues.

5.11. Thermogravimetric analysis (TGA)

Thermogravimetric Analysis (TGA) assesses the variation of a sample during temperature alterations in an environment with controlled pressure (nitrogen atmosphere) and temperature. Such environment occurs in a thermal analyzer that has a gas outlet and a scale attached to monitor the variation of reminiscing mass. The technique assesses the stability and degradation of materials including polymers, such as collagen. As the mass of collagen is reduced by the raise in temperature the loss is associated to events such as hydrogen bonds rupture and loss of intermolecular water, then the degradation of protein chains and rupture of the collagen fiber occurs. The mass alteration curve (TG) do not always precisely demonstrate the point of maximum loss and other events due to the gradual character of the phenomenon and is necessary to overlap the derived curve $\partial m/\partial t$ (DTG) [29,33,138,183,184].

6. Collagen spectroscopic characterization

6.1. Ultraviolet (UV) absorption spectrum

One of the basic spectroscopic methods for collagen characterization is the ultraviolet (UV) absorption scanning. The triple helix structure of collagen reaches a maximum peak at around 230 nm, probably due to the presence of C=O, -COOH and CONH₂ groups in its polypeptide chains [23,29,39]. Proteins generally present maximum absorption in the UV region near 280 nm, as a result of the direct contribution of tyrosine, tryptophan and phenylalanine. However, the quantity of these residues in the collagen molecule is very low, as seen on Table 3, lowering the absorption in this wave length [5,39]. The detection of peaks inferior to a 280 nm reading suggests the presence of a lower number of aromatic acid compounds in the studied protein structure [23]. Some collagen samples extracted from freshwater fish residues were inferior to the ones obtained from marine fish, as identified in Table 3. Cartilaginous fish had UV absorption peaks of 230 nm, while marine invertebrates reached peaks near 218 and 236.5 nm. UV absorption technique can be used for the assessment of the purity degree of collagenous samples, as an absorption close to 230 nm indicated

the triple helical structure of collagen, that is, the closer to 230 the greater the chances of the material to be purified [21], this can be used in a simple way by several industrial segments to know the viability of the collagen extracted.

Oliveira et al. [5] detected maximum UV peaks around 211 nm for pepsin-soluble collagen (PSC) extracted from *Cichla ocellaris* (Peacock bass) skin, while Chinh et al. [49] observed UV absorption peaks of 192.7 nm in acid-soluble collagen (ASC) isolated from *Cyprinus carpio* (Common carp) scales. In the marine environment, Kumar and Nazeer [185] detected maximum UV absorption peaks between 230 and 240 nm in collagens extracted from *Magalaspis cordyla* (Horse Mackerels) and *Otolithes ruber* (Croaker) skin, without interference in absorption due to the extraction method used by the authors (ASC and PSC); which was reaffirmed when 231 nm UV absorption peaks were observed for collagen extracted *Gadus macrocephalus* (Pacific cod) skin by PSC and ASC [117]. Similar values were detected for *Nibe japonica* (Giant Croaker, 230 nm) [21], *Centrolophus niger* (Black ruff, 232 nm) [23]. Song et al. [52] detected maximum UV absorption peaks of 230 nm for *Pelodiscus sinensis* (Soft-shelled turtle), a marine reptile, while Arunmozhivarman et al. [28] and Yousefi et al. [29] detected maximum UV absorption peaks of 230–240 nm and of 225 nm, respectively, for collagen extracted from by-products of domestic birds' skin.

6.2. X-ray diffraction (XRD)

X-ray diffraction technique has been applied in aquatic and marine biotechnology to identify the collagen structure through its fibrils' evaluation and orientation in mineralized tissues [43,49,186]. Diffraction occurs when there is interference in a wave through scattering centers (contiguous crystalline layers) whose spacings are the same size order as the wavelength of the applied radiation. If the wave length of the incident X-ray beam is in the order of 1 Å, its passage through the adjacent atomic layers of crystals in a sample generates the X-ray diffraction (XRD) phenomenon, whose specificity is due to the composition and arrangement of atoms, as well as the spacing of the crystalline planes [187,188]. XRD follows the Bragg law, in which the difference between the X-rays incident on adjacent layers, represented by a multiple of the incident wavelength ($n\lambda$) is twice the interlayer distance (d) by the sine of the scattering angle (θ) of the diffracted rays: $n\lambda = 2d \cdot \sin\theta$ [187]. The peaks in the 2θ curves versus Intensity relate to characteristics such as distance between collagen fibers, polypeptide chains bonds and/or collagen triple helix diameter, which can be affected by the extraction and solubilization method and which, then, reveal the integrity of the collagen extracted [51]. The collagen X-ray diffraction pattern shows three peaks: peaks C (diffraction angles: 5–10°), A1 (diffraction angles: close to 20°) and A2 (diffraction angles: 30–35°). Peak C is the first peak, exhibiting a distance between molecular chains, a second peak (A1) is used for diffuse scattering, while the third peak (A2) represents the height of the unit, typical of the triple helical structure [51,186].

When used in collagen extracted from by-products of teleost fish, Zang et al. [162], El-Rashidy et al. [189], Sun et al. [117], Sun et al. [190], and Alves et al. [43] reported the presence of two peaks (sharp peak and wide peak) in the analysis of collagen extracted from the scales of *Cyprinus carpio* (Carp fish) (PSC: 11.87 Å and 4.48 Å), scales from *Oreochromis niloticus* (Egyptian Nile Tilapia) (PSC: 11.56 Å and 4.48 Å), *Gadus macrocephalus* skin (Pacific cod) (ASC: 11.63 Å and 3.96 Å; PSC: 11.47 Å and 4.07 Å), *Oreochromis niloticus* (Nile Tilapia) skin (ASC: 11.66 Å and 4.18 Å; PSC: 11.90 Å and 4.38 Å), skin of *Salmo salar* (Atlantic codfish) (ASC: 11.46 Å and 4.52 Å), respectively. According to Chen et al. [51], when the distance between fibers is greater at peak 1, collagen is more capable

Table 3

Comparison of thermal and spectroscopic parameters of collagen extracted from by-products of animal processing.

Collagen source	Extraction	Tissue	Thermal properties			UV-vis	Circular dichroism (CD)		Ref.
			Differential scanning calorimetry (DSC)		Other denatures		Positive peak (maximum)	Negative peak	
			(T_{max})	(ΔH)					
<i>Scomber japonicus</i>	PSC	Bone	–	–	27 °C	–	–	–	[24]
<i>Scomber japonicus</i>	PSC	Skin	–	–	30 °C	–	–	–	[24]
<i>Oreochromis niloticus</i>	ASC	Skin	51.59 °C	–	–	222 nm	220 nm	196 nm	[160]
<i>Oreochromis niloticus</i>	PSC	Skin	50.57 °C	–	–	222 nm	220 nm	196 nm	[160]
<i>Coryphaena hippurus</i>	ASC	Skin	–	–	29.5 °C	–	221 nm	–	[22]
<i>Coryphaena hippurus</i>	PSC	Skin	–	–	28.8 °C	–	221 nm	–	[22]
<i>Thunnus obesus</i>	ASC	Skin	–	–	32.1 °C	–	–	–	[60]
<i>Thunnus obesus</i>	PSC	Skin	–	–	33.7 °C	–	–	–	[60]
<i>Thunnus obesus</i>	PSC	Scale	–	–	31.6 °C	–	–	–	[60]
<i>Thunnus obesus</i>	PSC	Bone	–	–	32.3 °C	–	–	–	[60]
<i>Anguilla anguilla</i>	ASC	Muscle	26.94 °C	–	–	–	220 nm	198 nm	[193]
<i>Anguilla anguilla</i>	PSC	Muscle	27.31 °C	–	–	–	220 nm	198 nm	[193]
<i>Nibeia japonica</i>	ASC	Swim bladders	–	–	33.8 °C	–	–	–	[69]
<i>Nibeia japonica</i>	PSC	Swim bladders	–	–	33.8 °C	–	–	–	[69]
<i>Takifugu flavidus</i>	SB ¹	Skin	41.8 °C	–	28.4 °C	234 nm	–	–	[46]
<i>Cyprinus carpio</i>	ASC	Scale	116 °C	3.7 J/g	32.2 °C	192.7 nm	–	–	[49]
<i>Ctenopharyngodon idella</i>	PSC	Skin	39.75 °C	–	–	235 nm	–	–	[66]
<i>Ctenopharyngodon idella</i>	PSC	Scale	34.49 °C	–	–	235 nm	–	–	[66]
<i>Carassius carassius</i>	PSC	Skin	39.05 °C	–	–	234 nm	–	–	[66]
Hybrid sturgeon	ASC	Skin	26.83 °C	–	32.78 °C	–	220 nm	198 nm	[147]
Hybrid sturgeon	PSC	Skin	26.54 °C	–	32.46 °C	–	220 nm	198 nm	[147]
<i>Oreochromis niloticus</i>	ASC	Skin	–	–	36.1 °C	–	–	–	[7]
<i>Oreochromis niloticus</i>	PSC	Skin	–	–	34.4 °C	–	–	–	[7]
<i>Coelomactra antiquate</i>	GSC	Body	33.05 °C	0.3667 J/g	–	–	–	–	[89]
<i>Coelomactra antiquata</i>	PSC	Body	31.33 °C	0.451 J/g	–	–	–	–	[89]
<i>Acipenser schrenckii</i>	PSC	Skin	34.4 °C	–	28.5 °C	–	221 nm	–	[68]
<i>Acipenser schrenckii</i>	PSC	Swim bladder	40.1 °C	–	30.5 °C	–	221 nm	–	[68]
<i>Acipenser schrenckii</i>	PSC	Notochord	–	–	33.5 °C	–	221 nm	–	[68]
<i>Probarbus jullieni</i>	ASC	Skin	36.91 °C	0.873 J/g	–	–	220 nm	198 nm	[151]
<i>Probarbus jullieni</i>	UASC ²	Skin	36.14 °C	0.896 J/g	–	–	219.9 nm	198 nm	[151]
<i>Probarbus jullieni</i>	PSC	Skin	38.27 °C	1.236 J/g	–	–	220.8 nm	198 nm	[151]
<i>Probarbus jullieni</i>	UPSC ²	Skin	40.82 °C	1.420 J/g	–	–	221 nm	198 nm	[151]
<i>Lates calcarifer</i>	PSC	Skin	109.6 °C	–	36.8 °C	230.3 nm	–	–	[151]
<i>Oreochromis niloticus</i>	PSC	Skin	113.7 °C	–	37.6 °C	230.9 nm	–	–	[50]
<i>Ictalurus punctatus</i>	ASC	Skin	36.12 °C	0.672 J/g	–	–	221 nm	200 nm	[142]
<i>Ictalurus punctatus</i>	HSC ³	Skin	36.05 °C	0.651 J/g	–	–	222 nm	199 nm	[142]
<i>Ictalurus punctatus</i>	PHSC ³	Skin	35.57 °C	0.564 J/g	–	–	221 nm	199 nm	[142]
<i>Nibeia japonica</i>	ASC	Skin	–	–	34.5 °C	–	–	–	[96]
<i>Nibeia japonica</i>	PSC	Skin	–	–	34.5 °C	–	–	–	[96]
<i>Misgurnus anguillicaudatus</i>	ASC	Skin	–	–	36.03 °C	218 nm	217 nm	197 nm	[130]
<i>Misgurnus anguillicaudatus</i>	PSC	Skin	–	–	33.61 °C	218 nm	217 nm	197 nm	[130]
<i>Müichthys miiuy</i>	ASC	Swim bladders	–	–	24.7 °C	226 nm	–	–	[70]
<i>Müichthys miiuy</i>	PSC	Swim bladders	–	–	26.7 °C	226 nm	–	–	[70]
<i>Cyclopterus lumpus</i>	ASC	Skin	–	–	17.9 °C	232 nm	–	–	[44]
<i>Cyclopterus lumpus</i>	PSC	Skin	–	–	17.5 °C	232 nm	–	–	[44]
<i>Probarbus jullieni</i>	ASC	Scale	37.67 °C	0.865 J/g	–	–	220.8 nm	198 nm	[65]
<i>Probarbus jullieni</i>	PSC	Scale	37.83 °C	1.185 J/g	–	–	221 nm	198 nm	[65]
<i>Salmo salar</i>	ASC	Skin	–	–	–	–	222 nm	196–200 nm	[43]
<i>Prionace glauca</i>	PSC	Skin	33 °C	–	–	–	–	–	[166]

(continued on next page)

Table 3 (continued)

Collagen source	Extraction	Tissue	Thermal properties			UV-vis	Circular dichroism (CD)		Ref.
			Differential scanning calorimetry (DSC)		Other denatures		Positive peak (maximum)	Negative peak	
			(T_{max})	(ΔH)					
<i>Scyliorhinus canicula</i>	PSC	Skin	23.6 °C	–	–	–	–	[166]	
<i>Thunnus albacares</i>	PSC	Skin	30.6 °C	–	–	–	–	[166]	
<i>Xiphias gladius</i>	PSC	Skin	31.4 °C	–	–	–	–	[166]	
<i>Kondakovia longimana</i>	ASC	Skin	24.04 °C	–	–	–	–	[87]	
<i>Kondakovia longimana</i>	PSC	Skin	34.17 °C	–	–	–	–	[87]	
<i>Kondakovia longimana</i>	ASC	Muscle	23.75 °C	–	–	–	–	[87]	
<i>Kondakovia longimana</i>	PSC	Muscle	33.74 °C	–	–	–	–	[87]	
<i>Illex argentinus</i>	ASC	Skin	23.21 °C	–	–	–	–	[87]	
<i>Illex argentinus</i>	PSC	Skin	31.49 °C	–	–	–	–	[87]	
<i>Pangasius pangasius</i>	ASC	Skin	80.1 °C	–	37.8 °C	–	–	[57]	
<i>Sepia pharaonis</i>	ASC	Skin	82.85 °C	–	–	–	–	[80]	
<i>Sepia pharaonis</i>	PSC	Skin	73.13 °C	–	–	–	–	[80]	
<i>Catla catla</i>	ASC	Scale	35.9 °C	1.04 J/g	–	232.12 nm	–	[12]	
<i>Catla catla</i>	PSC	Scale	36.15 °C	0.8 J/g	–	231.89 nm	–	[12]	
<i>Labeo rohita</i>	ASC	Scale	36.5 °C	0.97 J/g	–	232.07 nm	–	[12]	
<i>Labeo rohita</i>	PSC	Scale	37.73 °C	2.65 J/g	–	232.11 nm	–	[12]	
<i>Oreochromis niloticus</i>	ASC	Skin	–	–	35.2 °C	–	221 nm	197 nm [190]	
<i>Oreochromis niloticus</i>	PSC	Skin	–	–	34.5 °C	–	221 nm	197 nm [190]	
<i>Axinella cannabina</i>	ICC ⁴	–	25.4 °C	1.27 J/g	24.3 °C	–	221 nm	193–196 nm [75]	
<i>Suberites carnosus</i>	ICC ⁴	–	32.9 °C	5.74 J/g	28.2 °C	–	221 nm	193–196 nm [75]	
<i>Polyodon spathula</i>	ASC	Skin	–	–	29.6 °C	–	–	– [155]	
<i>Polyodon spathula</i>	PSC	Skin	–	–	28.2 °C	–	–	– [155]	
<i>Fugu flavidus</i>	ASC	Skin	–	–	27.4 °C	–	–	– [155]	
<i>Fugu flavidus</i>	PSC	Skin	–	–	26.9 °C	–	–	– [155]	
<i>Cyprinus carpio</i>	ASC	Scale	–	–	37 °C	–	–	– [48]	
<i>Loligo vulgaris</i>	ASC	Squid mantle	–	–	22 °C	–	–	– [19]	
<i>Loligo vulgaris</i>	PSC	Squid mantle	–	–	21 °C	–	–	– [19]	
<i>Labeo rohita</i>	ASC	Skin	36.40 °C	1.01 J/g	–	–	–	– [148]	
<i>Labeo rohita</i>	PSC	Skin	35.48 °C	0.31 J/g	–	–	–	– [148]	
<i>Nezumia aequalis</i>	ASC	Skin	31.55 °C	–	–	–	–	– [170]	
<i>Chimaera monstrosa</i>	ASC	Skin	35.65 °C	–	–	–	–	– [170]	
<i>Etmopterus spp.</i>	ASC	Skin	28.55 °C	–	–	–	–	– [170]	
<i>Galeus spp.</i>	ASC	Skin	28.45 °C	–	–	–	–	– [170]	
<i>Scyliorhinus canicula</i>	ASC	Skin	33.15 °C	–	–	–	–	– [170]	
<i>Leucoraja naevus</i>	ASC	Skin	30.25 °C	–	–	–	–	– [170]	
<i>Clarias gariepinus</i>	ASC	Skin	29.3 °C	–	–	–	–	– [171]	
<i>Salmo salar</i>	ASC	Skin	20.06 °C	–	–	–	–	– [171]	
<i>Gadus morhua</i>	ASC	Skin	15.2 °C	–	–	–	–	– [171]	
<i>Oreochromis niloticus</i>	PSC	Scale	32.09 °C	–	–	–	–	– [189]	
<i>Catla catla</i>	ASC	Skin	30.69 °C	0.4584 J/g	–	210–240 nm	–	– [40]	
<i>Catla catla</i>	PSC	Skin	34.99 °C	0.4293 J/g	–	210–240 nm	–	– [40]	
<i>Labeo rohita</i>	ASC	Skin	35.18 °C	0.7715 J/g	–	210–240 nm	–	– [40]	
<i>Labeo rohita</i>	PSC	Skin	35.19 °C	0.5886 J/g	–	210–240 nm	–	– [40]	
<i>Rhincodon typus</i>	PSC	Cartilage	34.02 °C	–	–	239.1 nm	222 nm	197 nm [11]	
<i>Rhincodon typus</i>	PSC	Cartilage/	–	–	–	–	–	– [11]	
Polypeptide	21.50 °C	–	–	210 nm	246.5 nm	202.5 nm	–	– [41]	
<i>Esox lucius</i>	ASC	Scale	79.3 °C	–	28.5 °C	210–240 nm	–	– [41]	
<i>Esox lucius</i>	PSC	Scale	80.1 °C	–	27 °C	210–240 nm	–	– [41]	
<i>Ctenopharyngodon idella</i>	ASC	Skin	36.4 °C	–	–	–	–	– [150]	

(continued on next page)

Table 3 (continued)

Collagen source	Extraction	Tissue	Thermal properties			UV-vis	Circular dichroism (CD)		Ref.
			Differential scanning calorimetry (DSC)		Other denatures		Positive peak (maximum)	Negative peak	
			(T_{max})	(ΔH)					
<i>Ctenopharyngodon idella</i>	PSC	Skin	35.7 °C	-	-	-	-	-	[150]
<i>Ctenopharyngodon idella</i>	ASC	Scale	39.9 °C	-	-	-	-	-	[150]
<i>Ctenopharyngodon idella</i>	PSC	Scale	35.4 °C	-	-	-	-	-	[150]
<i>Ctenopharyngodon idella</i>	ASC	Swim bladders	38.3 °C	-	-	-	-	-	[150]
<i>Ctenopharyngodon idella</i>	PSC	Swim bladders	38.0 °C	-	-	-	-	-	[150]
<i>Brama australis</i>	ASC	Skin	78.0 °C	238.1 J/g	24.0 °C	-	-	-	[180]
<i>Oreochromis niloticus</i>	ASC	Skin	31.15 °C	-	-	-	-	-	[181]
<i>Ctenopharyngodon idella</i>	ASC	Skin	36.74 °C	-	-	-	-	-	[181]
<i>Hypophthalmichthys molitrix</i>	ASC	Skin	36.88 °C	-	-	-	-	-	[181]
<i>Doryteuthis singhalensis</i>	ASC	Outer skin	-	-	35.70 °C	230 nm	-	-	[18]
<i>Doryteuthis singhalensis</i>	PSC	Outer skin	-	-	34.80 °C	222 nm	-	-	[18]
<i>Stichopus monotuberculatus</i>	PSC	Body Wall	30.2 °C	-	-	218 nm	-	-	[82]
<i>Chrysaora</i> sp.	PSC	Umbrella	37.38 °C	2.35 J/g	-	-	-	-	[120]
<i>Katsuwonus pelamis</i>	ASC	Spine	-	-	17.6 °C	220 nm	-	-	[63]
<i>Katsuwonus pelamis</i>	PSC	Spine	-	-	16.5 °C	220 nm	-	-	[63]
<i>Katsuwonus pelamis</i>	ASC	Skull	-	-	17.8 °C	220 nm	-	-	[63]
<i>Katsuwonus pelamis</i>	PSC	Skull	-	-	16.6 °C	220 nm	-	-	[63]
<i>Carcharhinus albimarginatus</i>	ASC	Cartilage	30.00 °C	-	30.00 °C	238.6 nm	221 nm	196-197 nm	[55]
<i>Carcharhinus albimarginatus</i>	PSC	Cartilage	31.25 °C	-	31.25 °C	237.7 nm	221 nm	196-197 nm	[55]
<i>Carcharhinus albimarginatus</i>	Gelatin	Cartilage	32.50 °C	-	32.50 °C	327.7 nm	221.5 nm	196-197 nm	[55]
<i>Thunnus albacores</i>	ASC	Swim bladders	32.97 °C	1.786 J/g	-	-	-	-	[71]
<i>Thunnus albacares</i>	PSC	Swim bladders	33.92 °C	0.354 J/g	-	-	-	-	[71]
<i>Saurida</i> spp. (Japan)	ASC	Scale	27.6 °C	0.44 mJ/mg	-	-	-	-	[67]
<i>Saurida</i> spp. (Vietnam)	ASC	Scale	27.4 °C	0.42 mJ/mg	-	-	-	-	[67]
<i>Trachurus japonicus</i> (Japan)	ASC	Scale	26.1 °C	0.29 mJ/mg	-	-	-	-	[67]
<i>Trachurus japonicus</i> (Vietnam)	ASC	Scale	28.1 °C	0.59 mJ/mg	-	-	-	-	[67]
<i>Mugil cephalis</i>	ASC	Scale	27.1 °C	0.28 mJ/mg	-	-	-	-	[67]
<i>Cypselurus melanurus</i>	ASC	Scale	29.2 °C	0.59 mJ/mg	-	-	-	-	[67]
<i>Dentex tumifrons</i>	ASC	Scale	28.2 °C	0.56 mJ/mg	-	-	-	-	[67]
<i>Ctenopharyngodon idellus</i>	SKA ⁵	Skin	35.6 °C	0.70 J/g	-	230 nm	-	-	[95]
<i>Ctenopharyngodon idellus</i>	SKP ⁵	Skin	35.8 °C	0.80 J/g	-	230 nm	-	-	[95]
<i>Ctenopharyngodon idellus</i>	SCA ⁵	Scale	34.8 °C	0.67 J/g	-	230 nm	-	-	[95]
<i>Ctenopharyngodon idellus</i>	SCP ⁵	Scale	35.2 °C	0.71 J/g	-	230 nm	-	-	[95]
<i>Ctenopharyngodon idellus</i>	BOA ⁵	Bone	36.0 °C	0.75 J/g	-	230 nm	-	-	[95]
<i>Ctenopharyngodon idellus</i>	BOP ⁴	Bone	36.4 °C	1.03 J/g	-	230 nm	-	-	[95]
<i>Acipenser schrenckii</i>	PSC-I	Skin	35.52 °C	-	-	-	221 nm	198 nm	[113]
<i>Acipenser schrenckii</i>	PSC-V	Skin	35.92 °C	-	-	-	221 nm	198 nm	[113]
<i>Acipenser schrenckii</i>	SSC ⁶	Skin	32.15 °C	-	-	-	221 nm	198 nm	[200]
<i>Acipenser schrenckii</i>	ASC	Skin	32.78 °C	-	-	-	221 nm	198 nm	[200]
<i>Acipenser schrenckii</i>	PSC	Skin	32.46 °C	-	-	-	221 nm	198 nm	[200]
Hybrid <i>Clarias</i> sp.	ASC	Skin	-	-	31.5 °C	-	-	-	[14]
Hybrid <i>Clarias</i> sp.	PSC	Skin	-	-	31 °C	-	-	-	[14]
<i>Scomberomorus niphonius</i>	ASC	Skin	-	-	15.12 °C	-	-	-	[15]

(continued on next page)

Table 3 (continued)

Collagen source	Extraction	Tissue	Thermal properties			UV-vis	Circular dichroism (CD)		Ref.
			Differential scanning calorimetry (DSC)		Other denatures		Positive peak (maximum)	Negative peak	
			(T_{max})	(ΔH)					
<i>Scomberomorus niphonius</i>	PSC	Skin	-	-	14.66 °C	-	-	-	[15]
<i>Scomberomorus niphonius</i>	ASC	Bone	-	-	18.02 °C	-	-	-	[15]
<i>Scomberomorus niphonius</i>	PSC	Bone	-	-	16.85 °C	-	-	-	[15]
<i>Acanthaster planci</i>	PSC	Body wall	-	-	33 °C	-	-	-	[90]
<i>Lates calcarifer</i>	ASC	Skin	33.33 °C	0.860 J/g	-	-	-	-	[149]
<i>Lates calcarifer</i>	ASC	Swim bladder	35.02 °C	0.918 J/g	-	-	-	-	[149]
<i>Evenchelys macrura</i>	ASC	Skin	-	-	38.5 °C	225 nm	230 nm	204 nm	[8]
<i>Evenchelys macrura</i>	PSC	Skin	-	-	35.0 °C	228 nm	230 nm	204 nm	[8]
<i>Rachycentron canadum</i>	ASC	Skin	38.17 °C	-	34.62 °C	219 nm	-	-	[39]
<i>Rachycentron canadum</i>	PSC	Skin	36.03 °C	-	33.97 °C	221 nm	-	-	[39]
<i>Diodon holocanthus</i>	ASC	Skin	29.64 °C	-	29.01 °C	210 nm	-	-	[9]
<i>Diodon holocanthus</i>	PSC	Skin	30.30 °C	-	30.01 °C	230 nm	-	-	[9]
<i>Nemipterus hexodon</i>	PSC	Skin	33.55 °C	0.819 J/g	-	230 nm	-	-	[112]
<i>Pangasianodon hypophthalmus</i>	ASC	Skin	35.5 °C	0.578 J/g	-	-	-	-	[159]
<i>Pangasianodon hypophthalmus</i>	PSC	Skin	35.3 °C	0.764 J/g	-	-	-	-	[159]
<i>Cyprinus carpio</i>	ASC	Scale	-	-	32.9 °C	-	-	-	[162]
<i>Cyprinus carpio</i>	PSC	Scale	-	-	29.0 °C	-	-	-	[162]
<i>Aluterus monoceros</i>	APSC ⁷	Skin	29.36 °C	0.60 J/g	-	-	-	-	[143]
<i>Aluterus monoceros</i>	YPSC ⁷	Skin	29.34 °C	0.28 J/g	-	-	-	-	[143]
<i>Aluterus monoceros</i>	PPSC ⁷	Skin	29.33 °C	0.83 J/g	-	-	-	-	[143]
<i>Chiloscyllium punctatum</i>	ASC	Skin	34.45 °C	0.661 J/g	-	230 nm	-	-	[53]
<i>Chiloscyllium punctatum</i>	PSC	Skin	34.52 °C	0.232 J/g	-	230 nm	-	-	[53]
<i>Chiloscyllium punctatum</i>	ASC	Cartilage	36.73 °C	1.553 J/g	-	-	-	-	[54]
<i>Chiloscyllium punctatum</i>	PSC	Cartilage	35.98 °C	0.847 J/g	-	-	-	-	[54]
<i>Carcharhinus limbatus</i>	ASC	Cartilage	36.38 °C	0.702 J/g	-	-	-	-	[54]
<i>Carcharhinus limbatus</i>	PSC	Cartilage	34.56 °C	0.949 J/g	-	-	-	-	[54]
<i>Sebastes mentella</i>	ASC	Skin	-	-	16.1 °C	-	-	-	[62]
<i>Sebastes mentella</i>	ASC	Scale	-	-	17.7 °C	-	-	-	[62]
<i>Sebastes mentella</i>	ASC	Bone	-	-	17.5 °C	-	-	-	[62]
Other sources of collagen									
Chicken	UPSCII ⁸	Sternal cartilage	54.18 °C	-	44.97 °C	218.1 nm	-	-	[27]
Chicken	PSC	Fat lungs	90.16 °C	-	38.5 °C	-	-	-	[152]
Chicken	UPSC ⁹	Fat lungs	94.16 °C	-	35.3 °C	-	-	-	[152]
<i>M. gallopavo</i>	PSC	Turkey tendon	44.5 °C	-	-	-	221.5 nm	-	[183]
<i>Ujumuqin sheep</i>	ASC	Bone ovine	42.31 °C	1.11 J/g	-	231.3 nm	-	-	[36]
<i>Ujumuqin sheep</i>	PSC	Bone ovine	38.91 °C	1.91 J/g	-	231.8 nm	-	-	[36]
<i>Bubalus bubalis</i>	ASC	Skin	51.2 and 60.2 °C	-	-	231 nm	-	-	[31]
Chicken	SSC	Feet	-	-	-	-	213 nm	-	[138]
Chicken	ASC	Feet	-	-	-	-	213 nm	-	[138]
Chicken	PSC	Feet	-	-	-	-	213 nm	-	[138]
<i>Dromaius novaehollandiae</i>	PSC	Emu skin	-	-	31.5 °C	235.1 nm	-	-	[16]
Chicken	Papain	Feet	-	-	49.80 °C	-	-	-	[145]
Chicken	PSC	Feet	-	-	57.68 °C	-	-	-	[145]
Bovine	UPSC ¹⁰	Tendons*	-	-	38.2 °C	-	-	-	[30]

Pro -proline; Hyp- hydroxyproline; The difference of Tmax is correlated with the imino acid content (proline and hydroxyproline), body temperature and environmental temperature. ASC- Extraction acid-solubilised collagen, and/or Extraction pepsin-solubilised collagen (PSC).¹Sodium bicarbonate and electro dialysis (SB).

² Acid-soluble ultrasound-assisted method (UASC) and pepsin-soluble ultrasound-assisted method (UPSC).

³ Extraction of collagen with homogenization-aided (HSC) method, extraction of collagen with pepsin and homogenization aided (PHSC) method.

⁴ Collagen fibrils (ICC).

⁵ SKP = pepsin-soluble collagen of skin; SKA = acid-soluble collagen of skin; BOP = pepsin-soluble collagen of bone; BOA = acid-soluble collagen of bone; SCP = pepsin-soluble collagen of scale; SCA = acid-soluble collagen of scale.

⁶ Isolated using sodium chloride (SSC).

⁷ PSC extracted with the aid of albacore tuna pepsin (APSC), yellowfin tuna pepsin (YPSC) and porcine pepsin (PPSC), respectively.

⁸ Extraction using pepsin soluble and ultrasound treatment time 36 min (UPSCII36).

⁹ Pepsin-soluble collagen by ultrasound pre-treatment (UPSC).

¹⁰ Pepsin-soluble collagen by ultrasound pre-treatment (UPSC). * Musculus extensor communis, musculus flexor digitorum, musculus digitorum profundus.

of carrying drugs, and is therefore a type preferred by the biopharmaceutical industry.

6.3. Differential scanning calorimeter (DSC)

Differential scanning calorimeter (DSC) monitors enthalpy (ΔH) variations in the collagen sample, in relation to a base line generated by a thermally inert reference material (usually the element Indium). The sample and the inert material are maintained in the same temperature during essay thermal variation programming. The energy spent to maintain the sample in the same temperature of the reference material during the process is expressed as positive and negative peaks, in case energy is removed (exothermic peak) or added (endothermic peak), respectively. Endothermic peaks in samples are associated to conformational modifications and destruction of the triple helix, corresponding to the denaturation of the collagen fibrils and its values represent the maximum transition (T_{max}) temperatures. Endothermic peaks T_{max} values are directly proportional to the enthalpy (ΔH) e directly proportional to resistance against denaturation. Thus, pepsin-solubilized collagens (PSC) are commonly less thermostable as they present lower endothermic peak T_{max} values than collagen solubilized by acid hydrolysis (ASC), due to the removal of the telopeptide region by pepsin [29,39]. Studies show a positive correlation between collagen thermostability and the imino acid and amino acid (proline and hydroxyproline) content in the sample due to the increase in stabilizing hydrogen bonds formed by these residues [29,53,191]. Table 3 provides information about ΔH variations of collagen extracted from fishery and aquiculture by-products, as well as from animals processing in general.

6.4. Circular dichroism (CD)

Circular dichroism is one of the most sensitive spectroscopic techniques to determine and monitor protein structural alterations and the molecular order of collagen [55]. It can directly interpret the alterations in the secondary structure, even if the method is empirical. The far ultraviolet spectra (under 250 nm) of proteins are extremely sensitive, and the near UV spectra reflect the contributions of aromatic side chains, disulfide bonds and CD bands from prosthetic bands. Together, these measures provide information about the general structure of a protein molecule, as well as its local conformation around aromatic and prosthetic groups and disulfide bonds [192]. In practice, this technique can be used to evaluate the presence of the secondary structure of collagen through the differential absorption of left-handed and right-handed circular polarized light in an asymmetric environment [43].

The essay is performed in a spectrometer using a quartz cylindrical cuvette. For each reading, the collagen is immersed in acetic acid solution 0.5 M for its solubilization. The CD spectra are obtained by continuous wave length scanning and can be verified in a scale of 180 to 260 nm; or with a fixed spectrum length of 221 nm, which is indicative that the triple helix is preserved. The temperature can be one of the variables tested, and a spectrum value lower than 221 nm indicates that the structure was altered, probably denaturation. Results are expressed in molar residue ellipticity, $[\theta]$: $[\theta] = (\theta * 100 * M) / (C * l * n)$. Where " θ " is ellipticity in degrees, " l " is the optical path in cm, " C " is the concentration in mg / mL, " M " is the molecular weight and " n " is the number of amino acid residues in the protein [22,43,65,193].

Table 3 provides the widely used CD in aquiculture biotechnology and successfully applied in the structural characterization of collagen extracted from by-products from fishery processing (scales, skin, muscle residues from teleost fish), as described by Ali

et al. [65], Alves et al. [43], Akita et al. [22], and Cao et al. [193] for *Probarbus jullieni* (Golden carp) (positive peak 221 nm), *Salmo salar* (Atlantic Salmon) (positive peak 222 nm), *Coryphaena hippurus* (Mahi mahi) (positive peak 221 nm) e *Anguilla anguilla* (European eel) (positive peak 220 nm), respectively. When investigating marine sponge species (*Axinella cannabina* and *Suberites carnosus*), Tziveleka et al. [75] identified maximum peaks of 221 nm for both species. All results indicate the presence of intact collagen secondary structure. The biggest limitation of this technique is the fact that the algorithms used for the conversion of the data obtained in structural data are based in globular proteins, different from the helical nature of collagen [194].

6.5. Fourier transform infrared spectroscopy (FTIR)

The collagen Fourier transform infrared (FTIR) spectrum can be characterized as a set of regions known as amide bands e vibration bands of proline and hydroxyproline pyrrolidine rings [29,195,196]. These bands provide information about the secondary structure of polypeptide chains (amide bands) as well as the characteristic presence of imino acids amino acids (proline and hydroxyproline) in the structure. Amide I can be considered a marker of the secondary structure and it is linked to the stretching vibration in the C=O bond ($1600\text{--}1700\text{ cm}^{-1}$) forming hydrogen bonds between adjacent chains. The decrease in the amide I band for lower frequencies is linked to the increase in hydrogen bonds and the consequent increase in molecular organization [20,197,198]. The amide II band is linked to the CN elongation and NH deformation vibration ($1550\text{--}1600\text{ cm}^{-1}$), specifying the number of NH groups involved in hydrogen bonds with adjacent α -chains (the lower the frequency, the greater the number of bonds) and highlighting the degree of maintenance of the collagen helical structure. The amide II band is also related to the vibration of glycine CH_2 , which is an amino acid present in large amounts in collagen [20,29,198]. The vibration of glycine CH_2 is also attributed to the amide III band [199]. This band is mainly associated with NH deformation and CN elongation in collagen amide bonds [20]. Other characteristic collagen bands are amide A and B. The amide band "A" is related to the stretching vibration of the NH group ($3400\text{--}3440\text{ cm}^{-1}$) and when this group is involved in hydrogen bonds in the peptide chain, the frequency is reduced to approximately 3300 cm^{-1} . The amide band "B" ($2924\text{--}2928\text{ cm}^{-1}$) is linked to the asymmetric stretching vibration of $=\text{CH e }-\text{NH}_3^+$. The shift from amide "B" to higher frequencies denotes an increase in free $\text{NH}\text{--}\text{NH}_3^+$ clusters in the N-terminal lysine residues [29]. The intensity of the band relative to the vibration of the proline and hydroxyproline pyrrolidine rings (around 1440 cm^{-1}) shows the amount of these amino acids in the structure of the analyzed collagen sample. The pyrrolidine ring imposes conformational mobility restrictions in the collagen polypeptide chains strengthening the triple helix [5,112,195,198]. The extraction technique used can lead to structural changes in the protein, with differences in functional groups and inter and intra-molecular interaction through FTIR [143], mainly in extractions with high degrees of hydrolysis. FTIR has the advantage of being fast, reliable and precise [20,185], largely used for structural identification of collagens extracted from fishery and aquiculture by-products [200], as illustrated in Table 4, used also to identify collagen isolated from mammals and land birds. One parameter that shows the degree of maintenance of the original structure of the collagen molecule after the extraction steps is the absorption / transmittance ratio. The value of approximately 1.0 of this ratio (between the value of amide III peak and that of proline and hydroxyproline pyrrolidine rings - 1440 cm^{-1}) means maintenance of the triple helix in the collagen structure after extraction [176].

Table 4 (continued)

Collagen source	Extraction	Tissue	Amide A	Amide B	Amide I	Amide II	Amide III	A/T	Ref.
<i>Chiloscyllium punctatum</i>	ASC	Cartilage	3293–3306 cm ⁻¹	2920–2922 cm ⁻¹	1641 cm ⁻¹	1536–1544 cm ⁻¹	1454 cm ⁻¹	~1.0	[54]
<i>Chiloscyllium punctatum</i>	PSC	Cartilage	3293–3306 cm ⁻¹	2920–2922 cm ⁻¹	1633 cm ⁻¹	1536–1544 cm ⁻¹	1454 cm ⁻¹	~1.0	[54]
<i>Carcharhinus limbatus</i>	ASC	Cartilage	3293–3306 cm ⁻¹	2920–2922 cm ⁻¹	1633–1634 cm ⁻¹	1536–1544 cm ⁻¹	1454 cm ⁻¹	~1.0	[54]
<i>Carcharhinus limbatus</i>	PSC	Cartilage	3293–3306 cm ⁻¹	2920–2922 cm ⁻¹	1633–1634 cm ⁻¹	1536–1544 cm ⁻¹	1454 cm ⁻¹	~1.0	[54]
<i>Sebastes mentella</i>	ASC	Skin	3425 cm ⁻¹	2935 cm ⁻¹	1658 cm ⁻¹	1552 cm ⁻¹	1240 cm ⁻¹	~1.0	[62]
<i>Sebastes mentella</i>	ASC	Scale	3296 cm ⁻¹	2926 cm ⁻¹	1653 cm ⁻¹	1541 cm ⁻¹	1242 cm ⁻¹	~1.0	[62]
<i>Sebastes mentella</i>	ASC	Bone	3300 cm ⁻¹	2926 cm ⁻¹	1654 cm ⁻¹	1541 cm ⁻¹	1240 cm ⁻¹	~1.0	[62]
<i>Lates niloticus</i> (Young)	ASC	Skin	3434 cm ⁻¹	2924 cm ⁻¹	1650 cm ⁻¹	1542 cm ⁻¹	1235 cm ⁻¹	–	[176]
<i>Lates niloticus</i> (Adult)	ASC	Skin	3458 cm ⁻¹	2926 cm ⁻¹	1654 cm ⁻¹	1555 cm ⁻¹	1238 cm ⁻¹	–	[176]
Other sources of collagen									
Chicken	UPSCII36 ¹²	Cartilage	3311.04 cm ⁻¹	2927.68 cm ⁻¹	1637.63 cm ⁻¹	1547.43 cm ⁻¹	1456.45 cm ⁻¹	–	[27]
Porcine	ASC-PSC	Skin	3300 cm ⁻¹	2929 cm ⁻¹	1630–1666 cm ⁻¹	1550 cm ⁻¹	1240 cm ⁻¹	1.0	[34]
Chicken	ASC	Feet	3399.56 cm ⁻¹	2923.72 cm ⁻¹	1652.01 cm ⁻¹	1539.87 cm ⁻¹	1241.29 cm ⁻¹	–	[174]
Sheep	ASC	By-products	3325 cm ⁻¹	2924 cm ⁻¹	1659 cm ⁻¹	1553 cm ⁻¹	1231 cm ⁻¹	0.84	[32]
Lamb	ASC	By-products	3318 cm ⁻¹	2922 cm ⁻¹	1657 cm ⁻¹	1560 cm ⁻¹	1238 cm ⁻¹	0.85	[32]
Chicken	PSC	Fat lungs	3300 cm ⁻¹	2891 cm ⁻¹	1673 cm ⁻¹	1582 cm ⁻¹	1237 cm ⁻¹	–	[152]
Chicken	UPSC ¹³	Fat lungs	3316 cm ⁻¹	2889 cm ⁻¹	1675 cm ⁻¹	1579 cm ⁻¹	1237 cm ⁻¹	–	[152]
<i>M. gallopavo</i>	PSC	Tendon	3324 cm ⁻¹	2938 cm ⁻¹	1658 cm ⁻¹	1548 cm ⁻¹	1234 cm ⁻¹	–	[183]
Chicken	Papain	Feet	3464–3433 cm ⁻¹	2927–2852 cm ⁻¹	1639.42 cm ⁻¹	1555–1451 cm ⁻¹	1200 cm ⁻¹	–	[144]
<i>Ujumuqin sheep</i>	ASC	Bone	3307 cm ⁻¹	2925 cm ⁻¹	1656 cm ⁻¹	1550 cm ⁻¹	1238 cm ⁻¹	1.1	[36]
<i>Ujumuqin sheep</i>	PSC	Bone	3305 cm ⁻¹	2922 cm ⁻¹	1656 cm ⁻¹	1550 cm ⁻¹	1238 cm ⁻¹	1.0	[36]
Porcine	PSC	Skin	3315 cm ⁻¹	3073 cm ⁻¹	1641 cm ⁻¹	1552 cm ⁻¹	1244 cm ⁻¹	~1.0	[44]
Chicken	PSC	Skin	305.19 cm ⁻¹	2922.52 cm ⁻¹	1633.98 cm ⁻¹	1549.08 cm ⁻¹	1238.07 cm ⁻¹	–	[28]
<i>Coturnix japonica</i>	ASC	Feet	3306.65 cm ⁻¹	2924.75 cm ⁻¹	1633.71 cm ⁻¹	1550.99 cm ⁻¹	1238.38 cm ⁻¹	–	[29]
<i>Coturnix japonica</i>	PSC	Feet	3294.64 cm ⁻¹	2928.82 cm ⁻¹	1631.09 cm ⁻¹	1547.9 cm ⁻¹	1238.7 cm ⁻¹	–	[29]
<i>Ovis aries</i>	ASC	Tendon	3302 cm ⁻¹	2923 cm ⁻¹	1632 cm ⁻¹	1548 cm ⁻¹	1237 cm ⁻¹	–	[35]
<i>Bubalus bubalis</i>	ASC	Skin	3299 cm ⁻¹	2950–2919 cm ⁻¹	1628 cm ⁻¹	1540 cm ⁻¹	1234 cm ⁻¹	–	[31]
Chicken	ASC	Feet	3297 cm ⁻¹	2930 cm ⁻¹	1630 cm ⁻¹	1552 cm ⁻¹	1238 cm ⁻¹	–	[138]
Chicken	PSC	Feet	3308 cm ⁻¹	2932 cm ⁻¹	1629 cm ⁻¹	1548 cm ⁻¹	1242 cm ⁻¹	–	[138]
<i>Dromaius novaehollandiae</i>	PSC	Skin	3309 cm ⁻¹	2925 cm ⁻¹	1633 cm ⁻¹	1541 cm ⁻¹	1237 cm ⁻¹	–	[16]
Type I collagen from human placenta			3420 cm ⁻¹	2928 cm ⁻¹	1646 cm ⁻¹	1536 cm ⁻¹	1236 cm ⁻¹	–	[18]
Bovine	UPSC ¹⁴	Tendons*	3310 cm ⁻¹	3082 cm ⁻¹	1636 cm ⁻¹	1550 cm ⁻¹	1241 cm ⁻¹	1.20	[30]
The standard type IV collagen from human placenta			3421 cm ⁻¹	2959 cm ⁻¹	1644 cm ⁻¹	1578 cm ⁻¹	1249 cm ⁻¹	~1.0	[8]

ASC- Extraction acid-solubilised collagen, and/or Extraction pepsin-solubilised collagen (PSC). ¹Hot water method (HWM) and Sodium hydroxide method (SHM).

² Sodium bicarbonate and electro dialysis.

³ Extracted by isoelectric precipitation (PSC-IP).

⁴ Collagen fibres incubated for 72 h in collagenase Type I.

⁵ Extraction of guanidine hydrochloride soluble collagen (GSC).

⁶ CSC1, CP (prepared from *Bacillus cereus* FORC005) soluble collagen; CSC2, CP (prepared from *Bacillus cereus* FRCY9–2) soluble collagen.

⁷ Acid-soluble ultrasound-assisted method (UASC) and pepsin-soluble ultrasound-assisted method (UPSC).

⁸ The effect of acetic acid, NaCl, solid/solvent ratio and time on the extraction of collagen were studied by one variable at a time (OVAT) method.

⁹ Spicule-free insoluble collagen (SF-InSC), Soluble collagen (InSC), Intercellular collagen (ICC) and Spongin-like collagen (SIC).

¹⁰ Isolated using sodium chloride (SSC).

¹¹ PSC extracted with the aid of albacore tuna pepsin (APSC), yellowfin tuna pepsin (YPSC) and porcine pepsin (PPSC), respectively.

¹² Extraction using pepsin soluble and ultrasound treatment time 36 min (UPSCII36).

¹³ Pepsin-soluble collagen by ultrasound pre-treatment (UPSC).

¹⁴ Collagen extraction through ultrasonic-pepsin tandem treatment. *Musculus extensor communis, musculus flexor digitorum, musculus digitorum profundis. A/T: absorption/transmittance ratio.

6.6. Raman spectroscopy

Raman spectroscopy is a technique that allows observation of vibrational, rotational or other low frequency modes [201]. The principle of this technique is based on the inelastic dispersion (Raman) of monochromatic light by matter, commonly from a laser in the visible range, close to infrared (IR), interacting with the vibrational molecular waves and photons, thus displacing the photons to different directions [201–203], detected through a spectrometer with a double monochromator, having been first observed in 1928 by Chandrasekhara Venkata Raman [204]. Still, in this technique the peptide bond that originates different vibrational types is observed, the Amide I and Amide III modes [31,205,206], causing no damage

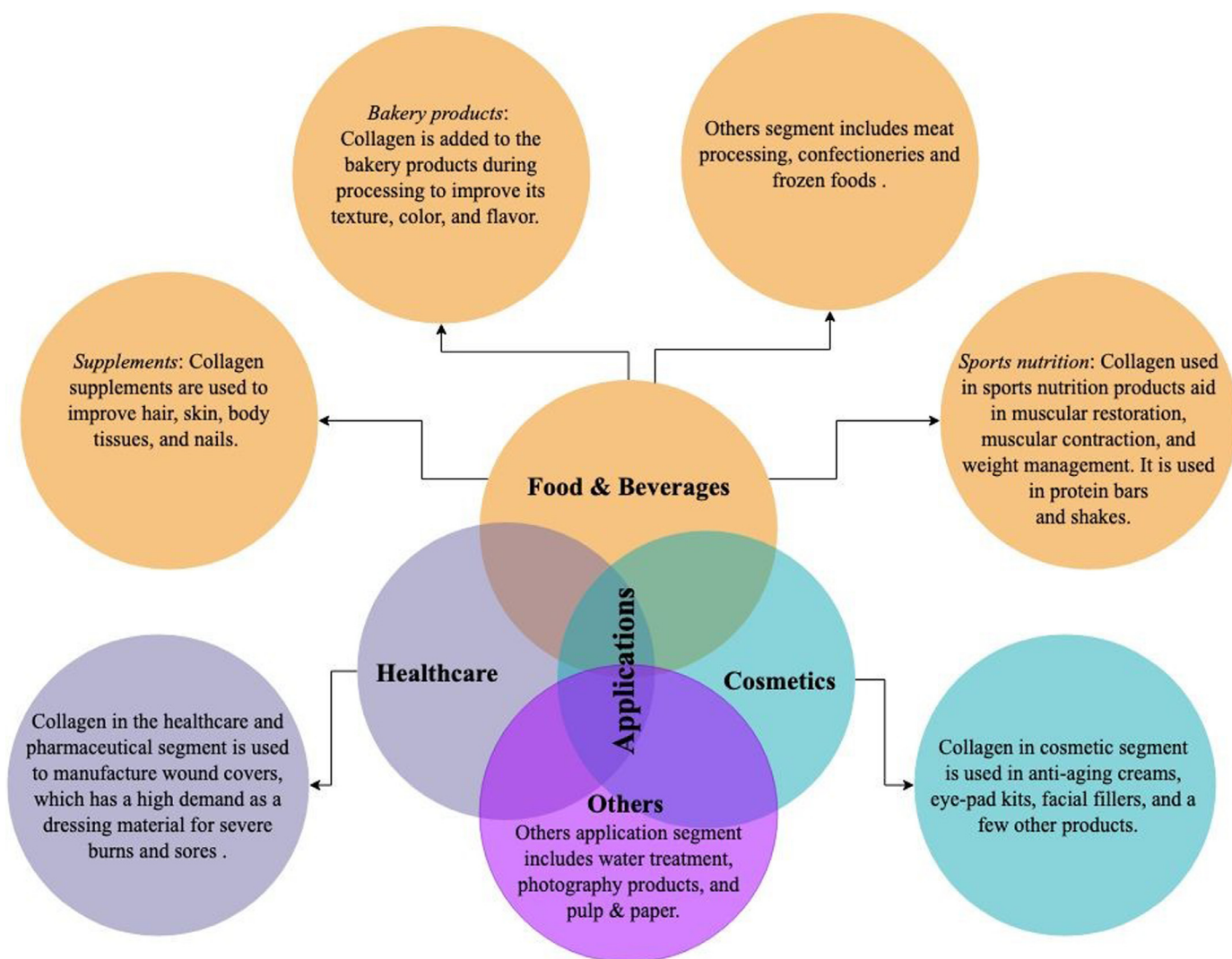
to the material and neither needing chemical markers for its identification [207].

Raman spectroscopy can be applied to biopharmaceutical polymers, in the area of regenerative medicine [204], and detection of chemical and biological compounds [208], through two methods: Raman microspectroscopy [206], using spectroscopic images to obtain the distribution of components and the orientation of collagen fibers in ECM [209]; and fiber-based spectroscopy, a valuable tool in the detection of tissue changes [203], enabling the identification of conformational changes [205,210], mainly caused by temperature fluctuations, as described for collagen extracted from by-products of scales of *Carassius auratus* (Gold fishes) [211].

Table 5

Companies operating in the collagen and derivatives (gelatin, peptides) market.

Companies operating in global collagen	Operating segment	Collagen-based products	Action
Nitta Gelatin Inc. (http://nitta-gelatin.com/)	Biomedical Biomedical	beMatrix® Cellmatrix®	Products for biomedical use and tissue engineering. Products for biomedical use and tissue engineering.
Rousset BV (https://www.rousset.com/)	Food, nutrition and health	Rousset® ResistaGel™	Gelling agent for the development of confectionery products.
	Food, nutrition and health	Rousset® AcidoGel™	Gelling agent allows you to manufacture stable acid marshmallows.
	Food, nutrition and health	SiMoGel™	Functional ingredients.
	Food, nutrition and health	StabiCaps™	Functional ingredients.
	Food, nutrition and health	Peptan®	Collagen peptides with biofunctional properties in bone metabolism.
	Regenerative Medicine	X-Pure®	3D bioprinting, wound healing, implantable membranes and drug delivery.
	Food, nutrition and health	GELITA ® RTE-DRINK	Promotes natural, highly digestible, non-allergenic food.
GELITA AG (https://www.gelita.com/en)	Food, nutrition and health	PEPTIPLUS®	Highly specialized collagen peptides, increasing muscle mass and strength.
	Pharmaceutical	FORTIGEL®	Stimulates the body's own mechanisms for maintaining healthy joints.
	Pharmaceutical	TENDOFORTE®	Increases the health and quality of ligaments and tendons.
	Pharmaceutical (animal) Photographic technology	PETAGILE® IMAGEL®	Neutralizes wear and tear on the joints. Photographic gelatines, testing films, microfilm, graphic film and holography.
	Cosmetic and health	QYRA®	Increases the attractiveness, smoothness and elasticity of the skin.
	Cosmetic and health	VERISOL®	Restores skin moisture, prevents the formation of wrinkles.
	Cosmetic and health Health and beauty	BODYBALANCE® VERISOL®	Increasing muscle mass and strength. Stimulates the skin's metabolism, reduces cellulite, promotes faster nail growth.
Symatase (https://www.symatase.com/)	Pharmaceutical Pharmaceutical Pharmaceutical	HEMOTESE® COLLAPAT® II NEVELIA®	Local hemostatic. Osteoconductive. Keep cell adhesion signals and mechanical structure to support regeneration.
	Biomedical research	SpongeCol®	Tissue engineering applications.
	Advanced BioMatrix, Inc. (https://advancedbiomatrix.com/)	Biomedical research Biomedical research	PureCol® Lifeink® PhotoCol®
Jellagen Pty Ltd. (https://www.jellagen.co.uk/)	Biomedical	JellaGel™	Jellyfish collagen hydrogel for <i>in vitro</i> cell culture and tissue engineering.
	Biomedical	Jellagen® 3D	Proliferation and differentiation to develop functional matrices.
GELNEX (https://www.gelnex.com.br/pt/) Holista CollTech Ltd. (https://www.holistaco.com/index.html)	Food, nutrition and health	PEPTINEX®	Food supplement.
	Cosmetic and health	OVICOLL™98	Sheep collagen, active ingredient in cosmetics and cosmeceuticals.
	Cosmetic and health	OVICOLL™95	Active ingredient in cosmetics, including face cream, body lotions, shampoo.
LAPI GELATINE S.p.a. (http://www.lapigelatine.com/it/)	Food, cosmetic and health	OVINEX™	Functional, nutraceutical and cosmeceutical foods and drinks.
	Nutraceuticals and nutricosmetica	FISH EDIBLE GELATINE	Gelatine obtained by the hydrolysis of collagen present in fish skin.
	Nutraceuticals and nutricosmetica	PHARMA GRADE FISH GELATINE	Gelatine obtained by the hydrolysis of collagen present in fish skin.
Weishardt (https://www.weishardt.com/) QUIRIS Healthcare (https://www.quiris.de/en/)	Neutraceuticals and health	Naticol®	Food products, including confectionery, snacks, baked goods, soups and purées.
	Pharmaceutical	ELASTEN®	Strengthens the collagen structure of all skin layers, improves elasticity.
	Pharmaceutical	CH-Alpha® PLUS	Regeneration of collagen in the articular cartilage.
Collagen Matrix (http://collagenmatrix.com/)	Biomedical	OssiMend® Bioactive Moldable Strips	Bone grafts.
	Biomedical	OssiMend® Bioactive Moldable Strips	Bone grafts.
	Biomedical	TenoMend™	Orthopedic.
	Biomedical	DuraMatrix® Suturable	Dural repair.
	Biomedical	NeuroMatrix®	Repair of peripheral nerves.
GelcoPEP (https://www.gelcopep.com/pt/home)	Nutricosmetica and health	GelcoPEP® CHD	It improves the conditions of the organism.
	Nutricosmetica and health	GelcoPEP® PLUS	It improves the conditions of the organism.



The key products include:	
Gelatin	Gelatin is derived from collagen, which is sourced from bovine, porcine, poultry, and marine, and has widespread applications in food & beverage, healthcare, and pharmaceutical industries.
Hydrolyzed collagen	Hydrolyzed collagen is also referred to as gelatin hydrolysate, collagen hydrolysate, collagen peptides, and hydrolyzed gelatin. It is sourced from collagen found in connective tissue, skin, and bones of animals.
Native	Native collagen is used in skincare formulations to delay the formation of new wrinkles and aids in the reduction of marks formed due to skin burns and minor skin wounds.

Fig. 6. Global collagen market and the main industrial segments operating in it. Image prepared in Flowchart Maker and Online Diagram Software (app.diagrams.net).

7. The global collagen market

The forecast for the global collagen market is positive. According to a report by Grand View Research [38] it is expected a greater development for the sector, with an estimated growth of up to

US \$ 7.5 billion by 2027, making the collagen products market a segment of great economic visibility. Some of the main companies operating in the global collagen branch are: Advanced BioMatrix, Inc. (U.S.), Symatase (France), Rousselot (The Netherlands), ITAL-GELATINE S.p.A. (Italy), Juncà Gelatines SL (Spain), Ewald-Gelatine

GmbH (Germany), REINERT GROUP Ingredients GmbH (Germany), QUIRIS Healthcare (Germany), Trobas Gelatine B.V. (the Netherlands), Holista CollTech Ltd. (Australia), Tessengerlo Group NV (Belgium), and GELNEX (Brazil). Companies like Nitta Gelatin Inc. (Japan), Weishardt (France), LAPI GELATINE S.p.a. (Italy), GELITA AG (Germany), Collagen Solutions plc (U.K.), and Jellagen Pty Ltd (U.K.) operate with collagen-based products extracted from aquatic sources, signaling the real possibility of aquatic biopolymers being inserted in the formulation of new products biotechnological.

The knowledge about the basic properties of the collagen extracted is a way to direct it to the global protein market for future applications (Fig. 6). Collagens with higher solubility and great ease to retain water are desirable to constitute cosmetic formulations. This type of collagen can be extracted from fish skin and scales by-products [43,212–214]; collagens that have good biomechanical properties, good biocompatibility, good biodegradability, low immunogenicity, high versatility, high molar mass, high isoelectric point (above 7), and are able to form films, result in a viable option for the manufacture of biomaterials [38,115,213,215], such as isolates from marine invertebrates [92]; collagens with low molar mass, low isoelectric point (less than 5), low viscosity and cannot form films may be an excellent option as therapeutic agents due to their biological functions [10]. Collagen-based products include native collagen, hydrolyzed collagen, gelatin and synthetic collagen [38]. The main targets for collagens extracted from cattle, pigs and fish are grouped below:

- i) **Food & Beverage industry:** Focused on the preparation of functional foods and beverages, food supplements, sweets and desserts, meat processing and production of food biofilms [38]. The food sector uses hydrolyzed collagen as a source of bioactive compounds (collagen peptides), obtained through enzymatic hydrolysis of the triple helix. These polypeptide fragments can present several biological functions [70,72,84,216–220]. The identification of these peptides can be performed using spectroscopic techniques, such as CD and FTIR. The FTIR to identify a collagen peptide with an immunologic role and good cellular apoptosis tolerance extracted from cartilage of *Prionace glauca* (Blue shark) [17]; while Ennaas et al. [216] used CD to identify and characterize a peptide (Collagencin) with antibacterial (Gram+ and Gram-) properties, preventing the growth of *Staphylococcus aureus*. Some collagen-based food products are described in Table 5.
- ii) **Biopharmaceutical industry:** Focused on the production of sponges, adhesives [221], surgical compresses used in wound healing [69,222], biological dressings for the treatment of diabetic ulcers [223,224], associated dressings, such as collagen-chitosan [23,58], tissue re-epithelialization and revascularization [7,83,189,215,222], osteochondral regeneration [83,215], facial bone reconstruction and implants [225], microfibers [140], nanoparticles and drug delivery [93,115,183]. Some biomedical, pharmaceutical, and nutraceutical products are described in Table 5.
- iii) **Cosmetic industry:** Focused on beauty products, body protection and/or cosmetic corrections, acting as moisturizing, anti-wrinkling, anti-aging agents as well as ultraviolet (UV) ray blockers, increasing skin softness and shine, perfecting fibroblast production and skin extracellular matrix, also used in hair products, increasing strand resistance, which contributes to hair growth and strength [43,92,115,218]. This sector has been one of the most economically significant, and forecasts are for expansion, mainly due to new sources coming from aquatic environments [38]. Some collagen-based cosmetic products are described in Table 5.

8. Conclusions

This review provides an overview of extraction methods and of the main collagen characterization techniques, with focus in the fishery and aquaculture sources. It is a compilation of information available in scientific literature which can be useful to guide professionals in the aquatic biopolymers field. This paper broadly provides the main physicochemical and spectroscopic properties of aquatic collagen, its implications and industrial targets compared to collagen from mammals. Extraction methods are also reviewed as a decisive factor in preserving the characteristics of collagen. New extraction approaches are also cited, such as the use of sonication and extrusion. In view of the need to adapt to current conditions and contribute to the reduction of environmental damage, the use of collagen from residues of fishery resources becomes an important asset for sustainability and impact reduction. Lastly, this review provides the collagen global market tendencies. With this article, we hope to encourage the use of collagen from aquatic sources in new research that can establish this biopolymer in the collagen global market, considered their physical, biochemical, and densitometric spectroscopic extracted collagen are similar to mammals.

Declaration of Competing Interest

There is no conflict of interest.

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