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COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Repurposing fish waste into gelatin as a potential alternative for mammalian sources: A review

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Abstract

Mammalian gelatin is extensively utilized in the food industry because of its physicochemical properties. However, its usage is restricted and essentially prohibited for religious people. Fish gelatin is a promising alternative with no religious and social restrictions. The desirable properties of fish gelatin can be significantly improved by various methods, such as the addition of active compounds, enzymes, and natural crosslinking agents (e.g., plant phenolics and genipin), and nonthermal physical treatments (e.g., ionizing radiation and high pressure). The aim of this study was to explore whether the properties of fish gelatin (gel strength, melting or gelling temperature, odor, viscosity, sensory properties, film-forming ability, etc.) could be improved to make it comparable to mammalian gelatin. The structure and properties of gelatins obtained from mammalian and fish sources are summarized. Moreover, the modification methods used to ameliorate the properties of fish gelatin, including rheological (gelling temperature from 13–19°C to 23–25°C), physicochemical (gel strengths from ~200 to 250 g), and thermal properties (melting points from ~25 to 30°C), are comprehensively discussed. The relevant literature reviewed and the technological advancements in the industry can propel the development of fish gelatin as a potential alternative to mammalian gelatin, thereby expanding its competitive market share with increasing utility.

Abbreviations: BHT, butylated hydroxytoluene; BSE, bovine spongiform encephalopathy; DSC, differential scanning calorimetry; FAO, Food and Agriculture Organization; GCP, gelatin collagen peptide; HPP, high-pressure processing; MCP, marine collagen peptide; MTG, microbial transglutaminase; MW, molecular weight; NaCl, sodium chloride; PUFA, polyunsaturated fatty acids; RSM, response surface methodology; SEM, scanning electron microscope; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TEM, transmission electron microscopy; UVRT, ultraviolet radiation technology.

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chemical property, fish gelatin, mammalian gelatin, physical property

1 | INTRODUCTION

Gelatin, frequently utilized as a gelling agent in food systems, has multiple functions. It is usually applied in jelly production, clarification of fruit juice, processing of confectionary, soup, and dairy, as well as in encapsulation, edible films, glue, and photography (Huang et al., 2020). Gelatin is predominantly generated from the skin and collagen of connective tissues obtained from mammals. It is thermo-reversible with a melting point lower than body temperature that contributes to its well-known "melt-in-the-mouth" property, as collagen has a unique property of forming a gel when dissolved in water (Norziah et al., 2009). In addition, gelatin has diverse applications in the food industry, particularly in association with the enhancement of elastic character, consistency, and stability of food products (Wu et al., 2020). It can also provide biological active peptides after protease hydrolysis (Park et al., 2020). Lastly, it is widely used as an important hydrocolloid and gelling and thickening agent in food products.

The global annual production of gelatin is ~ 326,000 tons (Norziah et al., 2009). Porcine skin (46%) is the biggest source of gelatin, while the skins (29.4%), bones (23.1%), and miscellaneous parts (1.5%) from bovine sources are also important contributors to worldwide gelatin production (Laura et al., 2020). However, religious and social issues, as well as the risk of bovine spongiform encephalopathy (BSE), have limited the market demand for bovine and porcine gelatin in recent decades. The possible alternatives for gelatin from mammalian sources are freshwater and marine fishes, especially their skins, bones, and fins, which remains unexplored (Karim & Bhat, 2009). Moreover, the Food and Agriculture Organization (FAO) estimated that there was an annual fishery waste of 20 million tons worldwide, accounting for 25% of the total fishery production (Gómez-Guillén et al., 2011). Unfortunately, such wastes of fish processing are not utilized properly resulting in environmental pollution (Zhang et al., 2020b).

Fish gelatin contributes to an extremely limited market share as opposed to the gelatin obtained from the bovine and porcine sources. However, the number of fish species used for gelatin extraction expanded recently, as shown in the overview of a few important species in Table 1. Insufficient availability of the raw materials for fish gelatin, inferior gelatin quality, as well as poor rheology, odor, and bloom strength are important factors hindering the industrial development of fish gelatin worldwide (Karayannakidis & Zotos, 2015). Moreover, price volatility and poor control of the intrinsic quality traits of fish gelatin further limit the growth of this sector (Karim & Bhat, 2009). However, recent studies have revealed that improvement in the physicochemical and functional attributes of fish gelatin can be achieved by adjusting the contents of the coenhancers (i.e., salts, sugars, enzymes, magnesium sulfate, formaldehyde, glycerol, and transglutaminase) to similar levels as those of mammalian gelatin (Cho et al., 2005). The commonly discarded collagen-rich fishery waste can not only be utilized to increase its commercial value and create new business opportunities for the food industry but can also promote environmental sustainability by reducing its harmful effects (Karayannakidis & Zotos, 2015). It is estimated that global fish production will increase to 186 million tons in 2030, which certainly will weigh on the environment (Alfaro et al., 2015; The World Bank, 2013). Therefore, the investigation of intrinsic physicochemical properties, development of improved extraction methods, and studying the effect of processing conditions (such as pH, temperature, and salt concentration) on the quantity and quality of gelatin are urgently required. Moreover, the other research areas that need to be explored are also discussed in this study.

Therefore, this review (1) compares the intrinsic physicochemical properties of the mammalian gelatin to the fish gelatin, (2) discusses the current developments to improve extraction methods and processing conditions of the quantity and quality of gelatin, and (3) expounds the increasing utility of fish-based gelatin in the industry as a result of the technological advancement.

2 | COMPARISON BETWEEN PHYSICOCHEMICAL PROPERTIES OF MAMMALIAN AND FISH GELATIN

Gelatin has a high degree of flexibility in its polypeptide chains. The functional quality of gelatin largely depends upon its molecular character; it is particularly related to the species-specific amino acids and their distribution (Jellouli et al., 2011), especially those of the amino residues (alanine [Ala]; phenylalanine [Phe]; glutamic acid [Gln]; cysteine [Cys]; isoleucine [Ile]; tyrosine [Tyr]; lysine [Lys]; and arginine [Arg] residues) and imino-acids (glycine [Gly]; serine [Ser]; threonine [Thr]; aspartic acid [Asn];

TABLE 1 Classification of fish species used for gelatin extraction

Type	Name	Main sources	References
Warm-water fish	Catfish	Asian; European countries; North America	Rawdkuen et al. (2013)
	Megrim	Northeast Atlantic	Hanjabam et al. (2015)
	Nile perch	Africa	Hanjabam et al. (2015)
	Carp (Rohu)	India	Hanjabam et al. (2015)
	Shark	Japan, India	Hanjabam et al. (2015)
	Snapper (Threadfin bream)	Thailand	Hanjabam et al. (2015)
	Tuna	India	Cho et al. (2015)
	Tilapia	Thailand	Rawdkuen et al. (2013)
	Triggerfish	Indo-Pacific	Jellouli et al. (2011)
	Lizardfish	Thailand	Wangtueai and Noomhorm (2009)
Cold-water fish	Pollock	North America and the United Kingdom	Avena-Bustillos et al. (2006)
	Rainbow trout	Asia and North America	Tabare (2010)
	Haddock	North Atlantic Ocean	Avena-Bustillos et al. (2006)
	Cod	Northern Pacific	Åsli and Mørkøre (2012)
	Salmon	North Atlantic, Pacific Ocean	Avena-Bustillos et al. (2006)
	Hake (*Pollachius virens*)	North Atlantic Ocean	Casanova et al. (2020)

methionine [Met] and histidine [His] residues, etc.), as shown in Table 2 (Leuenberger, 1991).

An important property of gelatin is its thermal reversibility due to the nonrandom presence of imino acids (i.e., proline or hydroxyproline) in their sequence, which is a unique trait compared to other gel-forming agents, such as proteins and polysaccharides (Limpisophon et al., 2009; Zhang et al., 2020b). The presence of high molecular weight (MW) polypeptides in gelatin, along with its gelling and thickening properties, imparts effective hydrocolloid abilities (Kaewruang et al., 2013). In contrast to other hydrocolloids, which mostly consist of polysaccharides, gelatin is composed of easily digestible proteins and essential amino acids (except for tryptophan) (Mariod & Fadul, 2013).

The manufacture of gelatin from nonmammalian sources has been increasing in recent years (Núñez-Flores et al., 2012). Rawdkuen et al. (2013) found that gelatin obtained from fisheries accounted for almost 1.5% of the global production in 2007 – twice the contribution of fisheries since 2002. Gelatin can be produced from multiple fish species; however, a few species are preferably used for its production (listed in Table 1). Furthermore, gelatins produced from different parts of the fish body have been evaluated for their functional properties (Wasswa et al., 2007). For example, Nile perch gelatin showed similar properties to that of mammals as compared to the gelatin obtained from cold-water fish skin (Muyonga et al., 2004). The gelatin from Nile perch skin contains greater content of polypeptides and β peptides than that from its bone. Additionally, the gelatins from both sources contained α peptides of low MW. In general, the yield of gelatin from Nile perch skin was higher than that from bone, and the functional properties of skin gelatin were also better than the bone counterpart. However, it has been demonstrated that certain pretreatments could be used to prepare bone gelatin with superior functional attributes (Wasswa et al., 2007). Particularly, the pretreatment of liming significantly improves the functional characteristics of bone gelatin as it regulates the desired alkalinity without causing the collagen to swell (Jamilah et al., 2011). The physicochemical and functional properties of the fish gelatin enable it to be a superior alternative to bovine gelatin. Therefore, gelatin acquired from these fishes is a potential alternative ingredient for the food industry (Sai-Ut et al., 2012).

Apart from the solubility, color, odor, and taste, other important factors responsible for the quality of gelatin are thermal stability (melting enthalpies and gelling ability) and gel strength, both of which are correlated with the rheological, emulsification, foaming, and film-forming properties of gelatin (Rawdkuen et al., 2013). Particularly, its bloom value can be classified as either low (<150 bloom), medium (150−220 bloom), or high (220−300 bloom). Moreover, gelatin with a high viscosity is considered commercially preferable and hence sells at a higher price (Karim & Bhat, 2009). Lastly, gelatin is also one of the most researched biopolymers due to its film-forming ability, which is useful in protecting foods from moisture loss, limiting exposure to light and oxygen, and preparing

TABLE 2 Amino acid compositions in some fish gelatin compared to mammalian gelatin

Composition		Fish gelatin								Mammalian gelatin	
		Alaska pollock skin	Salmon skin	Catfish skin	Leather jacket	Reef cod skins	Rohu skin	Crap skin	Tuna skin	Pork skin	Bovine skin
Amino acid	Ala	10.38	12.49	12.40	9.5	9.3	1.16	3.54	11.4	11.39	11.2
	Arg	5.18	5.06	5.00	5.8	5.3	4.93	4.75	5.5	5.19	4.9
	Asx	5.21	5.12	4.59	ND	ND	2.56	2.61	42.5	4.63	3.01
	Cys	0.14	0.08	0.10	ND	ND	ND	ND	ND	0.16	ND
	Glx	7.17	7.25	7.22	ND	ND	ND	ND	7.4	7.27	ND
	Gly	35.74	35.54	34.01	33.3	33.6	24.93	20.99	33.5	32.34	27.69
	His	0.80	0.87	0.60	6	8	0.71	0.03	0.6	0.48	0.03
	Hcy	0.16	0.12	0.09	ND	ND	ND	ND	ND	0.02	ND
	Hyl	0.61	0.76	0.58	ND	ND	8.90	7.78	0.5	0.68	11.26
	Ile	1.07	0.97	1.17	1.6	1.9	0.15	0.40	0.9	1.01	0.98
	Leu	2.10	1.83	2.09	2.6	3.1	3.21	1.40	2.1	2.58	1.73
	Lys	2.78	2.47	3.10	1.5	1.2	2.83	4.20	2.7	2.83	3.29
	Met	1.13	1.00	0.49	1.3	1.4	2.43	3.94	1.1	0.54	1.43
	Phe	1.20	1.27	1.30	1.0	1.1	1.11	0.66	1.2	1.44	1.20
	Ser	5.85	4.73	3.61	5.1	5.4	4.69	4.34	4.1	3.07	3.01
	Thr	2.68	2.55	2.60	2.2	2.7	4.41	4.19	2.3	1.69	2.06
	Tyr	0.24	0.13	ND	0.8	0.8	0.48	0.21	0.3	0.39	0.08
	Val	1.67	1.41	2.19	2.6	2.5	2.62	2.14	2.3	2.30	1.88
Imino acid	Hyp	5.30	5.56	7.72	8.0	8.1	11.59	11.72	8.3	8.53	11.26
	Pro	10.09	10.79	11.14	9.4	9.9	8.9	7.44	11.7	13.47	12.44
Reference		Avena-Bustillos et al. (2006)			Renuka et al. (2019)		Ninan et al. (2010)		Haddar et al. (2012)	Vijayakumar et al. (2018)	Ninan et al. (2010)

biodegradable films for the development of active packaging material, which can be enriched with antioxidants and/or antimicrobial substances (Huang et al., 2020).

Mammalian gelatin has comprised a major proportion of the food and pharmaceutical industries over the past several decades stemming from its outstanding film-forming abilities and low melting point. Furthermore, owing to the hydrophilic properties of gelatin films, it exhibits high resistance against oxygen at low relative humidity (Jongjareonrak et al., 2010). This characteristic feature is ideal and necessary, as high humidity makes gelatin films permeable to oxygen (Byun et al., 2012). Contrastingly, the gelatin film produced from carp skin has superior characteristics as it has significantly lower oxygen and water vapor permeability than from that of mammalian skin (Shyni et al., 2014).

The chemical, physicochemical, and functional properties of fish gelatin have been elaborated on previously (Karim & Bhat, 2009). However, a precise comparison of the differences between the properties of mammalian and fish gelatins is warranted. The comparative analysis will aid in optimizing the processing conditions and manufacturing methods of superior-quality fish gelatin. Therefore, the physicochemical properties, including melting temperature, sensory properties (primarily the odor), viscosity, and textural properties (bloom strength), of mammalian and fish gelatin were compared in this review, as shown in Table 3.

2.1 | Melting temperature

The major difference between the gelatins obtained from either mammalian or fish sources is their gelation temperatures. Moreover, variations in the gel melting and gel setting temperatures between the gelatin from cold- and warm-water fishes have been noted. The gelatin from cold-water fishes requires low gel melting and gel setting temperatures, primarily due to its high hydrophobicity and low content of imino acid (proline and hydroxyproline); these have been linked with a lower tendency of forming an intermolecular helix (Gilsenan & Ross-Murphy, 2000). Although the amino acid profiles of gelatins from different mammalian sources are always constant, those from different fish species usually vary. In the mammalian gelatins, glycine accounts for ~one-third of the total amino acid

TABLE 3 Physical properties of various fish gelatin and mammalian gelatin

Class	Type	Physical property			Reference
		Gel strength (Bloom, g)	Gelling temperature (°C)	Melting point (°C)	
Fish gelatin	Hemiramphus far	ND	19.5	25	Wu et al. (2020)
	Cod	177.84	21.2	27.4	Park et al. (2021)
	Catla	264.6	13.7	23.3	Chandra and Shamasundar (2015)
	Red snapper	0.77	16.00	26.00	Jeya Shakila et al. (2012)
	Grouper	0.79	16.00	25.00	Jeya Shakila et al. (2012)
	Shark	About 206	About 20.8	About 25.8	Shyni et al. (2014)
	Rohu	About 124	About 13.8	About 18.2	Shyni et al. (2014)
	Croda	About 440	About 17.46	About 25.56	Norziah et al. (2009)
	Carp	About 200	About 19	ND	Shyni et al. (2014)
	Catfish	About 276	17	25	Liu et al. (2008)
	Croaker	170 g	ND	20.36	Koli et al. (2012)
	Perch	150 g	ND	19.23	Koli et al. (2012)
	Tilapia	211 g	17.7	25.8	Sinthusamran et al. (2017)
Mammalian gelatin	Bovine	216 g	23.8	33.8	Cho et al. (2005)
	Porcine	295 g	25.6	36.5	Cho et al. (2005)

Note: ND, not detected.

residues, whereas proline and hydroxyproline together constitute ~one-fifth and alanine alone accounts for ~one-ninth (Correia et al., 2013). Overall, the content of these four amino acids in the mammalian collagen contributes to ~two-thirds of the total amino acid residues during the manufacturing of mammalian gelatin (Hanjabam et al., 2015). By contrast, the fish collagens with remarkable variation in amino acid composition have low proline and hydroxyproline content and high serine and threonine contents. Similarly, in a differential scanning calorimetry (DSC) study, Norziah et al. (2009) reported considerably lower melting points of gelatins obtained from cod skin than those prepared from bovine and shortfin scad.

The functional properties of fish gelatin with low melting temperatures vary from those with high melting points. Chiou et al. (2008) proved that the tensile strength and elongation value of the gelatin film prepared from pollock and salmon are lower than that from mammals (bovine and pig), which is related to the reduced denaturation of the fish gelatin film. The melting temperatures and tensile properties of fish gelatin films are also remarkably influenced by glutaraldehyde crosslinks. In addition, gelatin films extracted from cold-water fish showed lower water-vapor permeability as compared to those from warm-water fishes and mammalian sources (Avena-Bustillos et al., 2006). Notably, the reduced water vapor permeability of fish gelatin films contributes to the decrease in water loss of refrigerated and frozen foods.

2.2 | Sensory properties

A fishy odor is the most important challenge associated with fish gelatin, which limits its utility. The problem of fishy odor is quite prominent in the case of salmon skin (Tongnuanchan et al., 2014). Gelatin from salmon skin is rich in proteins and essential fatty acids because salmon skin contains high amount of protein (30–35%) as well as omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid and docosahexaenoic acid (Ferraro et al., 2010). On the one hand, the PUFAs in fish gelatin are beneficial for health; on the other hand, PUFAs are susceptible to lipid oxidation, which ultimately leads to the production of fishy volatiles (Orrawan & Worapong, 2012; Sae-Leaw et al., 2016). The primary products of oxidation (lipid hydroperoxides) are degraded into secondary lipid oxidation metabolites, including alcohols, aldehydes, ketones, and furans, which is usually indicated by the thiobarbituric acid (TBA) content. Gelatin with a TBA content higher than 8 mg/kg is not suitable for human consumption (Kristinsson & Hultin, 2004). Moreover, the distinguishable fishy odor renders them inferior to bovine gelatin in organoleptic qualities.

Despite this, some studies reported that the gelatins obtained from fish sources were superior to those manufactured from mammalian sources. For instance, Choi et al. (2000), Jayathilakan et al. (2012), and Shyni et al. (2014) conducted comparative studies on gelatins prepared

from fish, bovine, and porcine sources concerning their physicochemical and functional properties. They collectively found that the odor scores of skin gelatins obtained from bovine and porcine sources were greater than those of fish gelatin, which indicated that the organoleptic qualities (off-odor and aroma) of the former two were inferior to the latter. Moreover, Cho et al. (2015) reported the enhanced melting point, as well as an increased release of desirable flavor and aroma in flavored fish gelatin desserts when compared to those of the same product prepared from pork gelatin with equal bloom strength.

Off-odor compounds have also been reported from marine collagen peptide (MCP) and gelatin collagen peptide (GCP). In a recently published report, methional, as well as dimethyl tri-and tetra-sulfides, were regarded as the characteristic off-odor compounds of MCP and GCP (Limpisophon & Schleining, 2017). In addition, the sulfurous, cool/refreshing, and medicinal odors of some unknown compounds in MCP and GCP were also associated with the formation of off-odors. Therefore, the processing conditions largely affected the production of these volatile flavoring compounds. Nonetheless, deodorization could be performed to remove the off-flavors. However, an earlier contradictory publication reported that activated-carbon treatment could not eliminate the off-odor compounds from MCP and GCP during the manufacturing process (Sae-leaw & Benjakul, 2015). The most important off-odor producing compounds are mainly sulfur-containing compounds, such as methanethiol, as well as dimethyl di-, tri-, and tetra-sulfides, which tend to increase during the manufacturing process (Limpisophon & Schleining, 2017). In addition, bacteria can also ferment some of the flavor-producing organic compounds. For example, *Lactobacillus plantarum* has been used to effectively deodorize the off-odor of MCP (Yazdimamaghani et al., 2015).

2.3 | Viscosity

Viscosity is an important functional property for process control. Gelation of a gelatin solution dually depends upon its viscosity in water and the temperature. Whether fish gelatin possesses both gelling and nongelling properties depends on the origin of the fish species, habitat, and amino acid composition. The gelatin prepared from cold-water fishes demonstrates a high viscosity, which is not a desirable attribute in many applications (Chiou et al., 2006).

As the concentration of hydrophobic and hydroxylated amino acids, gelatin viscosity, and distribution of MW are dependent on the specific fish species, the quality of foods that uses gelatin is also affected accordingly. Lim and Mohammad (2011) reported that the gelatins prepared from different fish sources exhibited variations in their rheological, physicochemical, and structural properties. In another study, Gómez-Guillén et al. (2002) reported that gelatins with higher gelling ability and thermostability were obtained from flat-fishes (sole and megrim), compared to those from cold-water fish species (cod and hake).

2.4 | Textural properties

A technologically important character of gelatin is its bloom strength. The composition of amino acids and their molecular distribution play a key role in the gelatin-based gel strength as well as its melting point. For instance, the orientation of the R-groups in the amino acids of intact collagen and gelatin-based gels provides strength to the triple helix structures. In addition, a high quantity of hydrophobic amino acids also affects the rigidity, though less so than in mammalian gelatin which generally has better gel strength than fish gelatin. Interestingly, the collagen obtained from warm-water fishes contains higher amino acids than that from cold-water ones (Boran et al., 2010).

Water temperature plays a crucial role in the processing of fish gelatin, as the use of either warm or cold water will result in different gel strengths. Similarly, the storage temperature of fish skin may also lead to disparate gelatin strength. Fernández-Dıáz et al. (2003) revealed that gelatin derived from skin stored at −12°C had weaker gel strength than that prepared from fresh skin. In addition, the gelling and melting enthalpies of dried channel catfish skin gelatin were distinctly different from gelatin prepared from frozen skin.

3 | STRATEGIES TO IMPROVE THE FUNCTIONALITY OF FISH GELATIN

The physicochemical, functional, technological, and sensory attributes of gelatin prepared from beef, pork, and/or fish are determined by the method of preparation used as well as the intrinsic character of the collagen from the respective sources (Hu et al., 2020). There are two approaches generally adopted to prepare gelatin commercially. The first is the alkaline process in which raw materials are pretreated with a cold alkaline solution for several weeks and then extracted at a neutral pH. Alkaline pretreatment causes the de-amidation of aspartic acid and glutamic acid, thereby increasing their concentrations (Eysturskarð, Haug, Ulset et al., 2009). The second approach is acid treatment, which is the primary means for gelatin preparation and the only means for pork gelatin preparation. The acid treatment method involves pretreat-

ing the raw material for several hours and then extracting it with mild hot water under acidic conditions (pH 4.0) (Zhou et al., 2020). Consequently, two different types of gelatin are obtained. These are commercially known as type-A and -B gelatins, with isoelectric points at pH 8–9 and pH 4–5, respectively (Gómez-Guillén et al., 2011). In addition, several modifications, and combinations of the mentioned two processes have also been adopted to attain the required product attribute, process efficiency, and gelatin quality.

3.1 | Understanding gelatin formation

3.1.1 | Collagen-based gelatin

During the process of gelatin formation from collagen, acid and/or alkaline hydrolysis irreversibly ruptures the covalent bonds of the fibrous structures of collagen via a mild degradative process. Hydrochloric acid [HCl], sulfuric acid [H_2SO_4], phosphoric acid [H_3PO_4], calcium hydroxide [$Ca(OH)_2$], and sodium hydroxide [NaOH] are extensively utilized to produce gelatin from mammals (Ninan et al., 2010). First, the soluble collagen upon denaturation in hot water (40°C) produces one, two, or three random gelatin molecule chains from the destruction of the triple-helical structures, predominantly because of the breakdown of hydrogen- and electrostatic-bonds, and hence produces a high viscosity solution. Second, the cooling of this solution gives rise to crosslinks and/or produces junction zones that partially form ordered triple-helices. Finally, the purification and drying of gelatin are performed. In the aging of the gelatin solution, water is extracted from the linear and flexible parts of the three-dimensional protein network, causing it to collapse into a rubber-like film that ultimately vitrifies via hydrogen bonding and forms crosslinks upon drying. In short, the gelling process involves the structural rearrangement of the triple helix structure of collagen, the transition at a certain temperature called the gelling point, and the melting of protein structures at the denaturation point of the structure. Especially, the gelling and melting enthalpies of gelatin depend upon the ratio of proline and hydroxyproline (imino acids) in the source- and pretreatment-dependent collagen molecules (Maki & Annaka, 2020). Mammalian gelatin with good gel-forming ability is usually obtained during the initial extraction process performed at the lower temperatures, whereas the subsequent extraction at the higher temperatures produces gelatins with inferior mechanical traits attributed to accelerated hydrolysis (Eysturskarð, Haug, Elharfaoui et al., 2009).

Gelatin formation can be understood from the perspective of its composition and structure. Collagen, the main structural unit of gelatin, is a right-handed helical rod, composed of three left-handed helices called α-chains intertwined with the so-called collagen triple-helix, which is a product of repeating units of the glycine-*X*-*Y* sequence of amino acids, where *X* is predominantly a proline, while *Y* is mostly hydroxyproline (Gómez-Guillén et al., 2011). The triple helix of collagen makes up a fibrous structure that arranges in bundles to form the connective tissue matrix. Stabilization of the structure is mainly achieved by hydrogen bonding of the right-handed triple helix. However, the crosslinks between the α-chains of the collagen fibers organize them into a quarter-staggered pattern (Eysturskarð, Haug, Elharfaoui et al., 2009). The MW of a single α-chain is ~95–100 kg/mol. The subunits of the α-chains of collagen are released during pretreatment and extraction of gelatin due to the breakage of peptide bonds in the primary structures. The crosslinks, which are stronger than the hydrogen bonds, exist between the inter-β-chain and inter-γ-chain, which are in turn composed of two and three covalently crosslinked α-chains, respectively (Yi et al., 2006). The differences in the characteristics of gelatin obtained from fish and mammalian sources could therefore be explained in terms of the distribution of MW, the composition of amino acids, as well as by $\alpha1/\alpha2$ collagen-chain ratio (Eysturskarð, Haug, Elharfaoui et al., 2009). Yi et al. (2006) reported that hydroxyproline plays the most important role in the stabilization of the -OH group in collagen. The thermal stability of collagen is also affected by the total glycine–proline–hydroxyproline sequence. Maqsood and Benjakul (2011) mentioned that the quantity of proline and hydroxyproline in fish skin was associated with the temperature of the habitat. The warm-water species exhibited higher content of these imino acids in their skin than that of their cold-water counterparts, which ultimately affected the thermo-stability of gelatin produced from fish collagen.

3.1.2 | Factors influencing gelatin structure

The composition of fish gelatins is considerably variable when compared with that of mammalian gelatins. This is due to the diverse origins of extraction with respect to the raw materials, such as skin, bone, and scales from cold- and/or warm-water fishes. The gelatin composition, especially the amino acid content, contributes to its diverse applications in multiple food systems and pharmaceutical industries. Therefore, the properties of fish gelatin need to be improved to replace the usage of the functionally superior mammalian gelatin, which can be manipulated by the adjustment of processing conditions and process parameters.

Other factors affecting the intrinsic properties of collagen include the breed of species, age, the pattern of feeding, storage factor, environmental condition, the concentration of gelatin solution, time and temperatures of gel maturation, gel drying temperature, pH, salt content, as well as the average MW and the distribution of MW (Boran et al., 2010; Koli et al., 2013). Some authors hypothesized that substances like salt, glycerol, and enzymes could modify the structure of gelatins to improve their rheological properties in industrial processing (An et al., 2010). These gelatin modifiers can be classified into two groups, namely electrolytes and nonelectrolytes. In general, the electrolytes affect the biophysical attributes of proteins, principally by manipulating their ionic force and system pH. These attributes include gelation, water-holding capacity, swelling capacity, solubility, and viscosity. The nonelectrolytes, which include sugars and glycerol, generally improve the strength of the gelatin-based gels (Krishna et al., 2012). The examples of the modification methods used to modify the biophysical attributes of fish gelatin are listed in Tables 4 and 5.

3.1.3 | An overview of methods used to modify gelatin function

The functionality of fish gelatin can be modified by a myriad of methods (Hernández-Briones et al., 2009). For instance, the addition of glutaraldehyde and/or formaldehyde can enhance the double bonds in collagen chains. Blending fish gelatin with pectin, κ-carrageenan, and chitosan can incorporate the properties of these biopolymers into the fish gelatin (Koli et al., 2011). In addition, the mixing of plasticizing agents, such as sucrose, sorbitol, glycerol, and polyethylene glycol (Sztuka & Kołodziejska, 2009), as well as mineral salts (Razzak et al., 2016) can enhance the mechanical attributes of fish gelatin films. The addition of crosslinkers, such as glyoxal, formaldehyde, and glutaraldehyde (Almeida & Lannes, 2013) and/or enzymes such as microbial transglutaminase (Bae et al., 2009) can improve the functional attributes of fish gelatin. However, synthetic crosslinking agents that exceed a certain dose are often toxic and carcinogenic and should, therefore, only be used in a limited capacity in food systems (Staroszczyk et al., 2014). Hence, enzyme crosslinkers are good alternatives to chemical crosslinking agents for food packaging (Bae et al., 2009). Apart from using only chemical methods, certain physical methods may also be used in combination to improve the functional attributes of fish gelatin (Yang et al., 2012).

Direct modification of fish gelatin has also been reported previously by Huang et al. (2017), which included enzymatic, chemical, physical, and certain complex modifications. However, adjusting the extraction conditions proved to be a better way to alter the functionality of fish gelatin. Moreover, comprehensive detail is available for methods of extraction condition adjustment, the addition of proteins or polysaccharides, addition of crosslinking agents, and modification by nonthermal processing technology, among others.

3.2 | Extraction condition adjustment

The functional attributes of fish gelatin can be influenced by the adjustment of the extraction conditions (Cho et al., 2006). Changes in the concentration of acid or alkali, extraction time, and temperature significantly alter the mechanical and functional properties of fish gelatins (Chiou et al., 2009; Devi et al., 2013; Hernández-Briones et al., 2009; Jamilah & Harvinder, 2002; Karim & Bhat, 2009; Park et al., 2020; Surh et al., 2006; Taherian et al., 2011). For instance, gelatin has been prepared from the skin of the Shaari Eshkeli fish by manipulating the concentration of acetic acid (Al-Saidi et al., 2011; Kristinsson et al., 2005). Furthermore, Wangtueai and Noomhorm (2009) reported that the concentration of NaOH significantly affected the extraction yield and viscosity of fish gelatin. Extraction temperature also remarkably affected the gel strength, whereas the extraction time affected the extraction yield, gel strength, and viscosity. Koli et al. (2012) further indicated that the skin obtained from tiger-toothed croaker fish served as a good source of fish gelatin upon optimizing the extraction method to increase the yield. This resulted in the successful preparation of fish gelatin with optimal characteristics similar to that of mammalian gelatins.

Extraction conditions remarkably influence the properties of fish gelatins, particularly the concentration of H^+ and OH^-. Tabare (2010) et al. (2010) improved the physicochemical attributes of gelatin from rainbow trout skin by optimizing the extraction conditions. They obtained optimum yield and favorable physicochemical attributes at optimum concentrations of H^+ (0.121 N) and OH^- (0.19 N) for a pretreatment time of 3 h. They revealed that the H^+ concentration significantly affected the distribution of the MW, thereby affecting the gel stability and melting point. Moreover, the OH^- concentration significantly affected the viscosity and extraction yield. Similarly, the pretreatment time also influenced the properties of the fish gelatin. Yang et al. (2007) used a two-step response surface methodology (RSM) design to optimize the pretreatment time for the production of fish gelatin from the skin of channel catfish. This method also improved the gelatin yield, as well as its other physicochemical properties including viscosity and gelatin strength. The best physicochemical attributes were obtained with NaOH and acetic acid pretreatments (0.20 mol/L for 84 min and 0.115 mol/L for 60 min, respectively) at 4°C, followed by extraction at 55°C for

TABLE 4 Examples of modifying properties of fish gelatin

Fish source	Method	Improved properties	Reference
Parupeneus heptacanthus	Addition of MTGase and coconut husk extract	Gel properties and in-vitro digestibility	Avtar-Singh et al. (2020)
Alaska pollock	Addition of basil and citronella essential oils	Structural, morphological, and thermal properties	Tongnuanchan et al. (2014)
Skins of silver carp	Adjust extraction conditions (alkali and acid treatment, water extraction)	Sensory and instrumental characteristics	Boran et al. (2010)
Baltic cod skins	Addition of proteins (chitosan)	Physico-chemical properties	Staroszczyk et al. (2014)
Cold-water fish skin gelatin	Octenyl succinic anhydride modification	Structural, functional, and emulsion stability	Zhang et al. (2020a)
Seabass (*L. calcarifer*)	Spray drying with citric acid adding pretreatment	Physico-chemical properties and fishy odour	Sae-leaw and Benjakul (2015)
Cod, Pollock, and haddock skin	Addition of sodium alginate	Foam and emulsion stabilization	Razzak et al. (2016)
Yellowfin tuna	Addition of NaH_2PO_4, $MgCl_2$, $CaCl_2$, and glycerol	Physicochemical properties	Karayannakidis and Zotos (2015)
Commercial warm-water fish	Addition of plant extraction (lignin)	Physical and functional characterization	Núñez-Flores et al. (2013)
Chinese Herring species	Addition of enzyme transglutaminase	Gel properties	Norziah et al. (2009)
Croaker, perch	Addition of coenhancers ($MgSO_4$, sucrose and transglutaminase)	Gel strength and melting point	Koli et al. (2011)
Skins of catfish	Addition of chitosan and calcium acetate	Physico-functional and mechanical properties	Jeevithan et al. (2013)
Carp scales	Addition of pectin and MTGase	Rheological behavior, gel properties and nanostructure	Huang et al. (2017)
Sole (*Solea* spp.) skins	Addition of glycerol and sorbitol	Sensory characteristics	Gómez-Estaca et al. (2009)
Tilapia skins	Addition of sucrose, glucose and fructose	Structural characteristics, functional properties, and emulsion stabilization ability	Zhang et al. (2020)

180 min. In another study, Yang et al. (2008) reported that pretreatment with alkali and acid improved the physical properties of channel catfish gelatin. In addition, acid pretreatment significantly improved the yield and viscosity and exhibited the highest gel strength in the gelatin from the channel catfish. Finally, the acid pretreatment group displayed a sponge-like aggregate in the nanostructure of gelatin. Similarly, Liu et al. (2008) adopted RSM to optimize the extraction conditions to obtain gelatin from the skin of channel catfish and reported higher gel strength and gelling ability along with lower thermo-stability than those of gelatin from porcine skin. They attributed this difference in the functional properties to the different amino acid compositions between the gelatin types. This improvement is significant enough for the gelatin produced from the skin of channel catfish under these optimized processing conditions to be considered as a probable alternative for porcine and bovine gelatins in the gelatin industry.

3.3 | Addition of proteins or polysaccharides

The manufacture of films is one of the main purposes of fish gelatin preparation. Fish gelatin films are produced from warm-water fishes (bigeye red snapper, brown stripe red snapper, tilapia, carp, catfish, and tuna) as well as cold-water fishes (Baltic cod, Alaska Pollock, and Alaska pink salmon). The properties of fish gelatins can be improved by blending them with other polymers, particularly proteins, such as casein, chitosan, and polysaccharides (Acevedo et al., 2015). In another study, Eysturskarð et al. (2010) reported improvements in the properties of gelatin by using plasticizers and crosslinking agents. They used monosaccharides such as mannose, glucose, and fructose as plasticizers and ribose sugars as crosslinking agents. The ribose sugars enhanced the crosslinking with proteins upon mild heating by facilitating the Maillard reaction. Moreover, blending with other biopolymers, such as pectin

TABLE 5 Examples of modifying properties of fish gelatin films

Fish source	Method	Improved properties	Reference
A commercial cod fish	Addition of sugars (lactose)	Barrier properties of films	Etxabide et al. (2015)
Alaska pollock and salmon	Adjust drying temperature	Barrier and mechanical properties of films	Chiou et al. (2009)
Alaska pollock and salmon	Addition of crosslinkers (glutaraldehyde)	Mechanical properties of films	Chiou et al. (2008)
Atlantic salmon	High-pressure processing	Mechanical properties of films	Ojagh et al. (2011)
Baltic cod skins	Addition of transglutaminase or 1-ethyl-3 carbodiimide (EDC)	Water vapor permeability of films	Sztuka and Kołodziejska (2009)
Later calcarifer scales	Gamma irradiation	Mechanical and thermal properties of films	Perkasa et al. (2013)
Fish gelatin (granules)	Sugars (ribose and lactose) addition and ultraviolet (UV) radiation	Physical properties of films	Bhat and Karim (2014)
Warm-water fish	Addition of nanoclay	Mechanical and barrier properties of films	Bae et al. (2009)
Dry granules	Ultraviolet irradiation and sugars (ribose and lactose) addition	Lipid oxidation ability of films	Bhat and Karim (2014)
Cod, haddock, and pollock	Addition of lignosulfonate	Physical properties of films	Núñez-Flores et al. (2012)
Commercial grade fish gelatin	Eelectron beam irradiation	Physical properties of films	Benbettaïeb et al. (2016)
Water fish skin	Addition of gallic acid and glycerol	Antioxidant and mechanical properties of films	Limpisophon and Schleining (2017)

and κ-carrageenan, also improved the functional attributes of fish gelatin (Jeevithan et al., 2013; Takeungwongtrakul & Benjakul, 2017). In another report, Rahman et al. (2008) developed fish gelatin after adding κ-carrageenan and reported that the degree of turbidities was directly manipulated by the concentration of κ-carrageenan. Other factors affecting the degree of turbidities included the pH, type of salt added, and ionic strength of the salt. These biopolymers were generally used in a variety of applications as a thickener, stabilizer, fat substitute, taste releasing agent, and structural component. In food applications, mixing these biopolymers with fats, minerals, vitamins, and water have improved the physicochemical, functional, and nutritional properties. Moreover, optimization studies using various statistical designs and kinetic studies have found the precise mixture of biopolymers to show improvement in the physical properties of gelatin. When biopolymers or polyelectrolytes were mixed, it was expected that the system would be phase-separated. Oppositely charged polyelectrolytes would be joined together into a complex, while equally charged polyelectrolytes would be segregated into different phases (Pérez-Mateos et al., 2009).

Chitosan, obtained from the deacetylation of chitin, is also a widely available biopolymer that possesses excellent functional properties. It imparts bacteriostatic and fungistatic properties to the food systems. Hosseini et al. (2013) observed that chitosan was biocompatible, biodegradable, and nontoxic. Furthermore, it was shown that introducing different levels of chitosan into fish gelatin, in turn, influenced the functional attributes of the gelatin films produced. For instance, the addition of chitosan into fish gelatin for producing composite edible films reduced water vapor transmission. They reported that the gelatin film prepared by the addition of chitosan:gelatin ratio of 40:60 exhibited the lowest solubility and water vapor permeability, thus serving as a potential replacement for mammalian gelatin in some applications. Gelatin films were also used in the pharmaceutical industry for the manufacture of soft and hard capsules, which were used as containers for various drugs as well as a delivery tool through the gastrointestinal tract (Hanani et al., 2012). Hence, in these cases, the reduction of solubility and water vapor permeability was a desirable attribute. Furthermore, the addition of chitosan can inhibit the myofibril degradation during storage as revealed by Feng et al. (2016), who coated fish gelatin with chitosan and reported reduced myofibrillar degradation, improved functional quality, and hampered deterioration of the gelatin obtained from the fillet of golden pomfret during cold storage at 4°C for 17 days. For instance, MALDI-TOF-MS analysis revealed that the chitosan coating prevented the deterioration of skeletal muscle and meat sarcoplasmic proteins, including myoglobin, tropomyosin, and myosin light chains, in fish muscle. Lastly, the chitosan coating significantly inhibited the microbial population on the fish fillet during storage

at 4°C. The best results were obtained with 0.4% chitosan coating and 7.2% gelatin for preserving the quality of fish fillets during cold storage.

3.4 | Addition of crosslinking agents

Enhanced gel strength is required to achieve the optimum quality of fish gelatin. Crosslinking agents such as enzymes and salts can be used to improve gel strength. Particularly, transglutaminase and tyrosinase have been used to enhance the crosslinking and corresponding gel strength (Purnomo et al., 2003; Sims & Bailey, 1992). The mechanical properties and the minimum water permeability are important characteristics for the development of gelatin-based films, which can both be significantly affected by crosslinking chemicals. Synthetic chemicals, such as glutaraldehyde and calcium salts, as well as some biomaterials, such as phenolic compounds and organic acids, including tannic acid and ferulic acid, could improve the crosslinking abilities of the gelatin-based films (Bhat & Karim, 2014). In another study, Gómez-Estaca, Giménez, Montero et al. (2009) also reported that mechanical and water-barrier properties were improved by the addition of crosslinking agents, such as glutaraldehyde, transglutaminase, formaldehyde, glyoxal, ferulic acid, tannin acid, and genipin.

Another means to enhance the mechanical, functional, and sensory attributes of food products is through the addition of salts. Salts are an important ingredient in the production of gelatin, which have been associated with the improved quality and safety of gelatin, which affect the electrostatic interactions in food matrices. The electrostatic interactions play an important role in the gel strength and in the development of the structure and texture of fish gelatin. NaCl, in particular, has been predominantly used in gelatin production. However, with the growing concerns about NaCl reduction in meat products, a combination of other substituents has been reported. Other sodium salts, such as sodium acetate, sodium bicarbonate, and NaCl itself, could also be used in fish gelatin processing to solve the odor problem. Zhou and Regenstein (2007) tried sodium acetate and found that its use improved the flavor and prolonged the shelf life of fish muscle. Sodium bicarbonate had also been adopted for masking the typical aroma in meat from terrestrially farmed animals (Razzak et al., 2016). NaCl is traditionally added in the curing process as a preservative because it can modify the water-holding capacity of meat proteins to improve the quality and texture (Sow & Yang, 2015). In gelatin manufactured from salmon skin, the accompanied lipid and protein oxidation results in a fishy odor. Therefore, washing salmon skin gelatin with various salt solutions, including sodium acetate, sodium bicarbonate, and NaCl solutions, may alleviate this problem. Orrawan and Worapong (2012) reported that the quality of salmon skin gelatin was improved by the reduction of fishy odors using 0.5, 1.0, and 1.5% (w/v) sodium acetate, sodium bicarbonate, and NaCl, respectively. Although various methods such as active carbon absorption, yeast and lactobacillus fermentation, as well as the addition of salts have been used to remove the fishy odor of gelatin, the best sensory attributes have been obtained by the active carbon absorption method.

It is also reported that various salts can affect the melting enthalpies and gel strength of gelatins prepared from warm-blooded animals (Gómez-Estaca, Montero, Fernández-Martín et al., 2009). Karayannakidis and Zotos (2015) concluded that the appropriate concentrations of NaH_2PO_4, $MgCl_2$, $CaCl_2$, and glycerol modified the physical properties of gelatin from yellowfin tuna skin. The best results of the physical properties were obtained when gelatin was produced with the modification of NaH_2PO_4. The addition of sodium chloride (NaCl) posed negative effects on the physicochemical properties and nanostructure of fish gelatin. Sow and Yang (2015) added NaCl into fish gelatin and reported a reduction in gel strength and loss of textural quality. Instead of producing a rigid gel network, 1.5% NaCl altered the molecular order, decreased the number of helices, increased the number of random coils/disordered structures, and increased the formation of large aggregates. An appropriate concentration of salt is, therefore, crucial to induce the desired structural interactions in gelatin and incur appropriate modifications of its characteristics. Furthermore, the dissociation of NaCl into Na^+ and Cl^- ions affects the electrochemical properties of gelatin. Hence, an excess NaCl concentration hinders the formation of the triple-helix structure due to the reduced formation of H-bonds and increased gelation time.

Enzymatic crosslinkers as well as their combination with co-enhancers can also serve as important gel stabilizing agents. In an earlier publication, Eysturskarð, Haug, Ulset et al. (2009) revealed an improvement of gelling and melting points of gelatin prepared from the skin of yellowfin tuna by chemical and enzymatic modifications, which was superior to gelatin obtained from tilapia skin and mammalian sources. Among the chemical and enzymatic methods used, the addition of glutaraldehyde was the best method due to its high reactivity with amino groups and comparatively low cost. Nevertheless, toxicity remains a major limiting factor in improving the quality of fish gelatin (Gekko & Fukamizu, 1991). Microbial transglutaminase (MTGases) is another enzyme that incorporates a covalent crosslink between the amino acid residues (glutamine and lysine) through the catalysis of an acyl-transfer reaction. The introduction of crosslinking by MTGases

improves the stability and strength of fish gelatins, thereby improving their overall functional properties (Huang et al., 2017). Important factors that need to be controlled to obtain the desired thermal reversibility and gelling properties include the concentration of the MTGases, their incubation time, and their degree of heat-inactivation, which can be optimized by applying various statistical designs (Eysturskarð et al., 2010). In addition, the combination of MTGases with co-enhancers, such as $MgSO_4$ and sucrose, can also significantly improve the physicochemical properties of gelatin. The addition of such a combination not only increased the gelling and melting points of the fish gelatins but also made significant improvements in their gel strength (Gilsenan & Ross-Murphy, 2000). In another study, Simon et al. (2003) revealed another mechanism of incorporating crosslinking at the γ-position in glutamine residues using MTGases; it involved replacing the amide ammonia in the glutamine residues with another amine. The newly introduced amine was an ε-amino group from lysine residues. The introduction of ε-(γ-glutamyl) lysine isopeptide bonds caused the formation of inter- or intramolecular covalent crosslinks into the proteins. This incorporation of these bonds had been previously reported to improve the physicochemical and functional character of proteins in other muscle-based foods, such as sausages and tofu. Moreover, the MTGase reaction can increase the oxygen permeability of fish gelatin films. This enzyme also improves the tensile strength and melting enthalpies of fish gelatin films. However, the addition of MTGase results in a decreased elongation percentage of fish gelatin films. Norziah et al. (2009) also indicated that the transglutaminase enzyme significantly improved the gel strength of fish gelatin.

3.5 | Nonthermal technical methods

Nonthermal technical methods are the most promising prospects for consumer convenience (Mei et al., 2014). First, ultraviolet radiation technology (UVRT) has been reported in some scientific publications to modify the collagen solution, film, and fiber properties (Jaswir et al., 2011). As compared to ionizing radiation, UVRT is a weaker form of radiation with a lower penetration power but is easier to operate and is cheaper. This technique also has great potential for modifying the physical and mechanical properties of protein-based films. It can polymerize several monomers and amino acids in collagen and gelatin and thus introduce crosslinks (Bhat & Karim, 2009). UVRT produces radicals at the aromatic residues of collagen and gelatin, which then bind to each other through the formation of crosslinks (Benbettaïeb et al., 2016; Bhat & Karim, 2014). For example, the gel strength of dried fish gelatin has been improved after treatment with UVRT (253.7 nm, 30 W, for either 30 or 60 min). In addition, the viscosity of dried gelatin granules decreased. Finally, the melting temperature of fish gelatin has also been shown to change remarkably after the application of UVRT (Bae et al., 2009). The micro-textural and -structural changes brought about in gelatin makes UVRT a potential alternative method to improve the quality attributes of fish gelatin. It is worth mentioning that contrary to the high-energy gamma radiation, like ionizing radiation, which might compromise their potential applications for protein-based films, UVRT is safe to use even in edible films due to its low energy (Otoni et al., 2012). Moreover, Perkasa et al. (2013) also correlated the crosslinking density of fish skin gelatin with the treatment time of UVRT. Short-time UVRT led to intermolecular crosslinking of gelatin, while long-time UVRT acted on the breakage of gelatin molecular chains. These two opposite processes resulted in entirely different physicochemical properties. Despite the potential benefits of gelatin UVRT, only limited research has been reported. Bhat and Karim (2009) reported that the gelatin films subjected to UVRT (366 nm) improved tensile modulus values as compared to those of nonirradiated ones. Similarly, Zhou et al. (2006) reported that the porcine gelatin microcapsules subjected to irradiation treatment with UVRT (254 nm) increased their melting enthalpies and reduced their solubility in water. Commercial warm-water fish gelatin has high gel strength after UVRT, but low viscosity; the former is attributed to a greater degree of crosslinking, while the latter is attributed to greater chain scission after UVRT. Similarly, Otoni et al. (2012) reported that the treatment of cold- and warm-water fish gelatin samples with UVRT induced crosslinking, which resulted in the improvement of gel strength and viscosities of both these samples (Gs et al., 2020; He et al., 2021).

High-pressure processing (HPP) technology has accrued much attention from scientists and industry to preserve foods and modify their functional properties. HPP destabilizes the weak bonds of food systems, such as H-bonds, electrostatic bonds, van der Waals forces, and hydrophobic interactions but does not affect the covalent bonds of biopolymers, generally because of their low-energy levels (Shimada et al., 1996). HPP has also been used to improve the characteristics of thermally treated gels prepared from different protein sources such as ovalbumin, muscle fish protein, and muscle meat protein (Ojagh et al., 2011). Montero et al. (2002) reported on gelatin-based gels prepared from the skin of cod and megrim at two temperatures (20 and 7°C) treated with 200, 300, and 400 MPa and subsequent cooling at 7°C for 16–18 h. In cod gelatins, the turbidity declined, while the gel strength improved with the increasing pressure levels. Furthermore, Davies et al. (2016) also applied HPP on a milk–gelatin mix-

ture and optimized their rheology and microstructure by improving the degree of aggregation, altering various levels of milk/gelatin, and perfecting the proper pressure and temperature of treatment.

3.6 | Others

The benefits of multiple technologies and additives can be integrated in the preparation of gelatin. Núñez-Flores et al. (2013) utilized three co-enhancers, namely $MgSO_4$ (as the electrolyte), sucrose (a nonelectrolyte), and transglutaminase (the enzyme), in their study and reported that the active modes of these co-enhancers were different in their characteristics to alter the functional properties of gelatin. Moreover, some antioxidants, such as butylated hydroxytoluene (BHT) and tocopherol, were added to improve the ability of fish gelatin to form films. For instance, Nilsuwan et al. (2016) evaluated the extracts of borage seeds and leaves for their polyphenol contents and antioxidant activities. Nevertheless, they also noted a decrease in the breaking force of these films as a negative property. In conclusion, the extracts of borage seeds and leaves exhibited higher antioxidant capacity in edible gelatin films than those of BHT and tocopherol. In another study, the addition of lignin to gelatin caused a certain microphase separation as reflected by structural analysis, which inhibited the interaction among the gelatin molecules (Luccia et al., 2005). Furthermore, Shyni et al. (2014) presented that the mechanical properties of gelatin-based gels were improved by eliminating the molecules with low MW. However, they also showed that the pH levels close to the isoelectric point of gelatin did not affect its mechanical properties. By contrast, in another study, Eysturskarð et al. (2010) reported a direct link of low MW with the mechanical properties of gelatin gels. More importantly, they reported a strong association of the mechanical properties with the fractions of α- and β-chains, as well as the presence of the molecules with high MW. They also positively correlated the bloom value with the α- and β-chains, as well as with the macromolecules in mammalian gelatin. Furthermore, positive correlations were also reported for the dynamic storage modulus with the fractions of the β-chains and macromolecules in the gelatin produced from cold-water fish. In agreement with the previously cited study, the fraction of small molecules exhibited a negative correlation with the mechanical properties of mammalian gelatin and a negative correlation of the dynamic storage modulus with the fractions of α-chains and small molecules in the gelatin from cold-water fish.

The odor problem associated with fish gelatin requires improved processing methods and the use of food additives. Optimization strategies have been developed by utilizing co-enhancers during the manufacturing process to reduce the off-odor and improve the desired functional and rheological attributes of fish gelatin. Pretreatments with citric and acetic acids have also been used to eliminate the off-odor of fish skin gelatin. After treatment with citric acid, the fish skin gelatin exhibited a reduced fishy odor as compared to that treated with acetic acid (Hosseini et al., 2013). The use of citric and acetic acids was associated with increased binding sites for reactive volatile compounds in gelatin, which ultimately resulted in the unavailability of these compounds for oxidation. This processing method could also influence the off-odor produced during the manufacture of gelatin. Compared with fish gelatin produced using freeze-drying, fish gelatin produced by spray drying has lower aldehydes, ketones, and alcohols, and a less fishy smell (Monsur et al., 2014). In addition, the lower TBA reactive substances (TBARS) and peroxide values in the spray-dried gelatin indicated reduced availability of volatile compounds for oxidation, thus enhancing the quality and safety of fish gelatins. Hurdle technology, that is, the combination of two or more technologies to enhance the safety and quality of products, can be used to control the production of off-odor and make the off-odor-producing volatile compounds unavailable. For instance, spray-drying along with appropriate pretreatments effectively reduced the production of fishy odor and flavor in fish gelatins (Sae-Leaw et al., 2016).

4 | TECHNO-FUNCTIONAL GAP ANALYSIS AND FUTURE DIRECTIONS

4.1 | Techno-functional gap analysis

According to a report by the FAO, the present worldwide production of fish is ~179 million metric tons, out of which ~156 million metric tons are available for human consumption (Oliveira et al., 2020). However, more than 25% is wasted (unutilized) every year in terms of the recoverable fish scale, fin, bone, and skin (Gómez-Guillén et al., 2011). Therefore, efforts are being made to manufacture gelatin from fish waste, though to date only 1.5% of the total gelatin production worldwide is derived from fish. This exciting market opportunity has been comprehensively explored, yet several technological gaps leave it unripe to obtain maximum benefit. These gaps include the techno-functional gaps related to gelatin production, quality, and analysis as compared to traditional gelatin acquired from bovine sources. Thermostability, unacceptable color, traceability, yield, and bloom strength have been addressed by researchers, but their implementation for fish gelatin is limited.

Thermostability of fish gelatin is weaker than that of traditional gelatin. This has been attributed to the amino acid profile of collagen, which varies between fishes depending on their habitat. Particularly, the proline, hydroxyproline, and glycine content in cold-water fishes varies from that in mammalian collagen. The functionality of fish gelatin is severely compromised because of low thermostability; hence, efforts are needed to improve it. Of interest, a study has been published that focused on improving the thermostability of porcine gelatin to improve the gel strength at different temperatures (Gómez-Guillén et al., 2011). The addition of transglutaminase in gelatin altered its thermostability by increasing the crosslinking degree under acidic conditions at high temperatures. The pH was initially adjusted to 6 for crosslinking purposes for 1 to 4 h, and then further adjusted to 5 (Du et al., 2021). Crosslinking facilitated the formation of covalent bonds between gelatin fragments, increasing the thermostability of the gelatin (Du et al., 2021). Moreover, the inclusion of anionic gums, such as Arabic, xanthan, and tragacanth gums, in gelatin increased the intermolecular crosslinking via electrostatic interactions. By contrast, the incorporation of nonionic gums increased the intermolecular crosslinking by modifying the gel viscosity. Such modification, as well as the increase of electrostatic interactions, reduced the fluidity of the gels, thus increasing the thermostability of fish gelatin. The cause of viscosity modification was attributed to the polymer interaction effect. The anionic groups of tragacanth and Arabic gums formed interactions with the cationic groups of gelatin, resulting in the formation of a stable network. Likewise, guar gum created a pseudo-junction zone in fish gelatin through intermolecular entanglement due to the disruption of the latter's helical assembly by reorganizing the water molecules in its structure. Xanthan gum imparted void filling and viscosity modification of fish gelatin for the improvement of the thermostability (Binsi et al., 2017). The modification of the moisture content through high-temperature treatment (135–140°C) promoted the formation of a polymer network in fish gelatin that altered the equilibrium state of fibrillar and globular proteins, as well as intermediates to increase the thermostability of fish gelatin (Iakubova et al., 2021).

The fishy odor and compromised color are serious defects of fish gelatin that decrease consumer acceptance. Clarity is an important aspect of high-grade gelatin to broaden its applicability. Gelatin is generally colorless and has a degree of whiteness close to 100%. The color of fish gelatin is regulated by different factors, including the type and amount of raw materials, fish species, as well as the methods and conditions of extraction (Siburian et al., 2020). In this regard, the treatment of gelatin with NaCl and 0–1.0% w/v activated carbon proved to be effective in removing the fishy odor and color of fish gelatin. The maximum clarity (93.3%) of color was obtained with the combination of 0.5% NaCl and activated carbon (Tinrat & Sila-Asna, 2017). In addition, 1.0–3.0% w/v β-cyclodextrin, 0.1–0.5% w/v diatomaceous earth, and 0.5–2.0% w/v powdered activated carbon were used at mild temperatures (30–50°C) for 10–60 min in the gelatin derived from tilapia *(Oreochromis niloticus)* skin. The optimized conditions increased the clarity whereby the fish gelatin turned colorless from the initially yellowish color (Zhang et al., 2017). Furthermore, the issue of a fishy odor of fish gelatin has been addressed by ensuring adequate filtration or by pretreatment with acid, alkali, or salt, including either H_2SO_4, citric acid, NaOH, or NaCl at the concentration of 2, 1, 2, and 15 g/L, respectively (Tohmadlae et al., 2019).

Traceability is a serious gap in gelatin production, not only from fish sources, but also traditional sources such as bovine, porcine, and others. Moreover, the use of bovine carrying prion proteins in fish gelatin has been linked to BSE-related problems that can cause fatal neurodegenerative disease (Hameed et al., 2018). Therefore, traceability of the collagen source is imperative; however, there is still a lack of effective traceability strategies for gelatin (Jiang et al., 2020). Recently, multielements and stable isotope methods were used to distinguish the gelatin samples. The element traceability method showed higher accuracy as compared to the stable isotope method because of the successful validation of many traceability indicators. Moreover, as the element traceability method is more convenient, results could be obtained quicker than by using the isotope traceability method. Furthermore, traceability systems involve acquiring information from various suppliers of the raw materials used in a production process. Since fish collagen is present in minute quantities, acquiring the information from various sources can be problematic.

The functionality of fish gelatin can be improved by the methods adopted in mammalian gelatin manufacturing. Particularly, for swift adoption, the structural attributes of fish gelatin, which affect its physicochemical traits, need to be clearly understood through effective analytical methods. Various analytical methods can be used for evaluation of the protein nanostructure of gelatin including Raman spectroscopy, circular dichroism spectroscopy, high-performance liquid chromatography, gas chromatography, size-exclusion chromatography multiangle laser light scattering, gel electrophoretic analysis, rheometer, spectrophotometer, and Fourier transform infrared spectroscopy. Additionally, the scanning electron microscope (SEM) and transmission electron microscopy (TEM) methods are two commonly used methods to observe the configuration of gelatin. However, complex pretreatments applied to the gelatin samples before SEM/TEM investigation often interfere with the results related to the structure of gelatin.

The fundamental quality determinants of fish gelatin are the gel strength or bloom value (70–270 bloom), gelling point (8–25°C), and melting temperatures (11–28°C), which play important roles in its gelling potential (Huang et al., 2019). The underrepresented use of fish gelatin is primarily associated with its inferior gelling strength compared to that of bovine gelatin. The gelling ability is dependent on the chemical profile of the fish source and the environmental conditions, such as the temperature of the habitats. Moreover, amino acid content, MW, and the configuration of the α- and β-chains also affect the gelling potential of fish gelatin (Karayannakidis & Zotos, 2016). The gelatin extracted from cold-water fish exhibits a gel strength ranging between 119.6 and 273.0 g, which is significantly lower than that obtained from warm-water fish (293.2–466.4 g), due to the low content of hydroxyproline (Nitsuwat et al., 2021). By contrast, mammalian gelatin possesses the highest gelling potential (100–300 bloom) that enables a more extensive scope of applications than fish gelatin. In this regard, various strategies have been utilized to increase the gelling strength of fish gelatin, including enzymatic modification (use of laccase, tyrosinase, and MTGase); chemical modification (Huang et al., 2019); phosphorylation by the application of phosphorus oxychloride, phosphokinase, sodium tripolyphosphate, and trisodium trimetaphosphate (Xiong et al., 2016); induction of crosslinking through aldehyde modification by introduction of covalent stable amide bonds between gelatin chains (Padrão et al., 2014); phenolic modification using ferulic acid, caffeic acid, tannic acid, gallic acid, and rutin to facilitate hydrophobic interactions among the aromatic rings and hydrophobic side chains of phenols and fish gelatin, respectively; physical modification with electrolytic or nonelectrolytic substances including salts like $CaCl_2$, $MgCl_2$, and NaH_2PO_4; and lastly, by mechanical treatments, that is, HPP, drying, irradiation, and ultrasound (Wu et al., 2015).

Fish gelatin has a lower yield (6–19%) than the gelatin acquired from mammalian origin (Muyonga et al., 2004). The gelatin obtained from Spanish mackerel *(Scomberromorus commersoni)* and Nile tilapia had 13.03% (Kusumaningrum et al., 2018) and 12.1% yield (Martins et al., 2018), respectively. A lower yield is obtained from fish scales than from fish skin due to their dense structure and less collagen content. The variation in gelatin content is attributed to numerous factors, including the collagen content of the raw material, extraction method, and conditions (time, temperature, pH, ionic strength, acid type, and pretreatment time). Sufficient extraction and pretreatment times aid in the demineralization of scales, leading to efficient gelatin extraction and improved yields (Feng et al., 2015). Moreover, collagen solubilization and swelling under acidic conditions during extraction also contribute to high yield (Martins et al., 2018). By contrast, low yield is related to gelatin loss through leaching during washing, indicating incomplete hydrolysis of the collagen (Kusumaningrum et al., 2018). Common gelatin extraction methods such as microwave extraction, sonication, superheated steam extraction, and water bath treatment have different extraction rates. For instance, Kim et al. (2020) obtained the highest gelatin powder yield using the superheated steam extraction method. One of the most utilized approaches to enhance the obtained gelatin content of fish is the use of enzymes during extraction. Enzymes, such as alkaline protease obtained from *Bacillus licheniformis*, result in a significantly increased yield of 13–46% when used at doses of 5–20 U/g. Protease influences the extraction rate by efficient hydrolysis of the covalent bonds, as well as by disrupting the crosslinks between the molecules of fish collagen (Kouhdasht et al., 2018). Other methods, including nonenzymatic reactions and the addition of ascorbic acid and fructose, resulted in a yield of $13.2 \pm 2.3\%$ (Guerrero et al., 2020).

4.2 | Recommendations

Despite the tremendous potential of converting large quantities of fish waste into fish gelatin, this potential market is lagging because of its issues in thermostability, the fishy odor and clarity, traceability, structural attributes, bloom strength, melting temperatures, and the yield of gelatin obtained from fish origin. Based on the discussion of these attributes in the review, the following recommendations can be useful:

1. The thermostability of fish gelatin should be enhanced through the improvement of intermolecular crosslinking, the adjustment of viscosity and intermolecular entanglement, the void-filling abilities of enzymes and natural gums, and by high-temperature treatments.
2. The fishy odor should be controlled, and the color of fish gelatin should be decolorized with the help of β-cyclodextrin, activated carbon, NaCl, and diatomaceous earth, which will improve the consumer acceptability of fish gelatin.
3. New methods for tracing the origin, source, and fate of raw materials and gelatin should be developed and validated. Thus far, the least amount of work has been published related to traceability systems. Stable isotope-based traceability methods are recommended, which can improve the traceability of suppliers of fish raw materials from various sources and geographical locations.
4. The quality characteristics of fish gelatin need to be tested using microscopic, spectroscopic, chromatographic, rheological, and other novel analytical

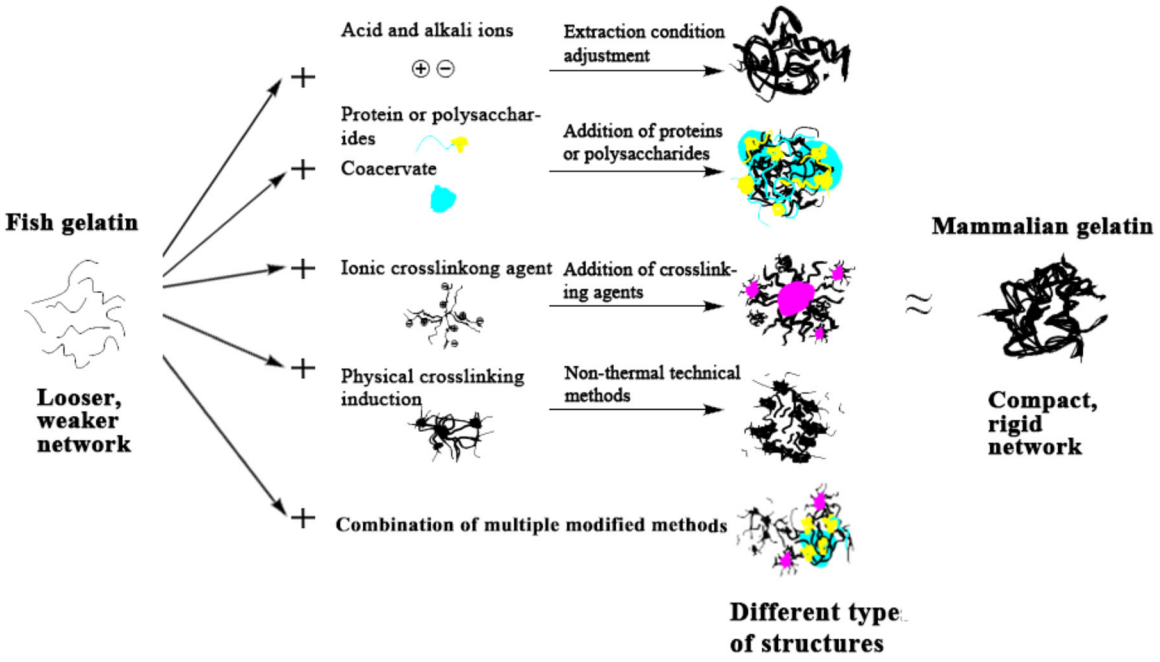

FIGURE 1 Narrowing the technofunctional gap between fish gelatin and mammalian gelatin (adapted from Sow et al., 2019 and Lin et al., 2017)

methods for guiding the optimization of its structural attributes, thus enhancing their market acceptability.

5 | CONCLUSION

Mammalian gelatin is currently leading the whole gelatin market; however, the commercial interest for fish gelatin is growing considerably. The reason for the shift is concerns related to the underutilization of waste generated from the fish-processing industry, as well as due to religious and cultural objections. Studies conducted on gelatin have demonstrated that there are clear connections between the functional properties and intrinsic qualities of gelatin with the materials, sources, and/or extraction conditions used during gelatin manufacture. This review discussed various methods for improving the physicochemical and functional attributes of fish gelatin and made suggestions for the optimum conditions (i.e., adjusting the gelling temperature from 13–19°C to 23–25°C, gel strength from ~200 to 250 g, and melting point from ~25 to 30°C). Using the latest production methods and analytical techniques discussed in this study, shown in Figure 1, fish gelatin production could be accelerated in the near future, thus enhancing its competitive market share along with its practical application.

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AUTHOR CONTRIBUTIONS

Huijuan Yang: Conceptualization; Resources. **Haifeng Wang**: Investigation. **Guangtian Cao**: Formal analysis. **Fei Tao**: Formal analysis. **Guanghong Zhou**: Project administration. **Qing Shen**: Writing – review & editing. **Hongshun Yang**: Software

CONFLICT OF INTEREST

The authors declare no competing financial interest.

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