Chapter 4

Tilapia (*Oreochromis aureus*) Collagen for Medical Biomaterials

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Abstract

Collagen is a natural polymer widely used in pharmaceutical products and nutritional supplement due to its biocompatibility and biodegradability. Collagen is a fibrous protein that supports various tissues, and its primary structure is formed by repeated units of glycine-proline-hydroxyproline. Traditional sources of collagen, such as bovine and pig skins or chicken waste, limit their use due to the dangers of animal-borne diseases. Thus, marine animals are an alternative for the extraction of collagen. The common name of *Oreochromis aureus* is tilapia, widely cultivated for sale as frozen fillets. During its processing, a large amount of collagen-rich wastes are generated. Therefore, the objective of this book chapter is to prove the potential of tilapia skin as an alternative source of collagen for the elaboration of biomaterials. Additionally to the literature review, experimental results of the extraction and characterization of tilapia skin collagen for use in medical dressings are presented.

Keywords: acid soluble collagen, marine byproducts, fish skin, valorization, biomimetic materials

1. Introduction

Collagen is widely used in the manufacture of medical materials due to its availability, versatility, compatibility, and degradability in advantage with other biomimetic biomaterials [1]. Traditionally, most of the collagen is extracted from the skins of cattle and pigs or chicken waste. However, these sources limit their applications due to the religious beliefs of some consumers and because of the risk of diseases such as bovine spongiform encephalopathy and aphthous fever disease [2]. During the production of commercial fish products, byproducts such as skin, bones, and scales rich in collagen are generated [3].

The isolation of collagen from byproducts from fish would reduce the environmental impact generated during its decomposition by providing an added value to these wastes. The collagen isolated from fish is easier to digest and absorb than that of terrestrial origin, due to the different hydroxyproline contents and a lower denaturation temperature [4]. However, the information reported on the characteristics and methods of extracting collagen is still not enough. Tilapia (*Oreochromis aureus*) is one of the main groups of fish grown and sold as whole frozen fish and frozen and fresh fillet. Tilapia skin is a byproduct that contains about 27.8% collagen and can be used for the extraction of collagen increasing the economic value of these industries [5]. Muyonga et al. have reported the extraction of collagen in acid medium of Nile perch [6]. Liu et al. established that the concentration of acetic acid and temperature of extraction of collagen have effects on the properties of collagen [7].

Tilapia skin collagen can be used to develop healing biomaterials and cosmetics or as food supplement due to its biological properties. In the literary review of this book chapter, the residues of the industrialization of tilapia (*O. aureus*) are presented as sources for the extraction of collagen. Basically, the collagen extraction and characterization methods are detailed with a theoretical and practical perspective for the fishing industries in order to generate biological products with high commercial value. The main purpose of this chapter is show the potential of soluble collagen in acid from tilapia skin as a biomaterial with medical applications.

2. Collagen

Collagen is the most abundant protein in vertebrates and contributes significantly to the hardness of connective tissues such as the skin, bones, cartilage, tendons, and blood vessels [8]. Due to its biological compatibility, biodegradability, low cytotoxicity, structural support, and hemostatic activity, collagen has been widely used in the food, pharmaceutical, cosmetological, and biomedical industries. Currently, at least 29 types of collagen have been identified, and these are classified according to their structure as fibrous, nonfibrous, and micro-fibrillary. Type I collagen is the most common, present mainly in the skin, tendons, and bones, while type II collagen is found in cartilage tissues, and type III collagen depends on the age of the tissue. The other types of collagen are only in very small amounts and in specific organs [9].

2.1. Chemical structure

It has been reported that collagen is composed of glycine (33%), proline (12%), alanine (11%), and hydroxyproline (10%) but is deficient in essential amino acids such as lysine and tryptophan [10]. The collagen monomer is called tropocollagen and is a cylindrical protein of 15 Å in diameter and 3000 Å in length, which is formed by three polypeptide chains or α chains of molecular weight of 100,000 daltons each [11]. The three chains are linked together through cross intermolecular bonds giving rigidity to the structure and poor solubility. Collagen is produced from the interaction of tropocollagen molecules [12].

The amino acid sequence of the polypeptide chain is characterized by the repetition of Gly-X-Y, where the X and Y positions are normally occupied by proline and hydroxyproline, respectively [13]. The glycine of the polypeptide chain becomes the helical center of the

molecule, because it only has a side chain of hydrogen. This structure is reinforced by pyrrolidine rings from proline and hydroxyproline [14].

In terms of amino acid composition and biocompatibility, the aquatic-type collagen has properties similar to mammalian sources. In both sources of collagen, glycine is one of the most abundant amino acids representing about 30%, while hydroxyproline is estimated in the range of 35–48% of the total amino acid content [15]. Glycine and hydroxyproline are important for the formation of the structure and characteristic stability of collagen. However, some differences in the amino acid content between aquatic species have been found. Swatschek et al. have reported that collagen isolated from marine sponges (*Chondrosia reniformis*) showed a content of glycine and hydroxyproline of 18.9 and 40%, respectively [16]. However, Silva et al. [17] reported values of 31.6% glycine and 47.36% hydroxyproline for collagen isolated from the same species. This discrepancy is due to some structural and chemical differences between the sources. In addition, glycoproteins in marine tissues generate differences in amino acid content, because they are associated with collagen and appear as impurities affecting the purity of collagen [16].

Compared with mammalian sources, aquatic collagen has a lower viscosity and thermal stability due to the difference between the content of proline and hydroxyproline in its structure [18]. Gómez-Guillén et al. [19] have reported that the content of proline and hydroxyproline in collagen isolated from cod and squid skin is similar. The structure of the collagen can be denatured under conditions of high temperatures forming gelatin. Gelatin is a mixture of peptides and proteins partially hydrolyzed from collagen molecules [20]. Gelatin is classified as the denatured form of native collagen with lower molecular weights than its predecessor [18]. Compared to gelatin, collagen exhibits superior characteristics such as greater enthalpy, structuring of fibrous networks, basic isoelectric point and high resistance to protease hydrolysis, greater mechanical resistance, and reversible extensibility, whereas the gelatin shows gel formation, great thermal stability, and unique rheological properties [21].

2.2. Natural sources

Collagen is one of the most common proteins in multicellular organisms and, due to its fibrous nature, provides structural rigidity to the connective tissues and internal organs. The waste generated during industrial food processing is the main source of collagen. Traditionally, the skin, bones, and cartilage of bovines and pigs are the materials for their isolation [22]. However, the application of these sources has limitations by certain religious and ethnic groups [18]. In addition, disease transmission is a probability, such as bovine spongiform encephalopathy and aphthous fever disease [2]. At the present, there is a growing interest in the valuation of industrial byproducts of aquatic origin as an alternative to conventional raw materials for the production of collagen.

During the industrial processing of fishery products, about 75% of the initial weight is discarded as waste, which are sources of collagen that can increase the profitability of the industry [23]. The skin, scales, fins, and bones of marine and freshwater fish are recognized as potential sources of collagen for the production of food, cosmetics, and pharmaceutical products [5]. Unlike collagen from terrestrial sources, that of aquatic origin is immune to diseases; of faster absorption due to its denaturation temperature, metabolic compatibility, molecular weight; and of low inflammatory response.

2.3. Biological properties

For the development of biomaterials, there are several desirable properties such as biodegradation, hemostasis, cell proliferation, immunogenic, and biocompatibility [24]. In addition to possessing all of them, collagen is resistant to traction with minimal extensibility. However, this depends on the amount and type of collagen, as well as the interaction with different glycoproteins and proteoglycans.

Ulery et al. collagen has an important function in coagulation, from the conversion of fibrinogen into fibrin, capturing platelets and forming clots [25]. Collagen as a hemostatic agent works as a mechanical and protective blockage from environmental factors, preserves epithelial cells, and increases the production and permeation of fibroblasts at the collagen-wound interface. Hemostasis is generated in less than 5 min; the process begins with the adhesion of platelets around the structure of collagen, activating a coagulation factor and epithelization. In the structure of collagen, the amino acid arginine participates in healing, decreasing stress in tissues and increasing the interaction of collagen with platelets. In addition, many studies show the influence of collagen on cell migration, adhesion, and differentiation [26, 27].

The biodegradation of collagen is carried out by the action of metalloproteinase enzymes. Type I collagen is hydrolyzed by collagenase (MMP-1) which has the ability to break down the triple helix of collagen, resistant to most proteases. Then, the resulting collagen fragments are degraded by gelatinases and other proteases [28].

The biocompatibility of collagen is a valuable aspect in most biomaterials, because when transplanting collagen to a damaged tissue, it does not leave strange residues for the organism during its degradation [24].

Collagen has low toxicity and a low probability to trigger an immune response, making it a suitable material for use as a biomaterial in the medical industry. However, a risk exists for people susceptible to collagen, which is why a serological test is currently available to identify people who are allergic to this protein [1, 25].

2.4. Methods of extraction of collagen of aquatic origin

The methodology for the extraction of collagen includes steps such as pretreatment of the raw material and isolation of collagen. The fish byproducts as collagen sources need a previous cleaning and size reduction, in order to facilitate the elimination of impurities and ensure the maximum collagen extraction. In addition, it is important to classify the waste generated by industrial processing such as the skin, scales, fins, and bones since they have characteristic elements that require different methodologies for the isolation of collagen [29]. **Table 1** shows various processing residues of aquatic species as sources of collagen.

For the removal of non-collagen proteins and pigments, an alkaline treatment is recommended, and diluted NaOH has been used the most because the performance of the collagen is not affected. However, increasing the NaOH concentration induces significant collagen losses [7]. Skierka et al. [34] propose using NaCl to remove non-collagen proteins from cod skin, but NaOH has shown greater efficacy than NaCl. The most effective method for the removal of fat from fish skin is with butyl alcohol [35]. The demineralization of scales and

Source	Species	Yield (%)	References
Scale	*Hypophthalmichthys nobilis*	PSC 2.7	[8]
Scale	*Ctenopharyngodon idellus*	ACS 16.1	[30]
Scale	*Oreochromis niloticus*	PSC 14.9	[5]
Bone	*Magalaspis cordyla*	ACS 30.5	[31]
		PSC 27.6	
Bone	*Otolithes ruber*	ACS 45.1	[31]
		PSC 48.6	
Skin	*Labeo rohita*	ACS 46.13	[23]
Skin	*Oreochromis niloticus*	ACS 39.4	[5]
Skin	*Misgurnus anguillicaudatus*	ACS 22.42	[32]
		PSC 27.32	
Skin	*Oreochromis niloticus*	ACS 27.2	[33]

Note: ACS, acid soluble collagen; PCS, pepsin soluble collagen.

Table 1. Potential sources of collagen of aquatic origin.

bones has been carried out with citric acid, HCl, and ethylenediaminetetraacetic acid (EDTA), achieving efficiencies greater than 90%. However, the selection of the chemical agent is of great importance due to the loss of collagen with the use of these acids [36].

After the removal of non-collagen proteins, demineralization, and defatting of the byproducts, the collagen is extracted. The properties and performance of collagen depend on the extraction procedures; therefore, it is important to establish the right conditions [23]. Collagen receives its name according to the methodology applied for its extraction.

Collagen soluble in salt can be isolated with solutions of NaCl and NaOH, although this method is not the most popular. In reference to Wang et al. [30], the authors isolated and characterized collagen from Amur sturgeon (*Acipenser schrenckii*) by several methods, with 0.45 M NaCl at pH 7.5 for 24 h, 0.5 M acetic acid, and hydrolysis with pepsin, and reported yields of 4.55, 37.42, and 52.80%, respectively.

Collagen soluble in acid is extracted with an acidic solution. This is the traditional method for extracting collagen, and hydrochloric, citric, acetic, and lactic acids have been used. Skierka et al. have reported higher performance when using organic acids such as acetic and lactic acid [34]. Collagen soluble in acetic acid has been isolated from different marine byproducts such as the skin [23], scales, bones [30], and fish fins [8]. To increase the collagen concentration, the supernatant is precipitated with NaCl. Then the precipitate is centrifuged and redissolved in 0.5 M acetic acid. Finally, it is dialyzed in acetic acid and lyophilized [32]. However, the purification costs and the process times are high, limiting their use in industrial processes.

To increase the yield of the isolated collagen, it is necessary to hydrolyze the collagen from insolubilities by means of enzymes. Enzymes such as trypsin, pancreatin, ficin, bromelain, papain, and pepsin have been used for this process. The latter has been the most commonly

used in marine byproducts [37]. Pepsin-soluble collagen obtained by hydrolysis with pepsin is called PCS or atelocollagen. This treatment separates the peptides specifically in the telopeptide region of collagen; these are non-helical ends, the hydrolysis being a more efficient process that decreases the toxicity caused by the telopeptides [29]. After hydrolysis, the collagen is centrifuged and dissolved in 0.5 M acetic acid, dialyzed, and lyophilized [38].

Kim et al. have reported that the extraction of collagen with ultrasound increases the performance, even when a lower concentration of acetic acid is used over than the traditional method [39]. However, Ran and Wang state that the ultrasound extraction method could break the hydrogen bonds in the collagen chains, causing denaturation of the protein and the enzyme used for its isolation [40]. In reference to Yu et al., various enzymes were used for the extraction of collagen, finding that the activity of papain was inhibited by structural changes in its activity, while pepsin did not change [41]. In addition, they reported that the application of ultrasound for long periods of time causes a rise in temperature leading to the denaturation of collagen.

Huang et al. have proposed an extrusion-hydro-extraction method for the extraction of collagen from tilapia (*Oreochromis* sp.) scales. Basically, the process consists of three main stages, preconditioning (citric or acetic acid), extrusion (360 rpm at 135°C), and hydro-extraction (25 and 50°C). The precise combination between heat and mechanical strength favors the extraction of collagen. The collagen isolated during this procedure was identified as type I, and the physicochemical properties of collagen revealed that it can be used for food, cosmetic, and medical applications [42].

3. Biomedical applications

Collagen, due to its multiple properties, has numerous applications in the food, medical, dental, cosmetic, and pharmaceutical industries. In addition, depending on its use, it can be processed in a wide variety of ways such as powders, injectable solutions, films, sponges, and hydrogels [29].

3.1. Food supplement

Collagen has been mixed with a wide variety of products and beverages, which is why collagen-based food supplements are the most common on the market. Collagen synthesis decreases with age, and the tissues lose flexibility and thickness, reason why collagen supplements are mainly used for skin care. In addition, the consumption of collagen increases the gain of muscle mass, decreases the recovery time of the muscles, and helps to rebuild damaged joints [21].

In the food industry, collagen is used as an additive for the improvement of the rheological properties of charcuterie and as a guarantee of the presence of nutritive fibers of animal origin [43]. Collagen functions as a barrier that controls the migration of oxygen, providing permeability to water vapor and prolonging the shelf life of food [21]. These films or edible coatings can be applied by wrapping, dipping, brushing, or spraying the food. Currently the functional beverage sector has increased its demand, requiring the implementation of new

ingredients to meet the various nutritional needs. According to Bilek and Bayram, they have developed functional drinks from orange, apple, and grape juice, mixed with hydrolyzed collagen. Due to its characteristic amino acid content, hydrolyzed collagen is a promising ingredient in the food industry, specifically for the production of beverages because it increases the protein content and bioavailability of collagen [44].

3.2. Cosmetology

Collagen is one of the main components of the skin and is responsible for its appearance and physical condition. The collagen present in the skin decreases with age and with prolonged exposure to ultraviolet irradiation. Therefore, it is important to compensate these losses by consuming products rich in this protein for cosmetic and pharmaceutical purposes [45].

Compared to high molecular weight collagen, cosmetic formulations use hydrolyzed collagen. This is due to its superior solubility at neutral pH and ease of penetration into the dermis and because it acts as a water-binding agent inside the skin. Thus, its addition in cosmetic creams increases its wetting capacity, providing a moisturizing, softening, and glowing effect to the skin [12].

Collagens of aquatic origin have potential as excellent ingredients for the cosmetic industry, reason why creams and gels have been developed with a high moisturizing action, anti-wrinkles, and UV protectors [29].

3.3. Healing materials

Collagen of aquatic origin has proven its use an alternative material in the manufacture of medical dressings such as sponges and membranes for the treatment of wounds. It is also included in biomaterials of medical uses for ophthalmology, bone substitutes, and gels for the administration of drugs [12].

3.3.1. Scaffolds

Collagen matrices have the ability to absorb large amounts of exudates from wounds. This favors the formation of a biodegradable gel or sheet on the surface of the wound that maintains a humid environment, promotes healing, and provides protection against external mechanical forces [46]. Collagen sponges possess adequate characteristics for tissue regeneration due to their high porosity, permeability, low toxicity, cell adhesion, and biocompatibility [47].

For the production of collagen sponges, aqueous solutions are prepared and finally lyophilized. The porosity of the sponge is controlled by varying the rate of freezing prior to lyophilization and the concentration of collagen in the solutions [28].

Chandika et al. developed collagen sponges from the skin of Japanese halibut (*Paralichthys Olivaceus*) in combination with alginate and chitosan; these exhibited a porous structure, high capacity of swelling, and biodegradation suitable for its application in tissues [48]. Cheng et al. prepared biomaterials of type I collagen isolated from jellyfish (*Rhopilema esculentum*) for the treatment of wounds. In vivo studies indicate that collagen sponges exhibit rapid hemostatic properties due to their ability to absorb liquids [49].

Biomaterial	Compound	Source of collagen	References
Sponge	Collagen/alginate/chitosan cross-linking with glutaraldehyde	Skin japanese halibut (*P. Olivaceus)*	[48]
Sponge	Collagen	Jellyfish (*Rhopilema esculentum*)	[49]
Sponge	Chitosan/collagen/hydroxypatite	Marine sponge (*Ircinia fusca*)	[51]
Membrane	Collagen/hydroxypropyl methylcellulose E15	Skin marine eel (*Evenchelys macrura*)	[52]
Membrane	Collagen/glycerol	Skin silver carp (*Hypophthalmichthys molitrix)*	[53]

Table 2. Healing materials based on aquatic collagen.

The collagen content in a sponge increases its porosity and biodegradation. This effect was described by Ullah et al. [50] in collagen sponges isolated from tilapia (*Oreochromis* sp.) scales mixed with chitosan and glycerin for the regeneration of damaged tissues. **Table 2** shows various biomedical materials based on aquatic collagen.

3.3.2. Membranes

Collagen membranes or collagen films have an average thickness of 0.01–0.5 mm and are formed by drying aqueous solutions by aeration. The membranes provide a protective barrier from the environment, are resistant to handling during their application, and can be sterilized. Collagen membranes have been used in the treatment of skin lesions, corneal tissue, and reinforcement in compromised tissues and as a vehicle for encapsulated drugs with slow release [12, 28].

Veeruraj et al. [52] developed collagen membranes isolated from *Evenchelys macrura* for the administration of standard commercial medicines such as ampicillin. However, Perumal et al. prepared collagen-fucoidan membranes as a substrate for the growth of fibroblasts [54]. Liu et al. modified the collagen membranes by crosslinking with alginate to improve thermal stability and mechanical properties and decrease the rate of biodegradation [55].

3.3.3. Ophthalmologic inserts

Collagen being biocompatible and biodegradable has been used in ophthalmology, as grafts for the replacement of corneas, suture material, and ocular protective films. Raiskup et al. applied a corneal collagen implant with riboflavin for the effective treatment of progressive keratoconus, reducing astigmatism and corneal distortion [56]. In reference to Zhang et al. [3], the authors proposed a type I collagen sponge obtained from rat tail as a substitute for corneal tissue. Long et al. evaluated collagen-hydroxypropyl methylcellulose membranes, which had optical properties equivalent to human corneas [57].

3.3.4. Bone substitutes

In comparison with other biomolecules, collagen has the great advantage of forming biomaterials with network and porous structures, with a mechano-elastic behavior suitable for various biomedical applications [58]. Collagen participates in the treatment of the cartilage and bone by cell proliferation and is able to regenerate tissue damaged by injury or wear [59]. The mechanical properties of bone substitutes are very important; collagen can be used in combination with other biomaterials such as hydroxyapatite, calcium phosphate, chitosan, and alginate, giving them different mechanical and biological properties than the original polymer [51].

According to Aravamudhan et al. [60], it has been reported that collagen-cellulose nanofibers with microporous structure are alternative candidates for the repair and regeneration of bone defects, increasing the bone density and quality of mice. Wahl et al. have reported a collagen-hydroxyapatite compound that has the potential to mimic and replace bone fragments of the skeleton [61]. On the other hand, Murphy et al. [62] established an optimum porosity of 325 μm in collagen-glycosaminoglycan sponges for its application in bone regeneration, increasing cell adhesion and filtration, in comparison with other pore sizes.

3.3.5. Drug releasers

Collagen gels can act as a matrix for the administration of drugs due to their properties such as fluidity, easy handling, and biocompatibility. According to Calejo et al. [63], polymer matrix from jellyfish collagen for the supply of proteins demonstrated the potential of aquatic collagen in the controlled release of drugs. On the other hand, Dinescu et al. reported collagen-sericin sponges for the supply of hyaluronic acid and chondroitin sulfate, which presented biological properties such as viability and cell proliferation [64]. Langasco et al. have reported that in vitro studies using collagen sponges with L-cysteine hydrochloride favor the slow release of drugs [65].

4. Extraction of tilapia skin collagen

4.1. Tilapia (*Oreochromis aureus*)

The tilapia species are native to Africa and the Middle East, among the most common cultivated species is the genus *Oreochromis.* These include the Nile tilapia (*Oreochromis niloticus*), blue tilapia (*Oreochromis aureus*), and tilapia from Mozambique (*Oreochromis mossambicus*); these species can crossbreed producing hybrids available for cultivation. Tilapia has been cultivated in almost all countries of tropical climate and subtropical regions. This species is of global importance in aquaculture due to its ability to adapt to different conditions and high reproductive rate [66].

Tilapia has the ability to grow in wide ranges of salinity, from freshwater to seawater, and can tolerate acid (pH 5) and alkaline (pH 9) systems, low levels of oxygen (<2 mg/l), and high

levels of ammonia (50 mg/l) [67]. Depending on the species, tilapia can be herbivorous or omnivorous, feeding on a wide variety of foods, including periphyton, phytoplankton, zooplankton, and balanced feed [68].

Tilapia is usually marketed in fresh-chilled form and frozen as whole or fillet. During the industrial processing of the fillets, large amounts of waste (skin, scales, and bones) rich in bioactive biomolecules are produced. These byproducts can be used for the extraction of collagen, which decreases the environmental impact and increasing the economic value of the company [5].

4.2. Preparation of tilapia skin

The skins of *Oreochromis aureus* from industrial processing were peeled and cut into pieces smaller than 2 cm and then stored frozen until use. For the removal of non-collagen proteins, the skins were immersed in a 0.1 N NaOH solution for 48 h, changing the solution every 24 h according to the method described by Zeng et al. [5]. Then, the skins were defatted with 10% butyl alcohol for 48 h with a change of solution every 24 h. Subsequently, the skins were washed with distilled water for waste disposal and dried for storage.

4.3. Isolation of acid-soluble collagen

The previously treated tilapia skins were submerged in 0.5 M acetic acid (1:50, w/v) for 3 days with gentle agitation, according to the method of Singh et al. [69] with some modifications. The mixture was then filtered through a 1 mm mesh, and the filtrate was lyophilized at −50°C with a maximum vacuum of 0.5 mBar. For the purification of the collagen, the lyophilized sample was resuspended in 0.5 acetic acid (1:30, w/v) for 2 days; then the solution was filtered through a mesh with a pore less than 1 mm. Finally, the filtrate was lyophilized and stored as collagen soluble in pure acid. The proximal composition was determined according to the AOAC [70] methods for moisture, ash, and proteins (Kjeldahl factor 6.25).

The process of extracting collagen from tilapia (*Oreochromis aureus*) skin is shown in **Figure 1**. The collagen extracted from tilapia skin has a uniform whitish color without any impurities. The average moisture content of the lyophilized collagen was 5.46 ± 0.49%, which determines its storage stability and influences its nutritional characteristics. In reference to the inorganic fraction, it was found that the ash content was 0.55 ± 0.02%; this indicates effectiveness of the method to eliminate minerals. Likewise, the average protein content was 87.54 ± 3.61%.

The yield of lyophilized collagen was estimated based on the weight of dry tilapia skins. Therefore, the yield of acid-soluble collagen was 11.37 ± 0.88% on a dry basis. The yield was lower than that reported by Chen et al. [33] with 27.2%. These differences may be due to the state of maturity of the fish, environmental conditions of its development, as well as the extraction method.

Also, it has been reported that collagen is not completely solubilized during extraction with acetic acid. Tamilmozhi et al. [71] reported trials with skins of *Istiophorus platypterus*. Hickman et al. established that molecules that solubilize in acid medium are monomeric [72].

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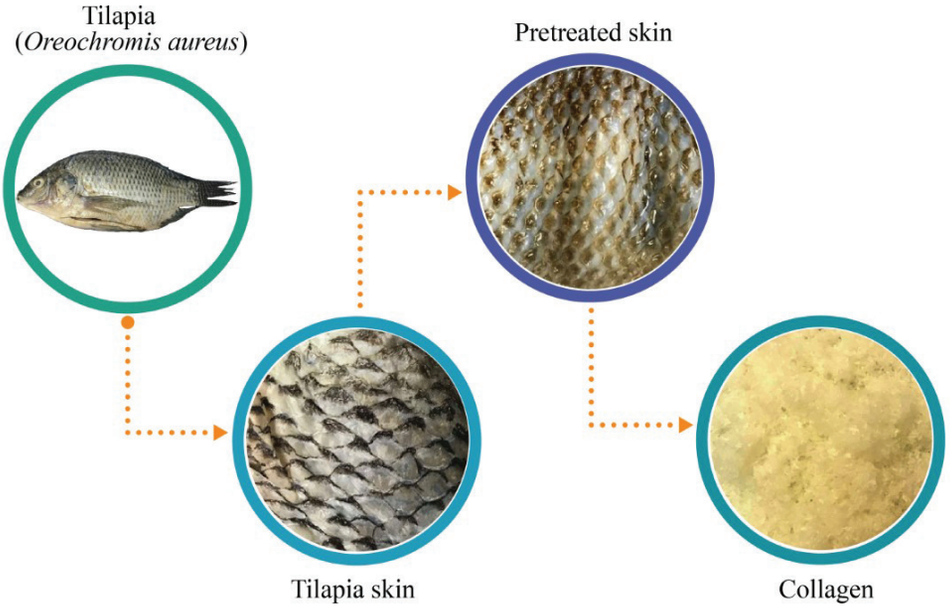

Figure 1. Diagram of extraction of collagen from tilapia (*Oreochromis aureus*) skin.

4.4. Characterization of acid-soluble collagen

4.4.1. Fourier transform infrared spectroscopy

For the identification of collagen, FTIR spectra were recorded (Thermo Scientific FTIR, model Nicolet 5s Madison, WI, USA). The equipment consists of an ATR ID3 accessory for germanium crystal reflection. The spectral resolution was 4 cm^{-1}, and 64 scans were obtained in the range of 600–4000 cm^{-1}.

The IR spectra showed the five characteristic absorption bands of collagen (amide A, amide B, and amides I, II, and III) suggesting the composition of amino acids and a high portion of proline and hydroxyproline in the collagen molecule [73]. Amide A is located at 3301 cm^{-1}, and it is associated with the N–H groups involved in the hydrogen bonds of the peptide chain, while amide B was measured at 3066 cm^{-1}, and it is related to the asymmetric stretching of C–H [32]. The peak corresponding to amine I was found at 1642 cm^{-1}; this is presented with a characteristic wavelength range of 1600–1700 cm^{-1} associated with the stretching vibrations in the C═O group, being an indication of the secondary structure of the peptide [31]. Amide II was measured at 1545 cm^{-1}, attributed to the stretching of the carbonyl group coupled to a carboxyl group. Amine III was measured at 1233 cm^{-1}, established by the combination of the stretching vibration between C–N and the bending vibration of N–H. The values found during the study confirm the presence of a triple helical structure characteristic of collagen, all in accordance with results reported by Sun et al. for collagen extracted from tilapia (*Oreochromis niloticus*) skin [73]. The FTIR spectrum of the acid-soluble collagen is shown in **Figure 2**.

4.4.2. UV absorption spectroscopy

The UV absorption spectra of the collagen isolates were measured in a UV spectrometer (ThermoScientific, Genesys 10S UV–Vis, USA), by the procedure described by Liu et al. [55]

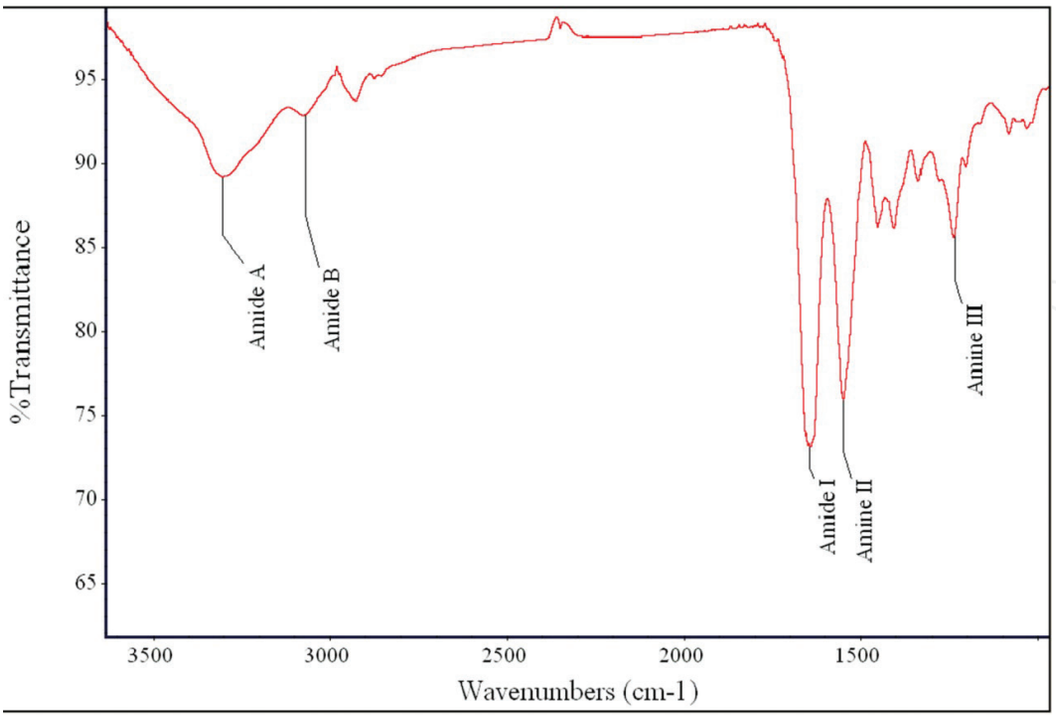

Figure 2. FTIR spectrum of collagen from tilapia (*Oreochromis aureus*) skin.

with some modifications. The lyophilized samples were dissolved in 0.5 M acetic acid to obtain a concentration of 1 mg/ml and centrifuged at 5000 rpm for 15 min. The absorbance of the supernatant was measured at different lengths in the range of 200–400 nm, with a range of 1 nm to record its characteristic spectrum and maximum absorbance.

The UV absorption of collagen is attributed mainly to the peptide bonds and side chains in its structure. The collagen isolated from the skin of tilapia showed a maximum absorption at 230 nm; this agrees with the characteristic absorbance of type I collagen [31]. They also coincide with different authors, who isolated collagen from different marine species [32, 35]. The UV–Vis spectrum of tilapia (*Oreochromis niloticus*) skin collagen is presented in **Figure 3A**.

4.4.3. Denaturing temperature of collagen

The denaturation temperature was determined according to the method of Sun et al. [73] based on the viscosity changes in relation to the temperature increase measured with an Ubbelohde viscometer. A solution of collagen at 1 mg/ml dissolved in 0.5 M acetic acid was prepared, the solution was loaded into the viscometer, and the temperature curves were recorded in the range of 10–45°C. The fractional viscosity for each specific temperature was calculated with the following equation: fractional viscosity = $(\eta_{sp}(T) - \eta_{sp}(45°C))/(\eta_{sp}(10°C) - \eta_{sp}(45°C))$, where η_{sp} is the specific viscosity, specific viscosity = $(t - t_0)/t_0 = \eta_r - 1$, where η_r is the relative viscosity, relative viscosity = t/t_0, where t is the time it takes for the collagen solutions to pass through the capillary of the Ubbelohde viscometer and t_0 is the time in which the solvent passes at the same temperature. The thermal denaturation curve was established by comparing the fractional viscosities and temperatures. The denaturing temperature is where the fractional viscosity is 0.5.

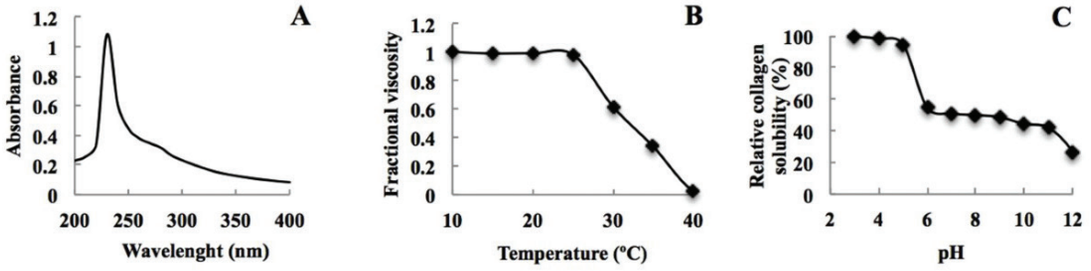

Figure 3. Characteristic UV spectrum (A), thermal denaturation curve (B), and solubility effect at different pH (C) collagen isolated from tilapia (*Oreochromis aureus*) skin.

The denaturation temperature found for acid-soluble collagen in tilapia skin was 32°C, similar to that reported by Sun et al. [73] for Nile tilapia skin, 35.2°C. However, for most marine species, these values range from 26 to 29°C [74] such as the following *Alaska pollack* (16.8°C), *Hypophthalmichthys molitrix* (29°C), and *Trachurus* (28.4°C) [6, 35, 74]. The connective tissue of the fish is continuously renewed, presenting a smaller amount of molecular crosslinks, which affects its thermal stability. In addition, the thermal stability of collagen correlates with the environmental temperatures present in the habitat of the species; this explains the difference between a higher Td of the collagen isolated from species of warm water compared to those of cold water, reason why subtropical and tropical fish present better thermal stability [75].

The denaturing temperature is the point where the collagen triple helix structure is deformed to a random spiral structure. This property is undesirable for the manufacture of biomaterials, because the denaturation drastically alters the physicochemical, biological, and mechanical properties of collagen. According to Fu et al. [76], this temperature can be measured by the viscosity changes caused by the heating of the collagen. **Figure 3B** shows the changes in the fractional viscosity with the increase in temperature for rehydrated collagen isolates in 0.5 M acetic acid from tilapia (*O. aureus*) skin.

The solubility of acid-soluble collagen was determined for different pH values (3–12) and for different concentrations of NaCl (0, 1, 2, 3, 5, and 6%) according to the method of Fu et al. [76] and Montero et al. [77]. All these solutions were centrifuged at 4000 g for 30 min, and the protein content of the supernatant was determined by the method of Bradford [78]. Finally, the relative solubility of collagen at different pH was calculated by comparison with the highest solubility presented at a given pH. Similarly, the relative solubility of collagen at different concentration of NaCl was calculated by comparison with the higher solubility at a given concentration of NaCl.

4.4.4. Effect of pH and concentration of NaCl on solubility

Collagen solutions from tilapia (*O. aureus*) skin showed a high solubility between the pH ranges of 3–5, while the maximum solubility was at pH 3. When increasing the pH to 6, the relative solubility of the collagen was 58.82 ± 4.04%; from pH 12 the solubility decreased to 32.35 ± 5.93%. According to Liu et al. [8], considerable decrease in solubility from pH 6 for collagen from different aquatic sources was reported. Chen et al. have reported a maximum solubilization at pH 3 and minimum at pH 7 for collagen of tilapia (*Oreochromis niloticus*) skin [33]. When the pH is equal to or close to the isoelectric point, the total charge of the protein

molecules is close to zero and results in precipitation [79]. The effect of pH on the collagen solubility of tilapia (*O. aureus*) skin is shown in **Figure 3C**.

The collagen showed greater solubility in NaCl concentrations between 1 and 2%, while at 3% of NaCl, a minimal decrease was observed (77.13 ± 9.27%). When increasing the concentration of NaCl to 6%, the solubility decreased to 34.60 ± 1.96%. The solubilities of shark (*Sphyrna lewini*) skin, bighead carp (*Hypophthalmichthys nobilis*), tilapia (*Oreochromis niloticus*), catfish (*Pangasianodon hypophthalmus*), and mackerel sardine (*Scomberomorus niphonius*) collagen decrease at concentrations greater than 2% NaCl [8, 34]. The decrease in collagen solubility with the increase in NaCl is due to the increase in hydrophobic interactions that favor the precipitation of proteins [80].

5. Conclusion

This literary review of collagen shows that this natural polymer possesses biological properties that benefit human health and justify its application as a medical biomaterial. Essentially, collagen is a biodegradable and biocompatible structural protein that has been used to enrich foods for athletes and novel cosmetics for skin care. Here, we propose a simple methodology for the extraction of collagen from the skin of tilapia (*Oreochromis aureus*) with properties similar to those of collagen from conventional sources.

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