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REVIEW ARTICLE

Fish Gelatin: Characteristics, Functional Properties, Applications and Future Potentials

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Abstract Gelatin, an important biopolymer derived from collagen, is widely used by various industries because of its functional and technological properties. The vast majority of commercial gelatin is derived from mammals, but due to many sociocultural and religious reasons, there is an increasing demand for alternative sources. The by-products generated by the fish-processing industry are a potential source for the production of gelatin. The high potential of fish gelatin production is due to the large quantities of collagen by-products generated. However, the main restriction to its use regards their lower rheological properties when compared to mammalian gelatin, which limits its range of applications. This review discusses the latest scientific literature about the main characteristics and functional properties of fish gelatin, which shows the main strategies used to improve its properties, as well as its applications and future potentials.

Keywords Fish · By-products · Gelatin · Properties · Applications · Future potentials

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Introduction

Several works have been developed in order to present alternatives to correct the protein deficiency affecting many people worldwide. One of these lines of research is directed toward the rational use of marine resources. Fish is an excellent source of protein because its muscles are made up of high nutritional value proteins as they contain high levels of essential amino acids [19].

Collagen is the main structural protein in the animal kingdom [28], and it is estimated that in fish processing the residue after filleting may be 75 % of the total weight, of which a large part consists of skin and bones with high quantities of this protein [39]. Such large amount of by-products could be used for a wide range of applications.

Collagen is characterized by its high content of glycine, proline and hydroxyproline, denatured in the presence of dilute acid standards and converted to soluble protein such as gelatin, when dissolved in heated solutions [56]. Derived from collagen, gelatin has many applications in food, pharmaceutical, photographic and other products. Most commercial gelatin is derived from mammals, obtained mainly from pigskin and cowhide, but due to many sociocultural and religious reasons, there is an increasing demand for alternative sources [24, 49, 56].

The use of fish by-products for gelatin production, as an alternative to that produced from mammals, raises some questions, such as the high diversity of aquatic species and the high deterioration susceptibility of this collagen, when compared with the mammalian collagen [33, 86].

This paper reviews scientific literature about fish gelatin obtained from different species. This work reports on the principal physical, chemical, structural characteristics and functional properties of fish gelatin, as well as its potential applications.

Industrialized Fish By-products

There is no definition for the components of by-products from the fishing industry, and they are usually designated as viscera, head, trimmings, bones and skin. The regulations generally divide them as by-products that can be destined for human consumption and for disposal [79].

During fish processing, there are many by-products generated by the industry. By-products obtained, dragged by the water treatment during the handling of fish, are basically solid and soluble solids or dispersed in the processing water. The solid products include heads, skin, bones, fins, pieces of muscle, scales, viscera and other parts [19].

In recent years, the interest in by-products from the fishing industry has gradually increased, now being considered as a potential source of resources for exploration, rather than as disposable waste.

Collagen

Collagen is a major structural constituent of vertebrates and invertebrates. It is a glycoprotein that contains small amounts of galactose and glucose [91]. It is found in abundance in mammals, and it is the largest protein component of skin, tendons, cartilage, bone and connective tissue [89].

The collagen molecule (tropocollagen) is a structure of about 2,800 Å and has a molecular weight of 300 kDa, composed of three polypeptide chains, called α chains of equal size, which have approximate molecular weight of 100 kDa, wound around a common axis [91] which form a triple-helix structure stabilized by hydrogen bonds. The intensity of cross-linking between the three polypeptide chains is highly variable, and it is directly related to the type of collagen, tissue, animal species, age and other factors [39].

Type-I collagen is found in all connective tissues, including bones and skin [70]. Type-II collagen is the major component of cartilage, and type III is predominant in vascular tissues, skin and intestines [91]. The existence of collagen types I and V has been reported in fish tissues, though the type-V collagen is found in substantially smaller quantities [67].

Collagen Versus Gelatin

Gelatin is obtained from the heating of collagen above the transition temperature of collagen's triple-helix structure, also called "super helix." The super helix is formed by a three peptide strand with a helix structure [89]. Such changes occur in a relatively narrow temperature range; firstly, the helical structure of the collagen molecule collapses; then, there is the unrolling of the molecular chains and the consequent reduction of molecular weight [77]. This treatment is necessary to disrupt the noncovalent bonds and disrupt the structure of the protein and thus to produce adequate swelling and rupture of intra- and intermolecular bonds, causing the collagen solubilization [39] and leading to gelatin conversion with increased hydration capacity [68].

Fish Gelatin

The extraction of collagen for gelatin production is traditionally carried out using bones and skins of mammals as raw material, especially bovine and pig. However, bovine-related health problems have increasingly been discussed such as Bovine Spongiform Encephalopathy [56, 69]. There are also restrictions on the mammalian gelatin in countries with Islam and Judaism religions, which only accept these gelatins provided if they follow the religion's required standards [24, 49].

The fish gelatin does not have such restrictions and additionally, and from the economic point of view, the use of fish by-products to obtain gelatin could be quite interesting for the fish-processing industry [33]. On the other hand, studies have shown that collagen from fish has been identified as a potential allergen, which could possibly become a problem for the use of fish gelatin in commercial products [46].

Fish gelatins have lower rheological properties than mammalian gelatins [24, 25]. The gelatin properties are influenced by two main factors: the characteristics of the initial collagen and the extraction process [52, 56]. Different fish species vary greatly in the amino acid composition of collagen [20]. However, the extraction process is very important because it determines the molecular weight distribution of gelatin [70].

There are other factors that should be considered for gelatin production from fish skin, such as the different structure of the triple helical collagen molecules, and also the high susceptibility of collagenous materials to degradation due to a lower content of nonreducible intra- and intermolecular cross-links [39, 72], in contrast to the more stable mammalian collagen [33]. The collagen from fish bones, however, has greater microbiological stability due to an increased incidence of cross-links, when compared to the collagen of other tissues such as skin [69].

Measures to control the quality of by-products are essential, given that besides the source and species, the properties of fish gelatin strongly depend on the conservation of raw materials [39]. Therefore, the use of by-products in the production of fish gelatin raises questions such as the high variety of aquatic species and also the high susceptibility of this collagen to deterioration, particularly when obtained from the skin, when compared to that from mammals [33].

Physical and Chemical Characteristics of Fish Gelatin

All types of gelatin have a similar composition, that is, water, small amounts of minerals and pure protein of connective tissue. However, depending on the material used, on the pretreatment process employed and the intensity of hydrolysis, various types of gelatin with different properties can be obtained [7].

Studies related to the use of fish to obtain gelatins have reported that the applicability, functionality and commercial value of this type of product are essentially dependent on their physical (viscosity, gel strength and stiffness, intumescence capacity and thermal stability) and chemical characteristics (moisture, ash and pH). These characteristics or properties can be especially affected by the amino acid composition, molecular weight and proportion of α chains [36, 42, 83].

Yield

The extraction yield of fish gelatin depends on the time and process of the extraction procedure [49]. According to Karim and Bhat [56], the mean extraction yield of fish gelatin ranges between 6 and 19 % (grams of dry gelatin per 100 g of clean skin on a wet basis), which is less than the mammalian gelatin. The low yield of fish gelatin may be due to the incomplete hydrolysis of collagen or the loss of collagen during the washing process [7].

Processing conditions (solvent, time and temperature) to produce the optimum yield of fish gelatin have been identified for specific types of raw material [56]. Jamilah and Harvinder [49] reported a yield of 5.39 % for gelatin extracted from black tilapia (*Oreochromis mossambicus*) and 7.81 % for gelatin extracted from red tilapia (*Oreochromis nilotica*). Similarly, Biluca et al. [16] achieved a yield of 5.85 % for gelatin extracted from catfish (*Clarias gariepinus*). Higher yield levels, of around 18 %, were achieved by Rahman et al. [77] in gelatin obtained from skin of yellowfin (*Thunnus albacares*).

pH values

The pH of gelatin solution reflects the chemical treatment used during the extraction stage [6]. The pH value directly affects the viscosity of gelatin, and alkaline pH levels (above 10) result in sharp viscosity drops, while acidic pH levels result in only a moderate reduction [46]. Biluca et al. [16] state that the higher pH may result from an efficient washing stage following the chemical treatments during the preparation of skins, before the extraction stage.

pH values are variable and dependent on the gelatin extraction process; thus, different pH ranges can be observed. Jamilah and Harvinder [49] reported pH values for gelatin extracted from the skin of black tilapia (3.81) and red tilapia (3.05); Alfaro et al. [5] reported pH value ranging from 3.85 to 4.38 for gelatin extracted from bones of king weakfish (*Macrodon ancylodon*); Biluca et al. [16] obtained pH values of 3.20 and 2.98 for gelatin from skin and bones of catfish.

Moisture

Commercial gelatins have moisture content between 9 and 14 %, with occasional samples outside this range [89]. Gelatin maintains a balance of 13 % moisture when maintained at 25 °C room temperature [21] and 46 % ambient relative humidity [28].

Gelatins with moisture content between 6 and 8 % are considered very hygroscopic, and this can complicate the determination of its physical attributes [28]. Studies conducted by researchers on gelatin extracted from different fish varieties have shown significant moisture content variations, such as 7.3 % for Nile tilapia (*Oreochromis niloticus*) [85] and 11.04 % for catfish (*Clarias batrachus*) [81].

Ash Content

The maximum ash content recommended for gelatin is 2.6 % [69, 89]. The nature of ash can be important; $CaSO_4$, for example, in gelatin can have excellent clarity; however, on dilution of the gelatin in a confectionery formulation, the ash can precipitate [28].

Kolia et al. [60] and Alfaro et al. [5] reported high percentages of ash for fish gelatins extracted from bone fraction. Values were of 2.70 and 3.80, respectively. This is attributed to the high content of mineral matter, mainly calcium content. According to Ward and Courts [89], the maximum ash content is often specified, yet not indispensable, except to indicate the calcium content, important information in some applications.

Color and Turbidity

The color of commercial gelatins usually ranges from pale yellow to dark amber [27]. Although the color of gelatin is an important commercial attribute, there is still no universally accepted method to measure it [28]. Factors such as fish species, raw material and extraction conditions influence the final color of gelatin [49]. The color can determine the application of gelatin.

The turbidity of the gelatin solution can, or not, be an important attribute depending on the application [28]. The turbidity and dark color of gelatin are commonly caused by contaminant substances that are not removed during the extraction procedure [60].

Heavy Metals

The determination of heavy metals in gelatin is complex due to the difficulty in its complete degradation [28]. According to Johnston-Banks [52], an edible gelatin must be free of heavy metal and organoleptically satisfactory.

Isoelectric Point

There are two main types of gelatin. Type A is derived from collagen with exclusively acid pretreatment, and type B that is the result of an alkaline pretreatment of the collagen. The isoelectric point of gelatin varies considerably according to the pretreatment used.

The isoelectric point of acid-processed gelatins is in the pH range of 6.0–9.5, while the alkali-processed ones are between 4.8 and 5.2 [89]. The highest isoelectric point of acid-processed gelatins is due to limited hydrolysis of the side chains of asparagine and glutamine. However, in the alkaline process, the side chains of these amino acids are easily hydrolyzed to aspartic and glutamic acids, resulting in gelatins with lower isoelectric points [28].

The isoelectric point of gelatin can be crucial for its application, depending on the pH range of the product it is intended for [89].

Amino Acid Composition

Gelatin obtained from different fish species varies greatly in amino acid content. According to Gómez-Guillén et al. [39], the physical properties of fish gelatin can be changed due to amino acid composition, the relative content of molecular weight components and also the presence of protein fragments. The amino acid composition of gelatin is predominantly 33 % glycine, 20 % proline and hydroxyproline and 11 % alanine [80]. Muyonga et al. [69] compared the content of proline and hydroxyproline found in gelatins and stated that gelatin extracted from mammals have about 30 % of these imino acids, while fish gelatin from warm-water fish present from 22 to 25 % and cold-water fish about 17 %. Karin and Bhat [56] reported better viscoelastic properties in fish gelatin of warm-water fish such as tilapia (*Oreochromis* ssp.) have been attributed to a higher content of alanine, proline and hydroxyproline.

According to Sarabia et al. [80], fish gelatins from warm-water fish contain about 70 hydroxyproline residues and 119 proline residues per 1,000 amino acid residues. The higher content of imino acids leads to physical properties similar to gelatin extracted from mammalians, with higher melting and gelation temperatures [45].

Cold-water fish gelatin, however, has a lower content of imino acids (proline and hydroxyproline) exhibiting lower rheological properties [22, 39, 56]. This type of gelatin has higher levels of glycine, serine, threonine, aspartic acid, methionine and histidine amino acids, and virtually the same proportion of alanine, glutamic acid, cysteine, isoleucine, tyrosine, phenylalanine, homocysteine, hydroxylysine, lysine and arginine when compared to mammalian gelatin [10]. Gelatin is not a complete protein. It lacks the essential amino acid tryptophan and is deficient in sulfur-containing amino acids such as cysteine and methionine [69].

Norland [72] and Kolodzieska et al. [61] state that the lower contents of proline and hydroxyproline in cold-water fish gelatin leads to lower rheological properties, which make them unsuitable for replacing mammalian gelatin, limiting its use in many applications [56] and requiring changes in its properties for industrial applications, particularly in food.

Molecular Weight Profile

The physical properties of gelatin depend not only on their amino acid composition but also on the relative contents of chains α, β and γ and high molecular weight aggregates, and also the presence of protein fragments of low molecular weight [39, 52]. Large amounts of β- and γ-chains have been shown to negatively affect some of the functional properties of fish gelatin, such as lowering viscosity, lowering melting and setting points, resulting in a longer setting time [69].

The severity of the extraction treatment is critical to the functional properties of fish gelatin [56]. Gelatin from extractions performed at high temperatures exhibits lower molecular weight profiles than gelatin from extractions at lower temperatures [69].

According to Johnston-Banks [52], gel strength of fish gelatin is proportional to the sum of α-chains, their dimers (β-components) and peptides, whereas the viscosity, the fixation rate and the melting point increase with increased amount of high molecular weight compounds. Therefore,

Table 1 Emulsifying properties, foaming capacity and foaming stability of different fish gelatins

Fish	Emulsifying properties				Foaming capacity (%)		Foaming stability (%)		References
	Skin		Bones		Skin	Bones	Skin	Bones	
	EAI m^2/g	ESI min	EAI m^2/g	ESI min					
Farmed giant catfish (*Pangasianodon gigas*)	NR	NR	NR	NR	130	NR	35	NR	[54]
Grey triggerfish (*Balistes capriscus*)	21.4	42.7	NR	NR	123	NR	117	NR	[50]
Tiger-toothed croaker (*Otolithes ruber*)	NR	NR	NR	NR	17	15	14	10	[60]
Pink perch (*Nemipterus japonicus*)	NR	NR	NR	NR	15	13	10	8	
Silver carp (*Hypophthalmichthys molitrix*)	55	80	NR	NR	NR	NR	NR	NR	[95]
Cuttlefish (*Sepia officinalis*) (gelatins obtained using different levels of pepsin 0, 5, 10, 15 U/g)	25.9	24.3	NR	NR	174	NR	128	NR	
	25.4	25.3	NR	NR	152	NR	126	NR	[55]
	27.6	39.8	NR	NR	111	NR	106	NR	
	27.6	40.5	NR	NR	100	NR	100	NR	

while the amino acid composition is determined primarily by the species, the molecular weight distribution of gelatin is dependent on the extraction process [69].

Functional Properties of Fish Gelatin

The properties of fish gelatin have been extensively studied, especially with regards to their sensory, emulsifiers, film formation and rheological properties [1, 5, 24, 97].

These properties can be affected by many factors, among which the extraction process and collagen characteristics stand out [52]. The extraction method (acid or alkali treatment) will influence its properties [45], as well as the parameters used in the extraction process (temperature, time and pH) [56].

Surface Properties

The amphoteric characteristic and hydrophobic areas in the peptide chain makes gelatin an emulsifying agent and with varied applications, such as applications in the manufacture of candy, oil-in-water emulsions such as low-fat margarine, salad dressings, milk cream and others [2, 55, 60].

The emulsion activity index (EAI) is usually determined by the turbidity of the emulsion at wavelength of 500 nm. The determination estimates the ability of the protein to assist in the formation and stability of the newly formed emulsion [95], whereas the emulsion stability index (ESI) represents the difference of EAI at 0 and 10 min at 500 nm [55].

The emulsifying capacity of gelatins (EAI and ESI) obtained from different fish species is shown in Table 1.

The formation of foam is generally controlled by the transport, penetration and reorganization of protein molecules in the air/water interface. The protein should be able to rapidly migrate to the air/water interface, where its unrolling and rearrangement take place [54]. The foam formation ability of gelatin is influenced by the protein source, the intrinsic properties of the protein, the protein composition and conformation in solution [58].

The foam stability of protein solutions is usually positively correlated with the molecular weight of peptides. Furthermore, the foam stability depends on the nature of the film and indicates the degree of protein–protein interactions within the matrix [50, 60].

The properties of the gelatin foam are important and very useful in foods such as marshmallows [60]. Table 1 shows the ability of foam formation and stability of different fish gelatins.

Film Formation Properties

Gelatins have been extensively studied due to their film-forming ability [9, 43, 48]. Studies on the production and characterization of films using fish gelatin are relatively recent.

Fish gelatins have good film-forming properties, obtaining transparent, almost colorless, water-soluble and highly extensible films [3, 10, 13, 40, 51].

However, in general, fish gelatin films exhibit lower water vapor permeability (WVP) values and lower mechanical properties when compared to films obtained from bovine or pig gelatins. This is due to the different amino acid compositions of gelatins, particularly the proline and hydroxyproline contents [29, 30, 63, 66, 84].

The improved characteristics of fish film may be due to the mixture of gelatin with other proteins and polysaccharides, or the addition of cross-linking agents. Additionally, different plasticizers can be used to improve the mechanical properties of gelatin films [74, 75, 88]. The improvement of these properties occurs when the molecular structures are used to contribute to reduce the intermolecular forces of the protein chains and can thereby reduce their hydrophilic characteristic or promote the formation of strong covalent bonds in the protein network of the film [90]. Approaches that propose to improve the formulation and preparation conditions of the film are also used, such as the use of organic acids or specific pH conditions in the preparation of the protein film and also irradiation [12, 15].

To broaden the antimicrobial and antioxidant activity of films from fish gelatin, several agents have been associated.

Gelatin films from catfish (*Ictalurus punctatus*) with the addition of clove essential oil reduced the counts of *Pseudomonas fluorescens, Lactobacillus acidophilus, Listeria innocua and Escherichia coli* in vitro and also the total count of bacteria in refrigerated raw salmon for 11 days [38].

The use of plant extracts such as citrus essential oils, including bergamot, kaffir lime, lemon and lime [87], green tea [35, 64], oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) extracts [37] added to the films obtained from the fish gelatin has shown significant antioxidant activity.

Rheological Properties

One of the main limitations on the use of fish gelatin is due to its low rheological properties when compared to mammalian gelatin [17, 24, 25], which limits its range of applications [6]. Fish gelatin generally exhibits lower gelation and melting temperatures and also lowers gel strength than the gelatins from mammals [65, 72].

Gel strength is an important property of gelatin, which is responsible for determining the quality of the product [36]. The gel strength or Bloom is defined as the strength, in grams, required to penetrate the mass of 4-mm gel prepared at a concentration of 6.67 % of gelatin kept refrigerated at 10 °C for 17 h [21]. For the determination, the gelatin samples are prepared and transferred into standard 150 ml Bloom jars. The characteristic dimensions of the flat-bottom jar are 85 mm of total height and 65 mm of shoulder height with an outer diameter of 66 mm, 59 mm inner diameter and 41 mm inner diameter at the neck [8].

The Bloom is one of the most important functional properties of gelatin as it is directly related to the resistance to degradation [45]. According to Kasankala et al. [57], the gel strength of fish gelatin may be affected by factors such as molar mass, concentration of gelatin solution, the temperature and aging time of the gel and pH. Fish gelatin typically has a Bloom value ranging from as low as zero to 270 g [56]. Gel strength values reported for cold-water species are frequently around 100 g or even lower, whereas gelatin from warm-water species normally registers values higher than 200 g [42].

After gelatin gel strength, viscosity is the second most important commercial property [60]. The viscosity of gelatin solutions varies with sources in terms of molecular weight and molecular size distribution of proteins [89]. According to Cole [28], the lower the average molecular weight of gelatin, the lower the gel strength and viscosity of the solution.

According to Johnston-Banks [52], commercial gelatins may present viscosity variations from 2.0 to 7.0 mPas, reaching 13.0 mPas for gelatins with specific applications. The standard method calls for the viscosity of a 6.67 % solution at 60 °C [28].

The viscosity differences found in fish gelatins can be explained by the extraction conditions, the variation among the fish species and the molecular weight [95].

The melting and gelling temperatures (the temperature at which a gelatin solution changes from solid to liquid and vice versa) of fish gelatin are relatively low when compared to bovine and pig gelatins (because they have smaller amounts of proline and hydroxyproline), which consequently limits its use [12, 42, 72]. However, Karim and Bhat [56] state that gelatin derived from tropical and subtropical species (warm-water) fish may have similar thermal stability to mammalian gelatins, and this feature is also dependent on the species, type of raw materials and processing conditions. Cho et al. [26] argue that an adjustment to the extraction temperature is a key factor in the processing of gelatins, as increasing the temperature from 60 to 75 °C causes a reduction in the rheological properties.

The higher melting point depicts better physical properties and indicates the possibility of obtaining gelatin with properties that are more similar to those obtained from mammalians [16]. According to Koli [60], the melting point tends to increase with aging time. Choi and Regenstein [24] and Tabarestani et al. [86] found a positive correlation between the melting point and molecular weight of gelatin peptides.

Table 2 shows the values reported by researchers for the main rheological properties of gelatin obtained from different fish species.

Improvement of Fish Gelatin Properties by Adding Agents

In order to improve the functional properties of fish gelatin, especially the rheological ones, several agents have been studied.

Table 2 Gelling point, melting point, gel strength and viscosity of various fish gelatins

Fish	Gelling point (°C)		Melting point (°C)		Gel strength (g)		Viscosity (cP)		References
	Skin	Bones	Skin	Bones	Skin	Bones	Skin	Bones	
King weakfish (*Macrodon ancylondon*)	NR	23.5	NR	23.5	NR	200	NR	NR	[5]
Farmed giant catfish (*Pangasianodon gigas*)	NR	NR	NR	NR	153		112.5	NR	[54]
Hoki (*Macruronus novaezelandiae*)	NR	NR	16.6	NR	197	NR	10.8	NR	[68]
Yellowfin tuna (*Thunnus albacares*)	18.7	NR	24.3	NR	426	NR	NR	NR	[25]
Alasca pollock (*Theragra chalcogramma*)	NR	NR	21.2	NR	98	NR	NR	NR	[97]
Sole (Solea vulgaris)	19	NR	19.4	NR	350	NR	NR	NR	[39]
Megrim (Lepidorhombus boscii)	19	NR	18.8	NR	340	NR	NR	NR	
Cod (Gadus morhua)	12	NR	13.8	NR	90	NR	NR	NR	
Hake (Merluccius merluccius, L.)	12	NR	14	NR	110	NR	NR	NR	
Rainbow trout (*Onchorhynchusmykiss*)	NR	NR	23	NR	459	NR	3.53	NR	[86]
African catfish *(Clarias gariepinus)*	NR	NR	22.1	21	NR	NR	1.13	0.66	[16]
Grey triggerfish (*Balistes capriscus*)	NR	NR	NR	NR	168.3	NR	NR	NR	[50]
Tiger-toothed croaker (*Otolithes ruber*)	NR	NR	20.3	19.5	170	150	10.53	8.30	[60]
Pink perch (*Nemipterus japonicus*)	NR	NR	19.2	19.0	140	130	8.47	6.8	
Silver carp (*Hypophthalmichthys molitrix*)	NR	NR	29	NR	NR	NR	NR	NR	[95]
Cuttlefish (*Sepia officinalis*) (gelatin obtained using different levels of pepsin 0, 5, 10, 15 U/g)	21.8	NR	25.4	NR	198	NR	NR	NR	[55]
	19.7	NR	23.5	NR	197	NR	NR	NR	
	15.3	NR	20.7	NR	162	NR	NR	NR	
	12.9	NR	19.2	NR	120	NR	NR	NR	

Gelatin modifying substances, such as salts, glycerol, glutaraldehyde, enzymes, sugars and others [6, 23], can be used to obtain desirable properties.

The rheological properties of fish gelatin can be improved by making use of protein–polysaccharide interactions. In recent years, these interactions have received considerable attention due to the many applications in food and in other areas [92]. The intermolecular arrangements can be attractive or repulsive and are guided by different variables such as pH, ionic strength, pressure, temperature and the structural characteristics and concentrations of biopolymers [40].

Studies carried out with gelatin–gum Arabic mixtures and gelatin-kappa-carrageenan consider the electrostatic attraction as the main mixture stability factor [46, 92]. Complementing the knowledge about the interactions between biopolymers, Badii and Howell [11] suggest an interaction mechanism of gelatin–egg albumen in which a small portion of gelatin bonds noncovalently to ovalbumin and uses a conformation that minimizes its aggregation with other egg albumen proteins. These events then result in high gel strength for this mixture. Table 3 lists papers that used various agents to improve the functional properties of fish gelatin.

Some approaches are composed of covalent bond formation between lysine and glutamine residues of the polypeptide chain, stabilizing the molecular structure [92]. To this end, the transglutaminase (TGase) enzyme is used in different concentrations to modify the rheological properties of gelatin.

The endogenous TGase is an enzyme that is responsible for acyl-transfer reaction that occurs between γ-carboxamide groups of glutamine residues as acyl donor and ε-amine groups of lysine residues as acyl acceptor [34]. TGase activity is found to be widespread in cells and body fluids of a number of mammals [34]. However, presently, the commercial production and purification of TGase are limited to that from microorganisms [18].

The effect of TGase on gelatin depends on the enzyme concentration, incubation temperature and gelatin sources [61]. Generally, glutamine undergoes deamidation to glutamic acid under the harsh acid hydrolysis conditions [53]. Norziah et al. [73] reported the improvement of gel strength when used TGase. In this paper, the authors assume that the increase in gel strength is correlated with a decrease in intermolecular aggregation, leading to the gel network formation due to the occurrence of intramolecular bonds. Increasing the degree of cross-linking can also be achieved with aldehydes. This treatment results in increased melting point of gelatin [23].

Salts can be used to induce interactions in gelatin, changing its characteristics [6]. The increased gel strength of gelatin with the addition of some salts is attributed to promoting a greater number of electrostatic interactions, with the formation of suitable junction zones due to the proper unfolding of the structure [80].

Table 3 Agents employed to improve the functional properties of fish gelatin

Gelatin modification	Agents	References
Enzymes	Transglutaminase	[6, 32, 61, 73]
Aldehydes	Glutaraldehyde	[23]
Nonelectrolytes	Glycerol, sucrose	[6, 32]
Electrolytes	Salts	[6, 32]
Biopolymers	Gum	[94]
	Kappa–carrageenan	[56]
	Pectin	[78]
	Hydroxylpropylmethylcellulose	[22]
	Egg albumen protein	[11]

Other solutes can be used to improve the rheological properties of gelatin. It is known that electrolytes, in general, affect the biophysical properties (swelling, solubility, gelling, viscosity and water retention capacity) of proteins, depending on the ionic strength and pH of the system [56].

Nonelectrolytes such as glycerol and sugar usually lead to an increase in viscosity [6] and gel strength of gelatins [32]. Alfaro et al. [6] observed an increase in gelatin viscosity of tilapia with the addition of glycerol. The authors believe that this increase may be due to the change in the arrangement of the water surrounding the gelatin molecules, resulting in the breaking/formation of hydrogen bonds and exposure of hydrophobic sites of the protein chain. Choi and Regenstein [24] evaluated the effect of sucrose addition on various gelatins, noting that the increase in the added content results in increased gel strength.

Bioactive Properties of Fish Gelatin Hydrolysates

The enzyme-hydrolyzed collagen plays an increasingly important role in various products and applications. Over the past decade, a large number of studies have investigated enzymatic hydrolysis of collagen or gelatin for the production of bioactive peptides [42]. Bioactive peptides are biologically active proteins, and these peptides have been studied for food and drug applications, in order to extend their useful life and to prevent the development of some diseases [62, 78].

Hydrolyzed peptides from fish sources can act as antimicrobial agents, because besides inhibiting micro-organisms they can modulate the inflammatory response [71], but this is still a little researched function. The molecular weight reduction in peptide fractions, which was associated with the elimination of aggregates, better exposure of amino acid residues and their loads, as well as the acquisition structure, have been suggested as factors that facilitate interaction with bacterial membranes. The amino acid composition, molecular weight and species of microorganisms are the factors that may explain the antimicrobial function [31].

Studies with peptide fractions (ranging between 1 and 10 kDa and <1 kDa) of skin gelatin of tuna and squid were tested against 18 strains of these bacteria and among these *L. acidophilus and Bifidobacterium animalis subp. Lactis, Shewanella putrefaciens* and *Photobacterium Phosphoreum* showed higher susceptibility [41].

Studies also have shown that some peptides can exhibit antioxidant activity, but the mechanism is still not completely understood. There are reports that they act as inhibitors of lipid peroxidation and can chelate free radicals and metal ions [59].

The antioxidant activity is attributed not only to the presence of amino acids, but also its position in the peptide sequence, and the conformation, specificity of protease and the type of acidic, alkaline or enzymatic hydrolysis [4, 76], as well as the molecular weight [41].

Skin gelatin hydrolysates from tuna and from the tunics of jumbo flying squid (*Dosidicus gigas*) were evaluated for their antioxidant power, and it was found that the elimination of 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals was negatively correlated with the total content of hydrophobic amino acids and imino acids [41].

The antioxidant activity in squid skin hydrolyzate was higher in fractions, with lower molecular weight [4]. Similar results were observed in cobia skin hydrolysate (*Rachycentron canadum*), which have smaller molecular mass values [93]. The giant squid (*D. gigas*) peptide hydrolysates obtained using commercial enzymes resulted in a twofold increase in activity compared to Ferric Reducing Antioxidant Power (FRAP). The type of commercial protease did not affect the antioxidant activity [4].

The use of bioactive peptides obtained from the marine-derived on antihypertensive activity has been shown effective [71]. The action of these peptides appears to be different than the synthetic drugs. Such peptides improve blood oxygenation and circulation in the heart, liver and kidneys. In vivo studies with administration of peptides from marine source in rats confirmed the antihypertensive action [71, 82, 96].

Applications of Fish Gelatin

Gelatin is an important biopolymer and is widely used by the food, pharmaceuticals, cosmetics industry and for photographic applications, due to its functional and technological properties [56].

According to Gonçalves [44], the technological properties of fish gelatins are widely used in various areas, having found similar applications to those of traditional bovine and pig gelatins, especially those produced from warm-water fish applications. The gelatin from cold-water fish has a restricted activity as gelling because of its low gelling power, due to the low hydroxyproline concentration.

In the food industry, gelatin is an extremely versatile ingredient [39] and can be used to improve the consistency, elasticity and stability of food, which is used in the production of sweets, edible films, encapsulation, clarification of fruit juices, dairy processing, soups and others [84]. Because gelatin has few calories, it is usually recommended to be used in foodstuffs to improve the protein levels and is also used to reduce the carbohydrate levels in foods formulated for diabetic patients [56].

In the pharmaceutical industry, gelatin is often used in the manufacture of various products such as capsules, ointments, cosmetics, tablet coatings and emulsions [40]. Furthermore, fish gelatin can be used to produce various microencapsulated foods and dried products such as vitamins and other pharmaceutical additives [98]. It can also be used in the photographic industry, which uses the unique combination of gelling agent and surface activity to suspend particles of silver chloride or light-sensitive dyes [14].

Gelatin is one of the most versatile biomaterials obtained and has been used due to its excellent film-forming capacity [47]. According to Benjakul et al. [14], fish gelatins which do not form gel at room temperature can be used in other applications that do not require high gel strength, such as for preventing syneresis and for changing food texture. These gelatins can be used in short-life products such as frozen or chilled foods [44].

Future Potentials

According to Goméz-Guillén et al. [40], gelatin production has increased in recent years, in which the main sources are the pig skin, bovine leather and pig and bovine bones. Other sources that include fish gelatin are produced at significantly smaller scales.

Some research groups have strived to determine the characteristics of fish gelatin, and also determine the optimal conditions of the production process. However, further technological development is needed in order to expand the production volume of fish gelatin. Studies are also needed to improve the functional properties of fish gelatin by adding different agents such as solutes, cross-linking adhesion promoters, bonding agents, hydrocolloid mixtures, among other properties.

The use of fish gelatin has much potential since it is an alternative to traditional gelatins, given that a portion of the population does not eat mammal-derived products. One should also consider that the production of gelatin from by-products of the fishing industry proves to be a viable option to make use of the available water resources, resulting in increased profits for the processing industries and also reducing environmental pollution problems.

Conclusion

There is an increasing demand for alternative sources for gelatin production. Most of the gelatin produced uses the bones and skins of mammals as raw material, especially bovine and pig. However, due to health or religious restrictions to mammalian derived gelatin in some countries, alternative sources such as fish have increasingly been demanded. The use of fish by-products for the production of gelatin has several advantages, namely:

- Fish gelatin has no sanitary and religious restrictions;
- The fishing industry generates large amounts of by-products with high collagen contents;
- The use of fish by-products to obtain gelatin is economically interesting for the processing industries;
- The reduction of environmental pollution problems caused by the improper disposal of by-products derived from fish processing;

The production/use of fish gelatin should also take into consideration some issues such as:

- The large number of aquatic species and the different functional properties of gelatin derived from each species;
- The lower rheological properties of fish gelatin (especially cold-water fish) compared to mammalian gelatin;
- The high degradation susceptibility of fishery products;
- The availability of raw material coupled with the relatively low gelatin yield.

In recent years, there have been several studies conducted in order to improve the functional properties of fish gelatin, such as the use of nonelectrolytes, salts, mixtures with other proteins and polysaccharides, cross-linking adhesion promoters, among others. Improving the functional properties of fish gelatin, especially the rheological properties, would broaden its range of applications. The fish gelatins with lower rheological properties can be used in various applications, which do not require high gel strength and high melting point.

In the future, fish gelatin may become a competitive alternative biopolymer in the market; nevertheless, expanding its use is fundamentally correlated to higher technological development and the improvement of its functional properties.

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