CHAPTER 5

Fish Gelatin

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Contents

Abstract

Gelatin is a multifunctional ingredient used in foods, pharmaceuticals, cosmetics, and photographic films as a gelling agent, stabilizer, thickener, emulsifier, and film former. As a thermoreversible hydrocolloid with a narrower gap between its melting and gelling temperatures, both of which are below human body temperature,

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gelatin provides unique advantages over carbohydrate-based gelling agents. Gelatin is mostly produced from pig skin, and cattle hides and bones. Some alternative raw materials have recently gained attention from both researchers and the industry not just because they overcome religious concerns shared by Jews and Muslims but also because they provide, in some cases, technological advantages over mammalian gelatins. Fish skins from a number of fish species are among the other sources that have been comprehensively studied as sources for gelatin production. Fish skins have a significant potential for the production of high-quality gelatin with different melting and gelling temperatures over a much wider range than mammalian gelatins, yet still have a sufficiently high gel strength and viscosity. Gelatin quality is industrially determined by gel strength, viscosity, melting or gelling temperatures, the water content, and microbiological safety. For gelatin manufacturers, yield from a particular raw material is also important. Recent experimental studies have shown that these quality parameters vary greatly depending on the biochemical characteristics of the raw materials, the manufacturing processes applied, and the experimental settings used for quality control tests. In this review, the gelatin quality achieved from different fish species is reviewed along with the experimental procedures used to determine gelatin quality. In addition, the chemical structure of collagen and gelatin, the collagen–gelatin conversion, the gelation process, and the gelatin market are discussed.

I. GENERAL INFORMATION ON GELATIN

Gelatin is a term used for a class of protein fractions that have no existence in nature. Gelatin is derived from collagen, which is a natural structural protein, predominantly found in the connective tissues of animals (Balian and Bowes, 1977; Belitz and Schieberle, 2004; DeMan, 1999) although also found in many other tissues. Collagen is the most ubiquitous of animal proteins. Gelatin is one of the most widely used biopolymers and is added to foods, drugs, cosmetics, photographic films, and other products, including paints, matches, and fertilizers, as a gelling agent, foam stabilizer, and structure enhancer (Gudmundsson, 2002; Karim and Bahat, 2009; Yang *et al.*, 2007; Zhou and Regenstein, 2004). Gelatin is able to form a high viscosity solution in warm water, which sets to a gel on cooling. The chemical composition of gelatin is, in many respects, similar to that of collagen, its parent molecule. Gelatin is, however, not composed of one size of collagen fraction or peptide chain but is a combination of many fractions varying in size, including the whole α-chain of the tropocollagen molecule (a trimer of around 330 kDa that aggregates to form the larger collagen structures) and hydrolytic fragments of parts of the

α-chains of different lengths (Eastoe and Leach, 1977). Gelatin gels have relatively lower melting temperatures compared to the gels of other gelling agents (Williams, 2007). Gelatin gels generally have a melting temperature below 35 °C, that is, below human body temperature, which makes gelatin unique in terms of its sensory aspects, especially flavor release, which is particularly desired for some food applications (Baziwane and He, 2003; Boran and Regenstein, 2009; Choi and Regenstein, 2000). Other gelling agents such as starch, alginate, pectin, and agar are carbohydrates and their gels cannot melt below body temperature as most have much higher melting temperatures (Williams, 2007).

Gelatin is obtained from the skins and bones of pigs and cattle, but mostly from pig skin. However, there are alternative raw materials that can be used in gelatin manufacturing, including by-products from the chicken and fish processing industries. Fish skins have received attention from researchers as an alternative raw material having the potential for the production of large amounts of high-quality gelatin. Therefore, recent studies with fish skin gelatin have focused on the evaluation of different fish species as an alternative raw material for gelatin production and the quality of the extracted gelatins in comparison with commercial gelatins from conventional sources (Boran and Regenstein, 2009).

In this review, gelatin production and processing, the raw materials used in gelatin production, the technological attributes of gelatin, the gelatin market, and the market specifically for fish gelatin are discussed. In addition, the most critical factors affecting the quality of gelatin are discussed. For this purpose, the chemical structure of collagen is reviewed in detail to take a closer look at the possible factors affecting the properties of the resultant gelatin. The conversion process of collagen into gelatin and the gelation mechanism are discussed to show which driving forces are involved in gelation, which factors might affect the solution–gel and gel–solution transitions, and how extraction conditions might affect the final product, gelatin. The methods being currently used to determine the quality of gelatin are also reviewed.

A. The parent molecule: Collagen

Collagen is the most abundant protein in the animal body (DeMan, 1999). Collagen is part of the connective tissue in muscles and many other organs, including the skin, bones, teeth, and tendons. Collagen fibrils normally have a regular periodicity of 64 nm when stained for microscopy, which can be increased to 400 nm with *r* tension (DeMan, 1999). Collagen molecules are arranged head-to-tail, with a 35-nm gap between molecules, and are found in larger structures having staggered bundles, that is, adjacent collagen molecules are not aligned with each other. Charged and uncharged residues are found to be periodically clustered

along the sequence of collagen at about every 230 residues, which is around 64 nm, although this distance may vary somewhat among different tissue sources of collagen. This suggests that the collagen molecules are aligned such that the maximum electrostatic and hydrophobic interactions occur between different molecules (Fig. 5.1).

Collagen constitutes 20–25% of the total protein in mammals and has a unique amino acid composition, which includes two modified amino (imino) acids, hydroxyproline and hydroxylysine (Belitz *et al.*, 2004). Its molecular structure is mainly the multiple repetition of a ''Glycine-X-Y'' sequence, where ''X'' is often proline and ''Y'' is often hydroxyproline. Collagen has a unique triple helix structure that is based on a special helix of three polypeptide chains with high levels of imino acids. Each polypeptide chain is left handed and has three amino acids per turn. These three polypeptide chains, called α-chains, are supertwisted around one another and form a superhelix that is right handed (Nelson and Cox, 2005). The basic structural unit of the collagen superhelix is called tropocollagen. It has a molecular weight of approximately 330 kDa, with a length of approximately 300 nm and a diameter of 1.5 nm (Belitz *et al.*, 2004).

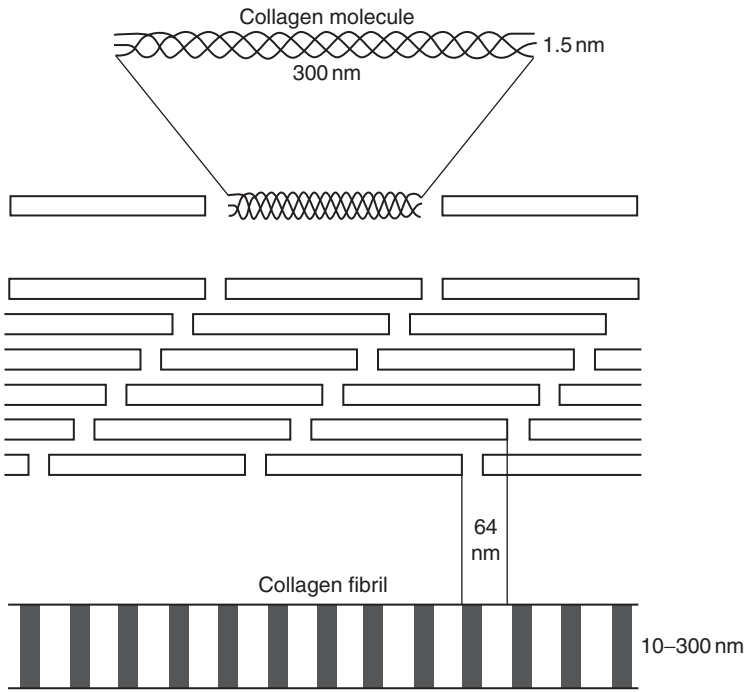

FIGURE 5.1 Schematic representations of collagen molecules and a collagen fibril.

When hydrolyzed, the collagen can give three different fractions: independent α-chains, which may have slightly different molecular weights, a β-chain, that is, two α-chains linked to each other by covalent bonds, and a γ-chain, that is, the three α-chains linked to one another by covalent bonds. These fractions differ in molecular size: α-chains correspond to a molecular weight of 80–125 kDa, β-chains correspond to a molecular weight of 160–250 kDa, and γ-chains correspond to a molecular weight of 240–375 kDa, which is very similar to the molecular size of collagen (Imeson, 1997).

Collagen typically contains about 35% glycine, 11% alanine, and 21% proline and hydroxyproline, the amount of which varies somewhat among different species although the high content of proline and hydroxyproline seems to be characteristics of collagen structure regardless of the source (Balian and Bowes, 1977). Hydroxyproline is a rare amino acid that is absent in nearly all other proteins and so its presence can be used to determine the presence of collagen in the presence of other proteins, and with proper calibration (i.e., the conversion factor is at a minimum species specific), the amount of collagen or gelatin (Engel and Bachinger, 2005) in the mixture. Another protein containing hydroxyproline is elastin, but the amount of hydroxyproline in elastin is very low and the amount of elastin in most tissues is also very low when compared to that of collagen (Nelson and Cox, 2005).

Collagen is generally considered to be an incomplete protein since the concentration of some essential amino acids is low in collagen and consequently, in gelatin (Belitz *et al.*, 2004; Nelson and Cox, 2005). However, when eaten as part of a meal, the contribution of gelatin to the amino acid ingestion needs to be considered.

The amounts of the aromatic and sulfur-containing amino acids are low (0–0.6%) in collagen, that is, tryptophan and cysteine are mostly absent in collagen (Balian and Bowes, 1977). Cysteine is usually absent in collagen, therefore, there are usually no disulfide bonds involved in collagen structure although there are some collagens that have cysteine (Engel and Bachinger, 2005). For those, disulfide bonds are also involved in the formation of intermolecular cross-links (disulfide bonds) and provide additional stabilizing effects for the structure. The structure of collagen provides an explanation of why glycine is the most abundant amino acid and why proline and hydroxyproline are found so often in collagen. Only glycine residues, as the smallest of the hydrophobic amino acids, can fit into the very tight central core between the individual α-chains, while proline and hydroxyproline residues permit sharp twists of the collagen helix allowing for the relatively low three amino acids per turn (Nelson and Cox, 2005).

The collagen molecule is primarily stabilized by hydrogen bonds between the backbone amino group of glycine and the backbone carboxyl

group of a residue in the X position of a neighboring α-chain, which is usually a proline. Proline in the Y position is generally hydroxylated posttranslation into hydroxyproline, which also plays an important role in the formation of intra- and intermolecular hydrogen bonds. Hydroxyproline is, therefore, important for both the structure of the collagen molecule and of the collagen fibrils (Brinckmann, 2005).

During maturation or aging of the living animal, collagen fibers strengthen and are further stabilized primarily by complex covalent bonds. Lysine, hydroxylysine, and histidine residues are heavily involved in the formation of these covalent bonds, that is, aldimine bonds between lysine and lysine or hydroxylysine (Balian and Bowes, 1977; Belitz *et al.*, 2004; Engel and Bachinger, 2005; Eyre and Wu, 2005; Nelson and Cox, 2005) that lead to the formation of desmosine and isodesmosine, which are unusual in that they involve the participation of four amino acids in the reaction. This is possible because of the rigid geometry of the collagen structures and the long times available for these reactions to occur.

All the fibril-forming collagen types (type I, II, III, V, XI, XXIV, and XXVII collagens) are cross-linked through a mechanism based on the reactions of aldehydes derived from some lysine (or hydroxylysine) side chains. Histidine might also participate in the formation of a trivalent cross-link by reacting with an aldimine bond formed between a lysine aldehyde and hydroxylysine residue.

With respect to tissue source, type I collagen is the most widely occurring collagen found in skin, tendon, bone, cornea, lung, and the vasculature, while type II collagen has a more specific tissue distribution being limited essentially to cartilage, while type III is found in relatively elastic tissues such as embryonic skin, lung, and blood vessels (Hulmes, 2008). For most nonfibrillar collagens (e.g., type IV, VI, and VII collagens), disulfide bonds may be the only source of intra- and intermolecular covalent bonds. There are usually no lysine-mediated cross-links in these collagens (Eyre and Wu, 2005). The best known nonfibrillar collagen is type IV collagen, which is a basement membrane collagen that forms specialized structures found at tissue boundaries, and in fat, muscle, and nerve cells. Collagen VI, on the other hand, is important in maintaining tissue integrity (Hulmes, 2008).

B. Collagen–gelatin conversion

There are several methods used by the industry to manufacture gelatin from collagen. The main purpose of the gelatin production process is to first remove unwanted materials that will interfere with the gelatin extraction and then to convert collagen that is insoluble in water into gelatin that is soluble in water, while obtaining maximum yield and superior functional properties (Hinterwaldner, 1977). In general, gelatin

is obtained using a sequence of three types of processing steps: pretreatments to remove noncollagen impurities and prepare the collagen for extraction, one or more water extraction steps to convert collagen into gelatin, and finally, a series of refinement and recovery processes to get a highly purified dried gelatin. A limited amount of gelatin has been marketed in liquid form to avoid the drying step (J.M. Regenstein, personal communication).

In the first step, raw materials are water washed to remove obvious impurities and then treated with alkali and/or acid to weaken the collagen structure by breaking intramolecular cross-linkages including covalent and hydrogen bonds and to release other impurities. Some size reduction may also be applied to increase the efficiency of the process. In the second step, the actual water extraction is performed at warm temperatures for an appropriate period of time. In the last step, extracted gelatin is subjected to several separation methods, including filtration, evaporation, and deionization, followed by drying and grinding (Hinterwaldner, 1977).

Gelatins are classified according to whether an acid or an alkali is used in the final preextraction step. If an acid solution is used as the final solvent, type-A gelatin (acid process) is obtained. In case of alkali as the final solvent, type-B gelatin (alkali process) is obtained (Hinterwaldner, 1977). Type-A gelatin's isoelectric point is higher compared to that of type-B gelatin, as a milder acid process does not remove the amide nitrogen of glutamine and asparagine, therefore, the resulting gelatin's isoelectric point might be as high as 9.4. If a more severe acid treatment is required, then some of the amide groups are hydrolyzed and the isoelectric point would be similar to that of the original collagen molecule, which generally lies between 6 and 8. Type-B gelatin's isoelectric point might be as low as 4.8, as the alkali process results in the loss of the amide groups (Eastoe and Leach, 1977).

In the acid process, the bones and skins are treated in a vessel containing a dilute solution of acid for a predetermined period of time. Then, the acid is washed out with cold water. In the alkali process, the demineralized bones (demineralization is mostly done with acid solutions to remove calcium and other salts from the bone to prepare the collagen-rich bone material known as ossein) are placed in liming pits and soaked in a lime suspension for longer than 60 days. For the hides or skins, a caustic soda solution is used for a shorter period of time. After this treatment, the raw material is washed thoroughly to remove any residual lime. The acid pretreatment is mostly used for skin, while the alkali pretreatment is mostly used for bones (Petersen and Yates, 1977).

The actual extraction method used for both acid and alkali pretreated raw materials is similar. The main extraction step is done using hot water at controlled temperatures, mostly higher than 40 °C, and it is the most

important step in gelatin production. In the industry, the extraction step is usually multiple extractions performed with gradually increasing temperatures beginning from 50 to 60 °C and going up to the boiling temperatures, usually in 5–10 °C temperature increment. Gelatins are collected so that the lower temperature fractions have minimal degradation and the higher temperature fractions have more variable molecular weights (Hinterwaldner, 1977). The dilute gelatin solution from the extraction process is clarified using lamellar separators (this equipment is built as a set of plates or discs that are arranged at such an angle that the solids can slide off into the sludge chamber, thus achieving clarification) and filtered using self-cleaning centrifugal filters or cellulosic filters. After that, gelatin solutions are deionized by passing through ion exchangers and concentrated, usually in a multiple effect vacuum evaporator. The concentrated solution is then sterilized by hot air in batch driers, and then cooled or chilled to rapidly form a gel. Then, the gel obtained is extruded to get gelatin noodles (i.e., continuous strands), followed by a final drying and grinding process. After all these treatments, gelatin granules or powder are obtained. Acid or alkali pretreatments designed to destroy or weaken cross-linkages between the α-chains or between tropocollagen molecules need to be adjusted not only in terms of concentration but also treatment time to avoid extensive degradation of collagen, which might result in lower quality gelatin. But at the same time, enough degradation is needed to be able to get a higher yield and acceptable gel strength (Holzer, 1996).

C. The mechanism of gelation

Hydrogen bonds certainly play an important role in gelation (Johns and Courts, 1977). Gelation can be considered as gelatin regaining its collagen structure, but this would not be exactly correct, because the conversion of collagen into gelatin is an irreversible process although gelatin can partially regain some collagen structure by recovering some cross-linkages. The greater the amount of cross-linkages recovered, the higher the gel strength and viscosity along with the melting and gelling temperatures (Belitz *et al.*, 2004). The concentration of α-chains and the cooling rate are the most important factors affecting the final gelation. At high gelatin concentrations intermolecular bond formation would occur with multiple strands, while the same process is more likely to occur as intramolecular bonds within a single strand at low concentrations. Similarly, slow rates of cooling allow more intra- and intermolecular cross-link formation, while rapid cooling does not allow that to happen (Belitz *et al.*, 2004).

D. The characteristics of gelatin

Gelatin is a gelling agent that is able to form thermoreversible gels, which means that when the gelatin gel is heated, it softens and again turns into a liquid. Then, it is able to return to the gel form when the solution is cooled again (Stainsby, 1977). Being able to melt below human body temperature makes its use very favorable in the food industry since gelatin is able to melt and release flavor when it is taken into the mouth, which is desired in terms of the sensorial properties of food products (Choi and Regenstein, 2000). Another important characteristic of gelatin is that its gel strength is relatively higher than most of the common gelling agents, which are usually carbohydrates and obtained from vegetable sources (Badii and Howell, 2006). The gap between melting and gelling temperature of gelatin gels is smaller than that of other gelling agents, which is desired for some particular applications, that is, food applications including jellies and custards (Jones, 1977).

E. Sources of raw material used in gelatin manufacturing

Pork skin is the most abundantly used raw material in gelatin production. About 45% of the world's total gelatin production is obtained from pork skin, followed by bovine hides with almost 30% (Karim and Bahat, 2009), and 23% of gelatin is obtained from bovine and porcine bones. Other sources include chicken and fish, but these account for only 1.5% of the world's annual gelatin production. In Europe, pork skin is the most abundantly used raw material for gelatin production, accounting for around 80% of the total, followed by cattle skin with 15% of the total gelatin production. The remaining 5% is from pork and cattle bones, fish, and chicken.

Recent studies have shown that fish skin, especially, might be an alternative source for gelatin production. Fish skin gelatins may provide better alternatives for some applications because of their, at times, relatively lower gel strengths and melting temperatures compared to that of mammalian gelatins. These characteristics may be desired in some food systems for ease of flavor release, leading to better sensory characteristics (Boran and Regenstein, 2009; Choi and Regenstein, 2000). In addition, obtaining valuable by-products from the fishery industry and reducing waste have made it an attractive research topic. As the issues of sustainability and the better use of harvested resources become more critical, the need to use fish waste more productively will only increase. Many fish species have been investigated as a raw material for gelatin extraction and the properties of the gelatins obtained from these sources have also been reported.

The waste from fish processing after filleting can account for as much as 75% of the total catch weight (Shahidi, 1995). It includes the heads, skin, and scales, guts/internal organs, frames (bone rack with adhering meat), and trim (pieces cut from the fillets during processing) (Regenstein, 2004). About 30% of such waste consists of skin, bone, and scale with high collagen content that could be used to produce collagen and gelatin (Gómez-Guillén *et al.*, 2002; Sadowska *et al.*, 2003; Young and Lorimer, 1960). Very little work has been done on fish bone gelatin (Muyonga *et al.*, 2004), while a little more work has been done on scale gelatin. Fish scales gelatin has been obtained from sardine (Harada *et al.*, 2007), Asian carp (Wang and Regenstein, 2009), and lizardfish (Wangtueai and Noomhorm, 2009).

F. The gelatin market

The world's total gelatin production is close to 350,000 tons annually, accounting for the market's economic value being over US$ 2 billion. Again, gelatin is used in many products, including foods, pharmaceuticals, cosmetics, photographic films, paints, fertilizers, and many more, as a gelling agent, stabilizer, and structure enhancer (Jones, 1977).

G. Industrial applications of gelatin

Gelatin's largest single use is in food products, more specifically, water gel desserts. Gelatin desserts might consist of several other gelling agents (i.e., agar, carrageenan) along with gelatin; however, gelatin gels usually melt at temperatures that are lower than the body temperature, which makes gelatin favorable compared to other gelling agents in food applications. In some parts of the world, water dessert gels are made from carrageenan and these have to be chewed as they do not melt in the mouth. Thus, though both are called water dessert gels, they have very different sensory properties.

Gelatin is used in dairy products as a stabilizer and as an ingredient to modify the texture. It is used in yogurt, ice cream, and other dairy products. Gelatin is added to yogurt to reduce syneresis and to increase firmness. Gelatin is an ingredient compatible with milk proteins and improves the sensory perception by not masking the product flavor as much as some other gums (Jones, 1977). The use of different concentrations of gelatin would provide the manufacturer with the possibility of obtaining a wide range of textures in food products. Gelatin is widely used in confectionery products, including soft gummy-type candies and marshmallows. Gelatin is the main gelling agent in gummy-type candies. Marshmallows usually contain about 3% gelatin, in which gelatin serves as a stabilizer and whipping agent (Jones, 1977).

Gelatin is also used in nonfood products, including drugs, cosmetics, photographic films, and paper and paint products. Pharmaceutical gelatin accounts for a significant proportion of the total production and it is used in the production of capsules, tablets, and pastilles (Wood, 1977). Gelatin is used for both soft and hard capsules. The gelatin protects the drugs during distribution and they are not released until after they are in the stomach. Gelatin acts as a binding agent in tablets. It is also used for tablet coating to reduce dusting, to mask unpleasant tastes, and to allow printing and color coatings for product identification.

Gelatin has been used for photographic emulsions for more than 100 years. It is still the principal constituent of the binder in the most commercial photographic films and papers (Kragh, 1977).

II. FISH GELATIN

In the last decade, gelatin extraction from fish skin has been intensively investigated. The physicochemical, textural, rheological, and sensory properties of extracted fish gelatin have also been studied in comparison with mammalian gelatin. The overall results suggest that fish skin might be an alternative raw material for high-quality gelatin production, eliminating religious concerns shared by the Jewish and Muslim communities and also providing an alternative and more lucrative way to use some fishery by-products (Boran and Regenstein, 2009), thus also improving the overall sustainability of the fishing industry. Some of the fish species investigated include Atlantic salmon (Arnesen and Gildberg, 2007), cod (Gudmundsson and Hafsteinsson, 1997), sin croaker and short fin scad (Cheow *et al.*, 2007), Alaska pollock (Zhou and Regenstein, 2004), big eye snapper and brown stripe red snapper (Jongjareonrak *et al.*, 2006), yellow fin tuna (Cho *et al.*, 2005), Nile perch (Muyonga *et al.*, 2004), black and red tilapia (Jamilah and Harvinder, 2002), grass carp (Kasankala *et al.*, 2007), and silver carp (Boran and Regenstein, 2009).

A. Common and potential sources

Gelatin can be obtained from various marine and freshwater sources. The species available for gelatin production are divided into three categories: marine invertebrates, sea mammals, and fishes. Based on their living environments, fishes are usually subdivided into four groups: hot-water fish, warm-water fish, cold-water fish, and ice-water fish. Cold-water fishes, such as pollock, cod, and salmon, account for a large part of the commercial fish capture. They are often processed into skinned and boneless fillets, leaving large amounts of fish skin, scales, and bones as waste or as a raw material that is sometimes wasted, sometimes used for

fish meal, occasionally for leather, and which would be appropriate for gelatin production. These by-products, especially the skin, usually contain a large amount of protein, most of which is collagen. Warm-water fishes account for most freshwater fish aquaculture production, and currently, many commercial fish gelatins come from these fish species.

B. Properties of fish gelatin

1. Physical attributes

a. Gel strength Gel strength is one of the most important quality characteristics used in the gelatin industry to differentiate gelatins. As measuring gel strength is very popular, there is a standard method. According to the standard method (Gelatin Manufacturers Institute of America, GMIA), gel strength must be measured at 10 °C on a gelatin sample prepared at 6.67% concentration of protein (w/v). Thus, if the gelatin is contaminated with other proteins, the amount of actual gelatin evaluated would be less that 6.67%. For practical applications, this does not matter as it allows the user to evaluate the ''gelatin'' they are using. For scientific purposes, however, this may underestimate the quality of the actual gelatin extracted. Another concern is with the protein determination itself. Because gelatin has a unique amino acid composition, a special conversion factor is needed for the Kjeldahl test, and work has shown that calibration of the Biuret or Lowry procedures may also require adjustments for an accurate measurement (Zhou and Regenstein, 2006).

The methods for dissolving gelatin in water is not standardized and there are variations in the procedures used, that is, different water temperatures, different durations of sitting at the higher temperature before cooling, with or without stirring, etc. Maturation time and temperature are standardized and are generally followed by most investigators, that is, 16–18 h at 10 °C. A particular jar is used for this measurement, called a ''bloom jar'' (Fig. 5.2), it requires about 155 ml of gelatin solution, which corresponds to about 10 g of gelatin. However, this particular jar cannot be regularly used in many scientific studies because it requires such a substantial amount of sample, which is often limited in scientific studies. Therefore, many scientists use other containers that differ in size and shape leading to significant differences in the results, making the data incomparable among the different studies. Because of sample limitations, in some cases, a lower percentage of gelatin is used, often 3.33%. The test settings are also standardized: It is the force required for a 12.7-mm diameter flat probe to penetrate 4 mm into the gel that is being lowered into the sample at a speed of 1 mm/s. This force, expressed in grams, is then reported as the gel strength. If done totally according to the official methodology, the resulting gel strength may be called the ''Bloom strength.''

FIGURE 5.2 Standard "bloom jar" provided by Texture Technologies Corporation (Scarsdale, NY).

There are different instruments that can be used to handle the probe and the different instruments may give different results (Table 5.1).

b. Viscosity Viscosity is generally measured using tubular glass viscometers as they are relatively inexpensive and easy to use compared to expensive and complicated computer-controlled instruments. Although advanced viscosity instruments might provide higher reproducibility and accuracy, the tubular glass viscometers also give high precision. Compared to gel strength, viscosity is not as well correlated with textural properties. The viscosity is mostly affected by molecular weight distribution.

Gelatin samples with high molecular weight fractions give high viscosity but that does not necessarily mean that their gel strengths would also be high. Gelatin samples from fish skin, for example, give unexpectedly high viscosity while giving low gel strength compared to that of pork skin gelatin due to the carefully controlled extraction conditions and consequently, the presence of higher molecular weight protein fractions (Boran and Regenstein, 2009). Arnesen and Gildberg (2007) reported that Atlantic salmon and Atlantic cod skin gelatin had higher viscosities than pork skin gelatin while giving lower gel strengths than pork skin gelatin. Generally, fish skin gelatins are expected to have a lower viscosity compared to that of gelatins obtained from porcine and bovine sources with

TABLE 5.1 Gel strength of a commercial gelatin measured using different instruments and probes in either a standard bloom jar or a 15-ml capacity small plastic jar

Measurement details	Average	SD
Standard bloom jar, TAXT2 texture analyzer, spherical probe	242	5.7
Standard bloom jar, TAXT2 texture analyzer, cylindrical probe	523	2.1
Standard bloom jar, Stevens texture analyzer, spherical probe	213	1.2
Standard bloom jar, Stevens texture analyzer, cylindrical probe	466	3.8
Small plastic jar, TAXT2 texture analyzer, spherical probe	320	9.2
Small plastic jar, TAXT2 texture analyzer, cylindrical probe	814	13.6
Small plastic jar, Stevens texture analyzer, spherical probe	294	2.5
Small plastic jar, Stevens texture analyzer, cylindrical probe	746	9.6

SD: standard deviation.Same sample (Knox Gelatin, Kraft Foods Global, Inc., Glenview, IL, USA) used for the measurements: 6.67% gelatin, dissolved at 50 °C for 30 min in distilled water, matured at 4 °C for 16–18 h. The measurements are done at 4 °C using the following settings: 4 mm penetration with 12.7 mm diameter probe (either spherical or cylindrical) with 1 mm/s penetration speed. Gel strength is given as *g* force required penetrating the probe onto the sample ($N = 3$).

similar molecular weight distributions. However, most investigators do not look at the molecular weight distribution beyond looking at the SDS-PAGE electrophoretigram. Because the distribution of peptides can be so great, much of it is ''smeared'' over the gel and cannot be easily quantitated. To do more is a lot of work and would likely be different for each preparation, so it has not been done critically.

c. Melting and gelling temperature Rheological methods have recently gained importance and have found applications in the determination of gelatin quality. Rheological measurements of both melting and gelling temperatures give highly reproducible results. A temperature sweep test is performed for this purpose. Heating or cooling is required to determine the melting and gelling temperature, respectively. The gelatin gel sample is prepared at a certain concentration and matured at a certain temperature for a certain period of time to standardize the procedure to be able to discriminate the samples based on their chemical differences (Chiou *et al.*, 2006; Cho *et al.*, 2006; Fernandez-Diaz *et al.*, 2003; Kasankala *et al.*, 2007). Prior to rheological determinations of melting and gelling temperatures, the droplet method was used as a standard method for determining the melting temperature. However, the rheological methods have replaced

this older method (Wainewright, 1977), which was less precise and more laborious. A drop of an organic solvent with a dye in it, originally carbon tetrachloride, but more recently chloroform, with the recognition of the toxicity of carbon tetrachloride, has been used. The temperature when the droplet fell is taken as the melting temperature—but is it the beginning of the fall, the middle or when it touches the bottom. This is not well spelled out. Obviously, the rate of heating will also affect the results both because of differences in heat penetration and in the accuracy of the reading; slower being better but taking longer!

The rheological test measured the stress/strain as a function of time and temperature. Again, an arbitrary heating rate is selected. The midpoint of the transition is usually considered to be the melting or gelling temperature. The amount of cooperativity of the system can be determined by the width of the curve (in degrees) usually measured from the 25% point of transition to the 75% point of transition. The greater the cooperativity (or uniformity of the material), the smaller the width observed.

Other rheological tests, including time sweep, frequency sweep, stress sweep, and strain sweep, have also found applications in determination of gelatin quality, as they allow researchers to discriminate the gelatin gels according to their strength and elasticity. Stress and strain sweep tests are used to determine the linear viscoelastic region of the gels. Frequency sweep tests are useful to determine if the gelatin gels change with the changing frequency of the stress applied. Time sweep tests are used to determine if the gelatin gels' viscoelastic properties change with time at a controlled temperature and at a set level of stress applied.

The recent literature on fish gelatin includes some examples of these tests used to make comparisons among gelatin samples from different sources. Chiou *et al.* (2006) used temperature sweep tests to determine the melting and gelling temperature of gelatin gels. They also used time sweep tests to show the increasing elastic modulus at different temperatures with an increasing concentration of glutaraldehyde added to the gelatin gels. Gudmundsson (2002) used frequency sweep tests successfully to differentiate the gelatin gels based on their elastic moduli and the temperature sweep tests to determine the melting temperature of blended fish gelatin gels. Zhou and Regenstein (2007) used temperature sweep tests to compare the melting temperatures of gelatin gels from different sources. In another study, Zhou *et al.* (2006) used strain sweep and frequency sweep tests to compare the gelatin gels from different sources based on their viscoelastic properties. Recent studies gave good examples of how rheological measurements had strong correlations with conventional parameters, including gel strength and viscosity (Gilsenan and Ross-Murphy, 2000; Gudmundsson, 2002; Zhou *et al.*, 2006).

2. Chemical characteristics

a. Amino acid composition Chiou *et al.* (2006) showed that differences in the amino acid composition have significant effects on the melting and setting temperatures of gelatin obtained from different sources. According to their results, the higher proline and hydroxyproline content of pork gelatin correlated with stronger gels having higher gelling temperatures. Proline and hydroxyproline are, however, not the only amino acids having significant effects on gelatin structure. The content of glutamic acid, aspartic acid, lysine, hydroxylysine, arginine, and histidine are also important in cross-link formation and electrostatic interactions. As collagen usually lacks cystine, there are no disulfide bonds in the collagen structure. Collagen is mostly stabilized by hydrogen bonds formed between side chains of the amino acids and water in addition to the twisted structure enforced by the high content of proline and hydroxyproline along with intra- and intermolecular cross-links (Engel and Bachinger, 2005).

b. Peptide size in relation to quality Collagen-containing tissues are treated with acid and/or alkali followed by a heat treatment in the presence of water to break the structure of the collagen fibrils irreversibly to obtain gelatin (Eastoe and Leach, 1977). While the molecular weight of the collagen molecule is about 330 kDa, gelatin is generally considered to be all collagen fractions with a molecular weight higher than an arbitrary minimum of 30 kDa. When most fractions are below 30 kDa, the product is usually called a gelatin hydrolysate, as such products are not able to form a gel, although these peptides are believed to participate in gel formation (Eastoe and Leach, 1977). A heat treatment of about 40 °C breaks hydrogen and electrostatic bonds in newly formed collagen molecules releasing single α-chains, but this is insufficient to break the cross-links and covalent bonds in the collagen structure of mature collagen (Eastoe and Leach, 1977). With treatments at higher temperatures, on the other hand, those covalent bonds, including intermolecular cross-links and peptide bonds, break down and therefore, smaller α-chain fractions are obtained (Eastoe and Leach, 1977). The position of the bond breaks determines the molecular weight and the number of polypeptide chains. As amino acid sequence and composition of collagens from different sources vary greatly, bond breaks appear to be relatively random and these random bond breakdowns are the main cause of molecular heterogeneity in gelatin (Eastoe and Leach, 1977).

The raw materials used in gelatin production contain a variety of substances that are the source of organic and/or inorganic impurities in gelatin. Noncollagen protein fractions, lipids, nucleic acids, and other cell

components are among the organic impurities. Inorganic impurities include naturally present minerals such as calcium, sodium, potassium, and iron along with those derived from substances added for gelatin preparation, that is, acid and/or alkali with their impurities (Eastoe and Leach, 1977). Finally, commercial gelatin products contain a substantial amount of water usually as the second largest component in the whole and its amount varies greatly based on the drying process applied, the nature of the raw material, and the temperature and relative humidity of storage. Generally speaking, the water in gelatin is between 9% and 14% with occasional samples outside of this range (Eastoe and Leach, 1977).

C. Other factors affecting quality

There are several factors that significantly affect the properties of gelatin (Cho *et al.*, 2006). The raw materials used in gelatin manufacture have obvious effects on gelatin, mostly originating from differences in the amino acid composition of the collagen of the raw material. Also, variations in processing conditions such as extraction time, extraction temperature, and concentration of acid or alkali dramatically affect the product (Boran and Regenstein, 2009; Cho *et al.*, 2006; Hinterwaldner, 1977; Zhou and Regenstein, 2005). For example, longer extraction temperatures and/or higher extraction temperatures cause excessive damage to the collagen molecule and the resulting gelatins form weak gels and have low viscosity. Similarly, excessive concentrations of acid and/or alkali can cause degradation of collagen structure giving a gelatin with lower functional values.

1. The effects of processing conditions

The extraction conditions greatly affect the quality of gelatin as shown by many researchers. Therefore, optimization of extraction is of great interest to obtain the best possible product by eliminating and/or minimizing the excessive effects of extraction treatments causing extensive damage to collagen molecules. The most important extraction parameters are acid and/or alkali treatments, more specifically, the concentration of acid and/or alkali and duration of the treatment; extraction temperature and duration, and the conditions of refining and drying processes. A general flowchart of gelatin extraction from fish skin is given later and it shows that those treatments greatly affect the quality of the gelatin (Fig. 5.3).

a. Alkali and acid treatments The gel strength of gelatin is greatly influenced by the concentration of acid and/or alkali, the duration of the acid and/or alkali treatment, and possibly the treatment temperature. A study

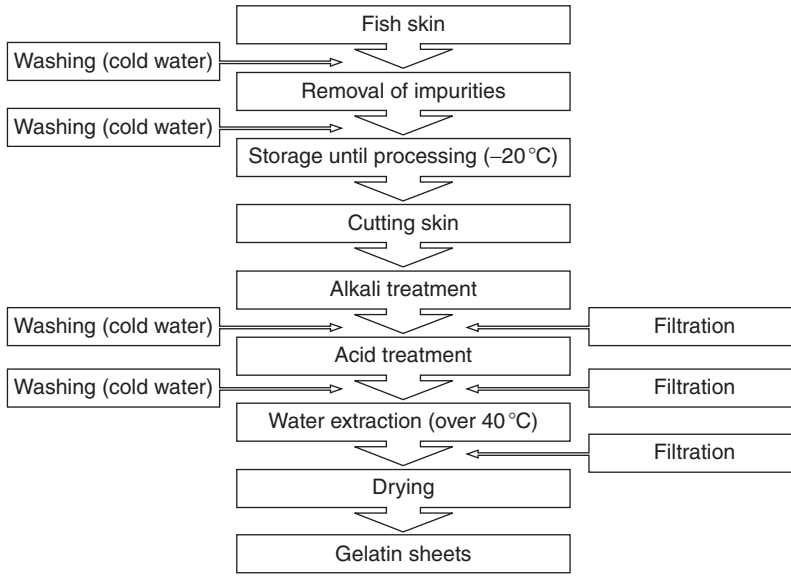

FIGURE 5.3 A general flowchart of gelatin extraction from fish skin.

by Gudmundsson and Hafsteinsson (1997) showed that high concentrations of alkali or acid increased the gelatin yield while decreasing gel strength. These results have also been confirmed by Zhou and Regenstein (2005). Another study done by Cho *et al.* (2006) showed that alkali concentration up to 1.5% increased gelatin yield significantly. Zhou and Regenstein (2004) confirmed that the concentrations of acid or alkali have a significant effect on gelatin yield, gel strength, and viscosity. Acid treatment is also important for the sensory aspects of gelatin, appearance and smell, as the acid treatment effectively removes odors and color that originate from the raw material (Boran and Regenstein, 2009; Zhang *et al.*, 2007). Alkali treatment is, similarly, important and responsible for removal of possible impurities from the raw material and also for weakening the collagen structure, leading to higher yield and superior quality. In addition, alkali treatment causes glutamine and asparagine to lose their amine groups, converting them to glutamic and aspartic acid residues, respectively, lowering the isoelectric point of collagen (Johns and Courts, 1977). Therefore, both acid and alkali treatments need to be optimized for pH, duration, and temperature of extraction. Zhou and Regenstein (2005) also did their pretreatment at low temperatures, which led to a much higher amount of the full α-chains. They suggest that the low temperature may have prevented any native proteases/collagenases from degrading the collagen prior to the extraction.

The main extraction step although normally done in water can be done at using mild acid or alkali conditions. Acid or alkali treatments are useful for a more effective extraction, increasing the yield and shortening the extraction time. Zhou and Regenstein (2005) showed that acidic conditions are more favorable for higher gelatin yield. However, acidic conditions also cause low gel strength, which is not desired in most gelatin applications. The isoelectric point of collagen is around 6–6.5, depending on the amino acid composition, specifically the content of the acidic and basic amino acids of collagen, which vary both due to source and to processing conditions, for example, due to the impact of processing on glutamine and asparagine. The isoelectric point of purified collagen is difficult to measure because collagen is difficult to isolate in its natural form as it is not readily soluble in water at room temperature and when it is dissolved with the help of heat treatment, collagen loses its natural state. Therefore, the isoelectric point measured does not reflect the physiological isoelectric point of the collagen, but many researchers agree on the approximate value of 7.0 for the isoelectric point of collagen under physiological conditions (Johns and Courts, 1977). Neutral extracts of untreated tissues of pork and rabbit skin, for example, had isoelectric points in the range of pH 5.6 and 6.8, respectively (Johns and Courts, 1977). A pH that is higher or lower than the isoelectric point results in higher extraction yield as collagen is less tightly bound at pH values different from its isoelectric point. The net charge of the collagen molecule is zero at the isoelectric point where there are an equal number of positive and negative charges on the molecule allowing it to form the maximum number of intermolecular salt bonds and electrostatic interactions, which strengthen and stabilize the structure of the collagen. However, the isoelectric point also reflects in part the binding of other ions that change the isoelectric point. The most accurate figure would be the isoionic point, which would be the extrapolation of the isoelectric point to pure water (where the isoelectric point measurement is difficult because there is no charge). According to the application in which gelatin is used, the effect of pH on gelatin needs to be carefully considered and the pH of the extraction solution needs to be adjusted to get a high quality gelatin. For example, as type-A gelatin has a higher isoelectric point, its use is favorable in those applications that require low pH at which the gelatin would be conducive to forming gel networks. Similarly, as type-B gelatin has a low isoelectric point, it is used in those applications that require a higher pH at which the gelatin is readily available for formation of the gel network.

b. Extraction temperature and duration Different temperatures and times are used in gelatin manufacturing but most extractions are between 45 and 60 °C. Temperatures from 50 until 80 °C can promote intramolecular bond formation between strands and consequently gelatin with stronger gelling ability can be obtained (Djagny *et al.*, 2001). Higher

temperatures over 80 °C, however, result in fracturing of intramolecular chains giving gelatin having a weaker gelling ability. Lower extraction temperatures, on the other hand, lead to low yields but a superior quality. Similarly, longer extraction times give better yield, while the extracted material suffers from low gel strength and viscosity due to excessive damage to collagen fractions with longer heating and possibly extraction of other proteins. Therefore, it is necessary to balance both extraction temperature and duration of the extraction, to get the best possible outcome. For this purpose, a few modern optimization studies have been done on gelatin extraction from skins of different fish species (Boran and Regenstein, 2009; Kasankala *et al.*, 2007; Zhou and Regenstein, 2004).

c. Storage and transportation There are many other factors affecting gelatin properties. Going into detail for each one of them is beyond the scope of this chapter. To be brief, every processing step, especially if heat is involved, has an effect on gelatin properties including yield, gel strength, melting and gelling temperatures, and viscosity. Raw materials are important with respect to purity and ease of processing. The actual type of the acid and/or alkali used in the pretreatment and/or the extraction is also important. Freshness and storage of raw materials, any possible microbial contamination or the presence of microbial or metabolic enzymes, the water content of gelatin, and transportation conditions are other factors that can affect the quality of gelatin.

D. Quality of fish gelatin compared to mammalian gelatins

Arnesen and Gildberg (2007) studied the skins of Atlantic salmon and Atlantic cod for gelatin production and reported that Atlantic salmon skin gelatins had higher gel strength and gelling temperatures than Atlantic cod skin gelatins. The gel strength of the salmon and cod were found to be 108 and 71 g, respectively, while their gelling temperatures were 12 and 10 °C, respectively. Arnesen and Gildberg (2007) also reported that the gel strength of the gelatins obtained increased with storage time and higher extraction temperature resulted in lower gel strength. Gudmundsson and Hafsteinsson (1997) also studied cod skin as a raw material for gelatin production, reporting that the proline and hydroxyproline content of cod (a cold-water species) skin gelatin (~18%) was lower compared to that of tilapia (a warm-water species) skin gelatin (~25%), resulting in relatively lower gel strength and viscosity. According to their results, tilapia skin gelatin gave 260 g bloom strength, while cod skin gelatin had 180 g bloom under the best extraction conditions reported. Choi and Regenstein (2000) compared various gelatin samples from different sources in terms of their physicochemical and sensory properties and reported that Alaska pollock gelatin had lower gel strength along with lower melting temperature

compared to that of pork skin gelatin. Alaska pollock gelatin melted at 24 °C while the pork skin gelatin melted at 29 °C (Choi and Regenstein, 2000). They also compared the sensory properties of gelatin gels prepared from Alaska pollock and pork skin gelatins and reported that a low melting temperature and gel strength might be useful in creating products with a faster and stronger flavor release. Chiou *et al.* (2006) studied Alaska pollock and Alaska pink salmon for gelatin production and the quality of the gelatin obtained in comparison with pork skin gelatin. They reported that Alaska pollock and Alaska pink salmon skin gelatins had lower melting and gelling temperatures along with lower gel strength compared to that of pork skin gelatin due to the lower proline and hydroxyproline content of skin gelatins obtained from these fish species. They reported that the pollock and salmon skin gelatins had gelling temperatures of 7 and 5 °C, respectively, while pork skin was reported to have a gelling temperature of 24 °C, which was attributed to the high content of proline and hydroxyproline of the pork skin gelatin (Chiou *et al.*, 2006).

Kasankala *et al.* (2007) studied grass carp skin as an alternative raw material for gelatin production and reported that the hydroxyproline content of grass carp skin gelatin (11.3%) was slightly higher than that of bovine skin gelatin (11.2%) and a little lower than that of pork skin gelatin (13.2%). They also reported high gel strength, melting and gelling temperatures for grass carp skin gelatin compared to that of gelatins obtained from other fish species. According to their results, the carp skin gelatin had a gelling temperature around 19 °C and a melting temperature around 26 °C, which was a little lower than that of pork skin (25 and 31 °C, respectively) and bovine gelatins (21 and 30 °C, respectively) (Kasankala *et al.*, 2007). Boran and Regenstein (2009) also reported similar results for skin gelatin obtained from silver carp, another Asian carp species, that is, it had high gel strength (600 g for optimized gelatin) possibly due to the high hydroxyproline content (~11%). Therefore, it does appear that the assumption that there is a strong connection between the content of hydroxyproline and proline and the physicochemical properties of gelatins continues to hold with the more recent research with fish gelatins.

III. METHODOLOGICAL CHALLENGES IN QUALITY DETERMINATION OF GELATIN

Both commercial gelatin powders and those produced on a small scale for research purposes have an amount of water that varies due to the differences in processing and drying methods (Eastoe and Leach, 1977). Water content of gelatin is important for both ease and duration of storage, as high water content favors microbial spoilage. In addition, higher water containing gelatin formulations can be sold for less. The drying method is

the major factor affecting the water content of gelatin products. Heat drying and freeze drying are two of the most common methods used to remove water from gelatin preparations. Heat drying is generally done at low temperatures between 40 and 60 °C for several hours to several days (Hinterwaldner, 1977). Freeze drying is a much faster method compared to heat drying and able to remove water while causing less damage to the gelatin, but it is generally more expensive.

The gelatin powder obtained is generally not tested for its water content, and even when determined, this information is not generally included in the calculations when preparing samples for testing, that is, the gelatin is simply weighed out. This might lead to a lack of agreement between data from different sources. To prevent confusion and to get comparable data, water content of gelatin samples should be determined and included in the calculations to make sure that the actual gelatin amount is the same in each sample being compared for their characteristics. After maturation, making a direct comparison of two gelatin samples with different amounts of gelatin for gel strength would be erroneous as the actual gelatin concentration of the samples is different.

IV. CONCLUSIONS AND SUGGESTED READINGS

Previous studies done on gelatin have shown that there are clear connections between gelatin's functional properties and the extraction conditions. While higher extraction temperatures and durations result in higher yield, the gelatin obtained is of poorer quality due to damage to the collagen fractions. Similarly, higher acid and/or alkali concentrations result in higher yield along with purer material, but the gelatin obtained lacks necessary functional properties. Therefore, an optimization of manufacturing process of gelatin is needed to get a final product with desired properties.

This chapter only covers general information on collagen and gelatin and a limited introduction to fish gelatin. For a thorough understanding of collagen and gelatin, additional sources should be consulted. For this purpose, an excellent book of contributed chapters edited by Ward and Courts (1977), ''The Science and Technology of Gelatin,'' covers almost every area related to gelatin and is still a very useful source of information for current collagen and gelatin producers and users, and for researchers.

REFERENCES

Arnesen, J. A. and Gildberg, A. (2007). Extraction and characterization of gelatine from Atlantic salmon (*Salmo salar*) skin. *Bioresour. Technol.* **98**, 53–57.

Badii, F. and Howell, N. K. (2006). Fish gelatin: Structure, gelling properties and interaction with egg albumen proteins. *Food Hydrocolloid.* **20**, 630–640.

Balian, G. and Bowes, J. H. (1977). The structure and properties of collagen. *In* ''The Science and Technology of Gelatin'', (A. G. Ward and A. Courts, Eds), pp. 1–27. Academic Press, New York.

Baziwane, D. and He, Q. (2003). Gelatin: The paramount food additive. *Food Rev. Int.* **19**(4), 423–435.

Belitz, H. D., Grosch, W., and Schieberle, P. (2004). Food Chemistry. 3rd revised edn. Springer, New York, pp. 579–586.

Boran, G. and Regenstein, J. M. (2009). Optimization of gelatin extraction from silver carp skin. *J. Food Sci.* **74**(8), E432–E441.

Brinckmann, J. (2005). Collagens at a glance. *In* ''Collagen: Primer in Structure, Processing and Assembly'', (J. Brinckmann, H. Notbohm, and P. K. Müller, Eds), pp. 1–6. Springer, New York.

Cheow, C. S., Norizah, M. S., Kyaw, Z. Y., and Howell, N. K. (2007). Preparation and characterization of gelatins from the skins of sin croaker (*Johnius dussumieri*) and short fin scad (*Decapterus macrosoma*). *Food Chem.* **101**, 386–391.

Chiou, B. S., Bustillos, R. J. A., Shey, J., Yee, E., Bechtel, P. J., Imam, S. H., Glenn, G. M., and Orts, W. J. (2006). Rheological and mechanical properties of cross linked fish gelatins. *Polymer* **47**, 6379–6386.

Cho, S. M., Gu, Y. S., and Kim, S. B. (2005). Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. *Food Hydrocolloid.* **19**, 221–229.

Cho, S. H., Jahncke, M. L., Chin, K. B., and Eun, J. B. (2006). The effect of processing conditions on the properties of gelatin from skate (*Raja kenojei*) skins. *Food Hydrocolloid.* **20**, 810–816.

Choi, S. S. and Regenstein, J. M. (2000). Physicochemical and sensory characteristics of fish gelatin. *J. Food Sci.* **65**(2), 194–199.

DeMan, J. M. (1999). Proteins: Animal proteins. *In* ''Principles of Food Chemistry'', pp. 147–149. Aspen Publishers, Inc., Gaithersburg, MD.

Djagny, K. B., Wang, Z., and Xu, S. (2001). Gelatin: A valuable protein for food and pharmaceutical industries: Review. *Crit. Rev. Food Sci.* **41**(6), 481–492.

Eastoe, J. E. and Leach, A. A. (1977). Chemical constitution of gelatin. *In* ''The Science and Technology of Gelatin'', (A. G. Ward and A. Courts, Eds), pp. 73–105. Academic Press, New York.

Engel, J. and Bachinger, H. P. (2005). Structure, stability and folding of the collagen triple helix. *In* ''Collagen: Primer in Structure, Processing and Assembly'', (J. Brinckmann, H. Notbohm, and P. K. Muller, Eds), pp. 8–24. Springer, New York.

Eyre, D. R. and Wu, J. J. (2005). Collagens crosslinks. *In* ''Collagen: Primer in Structure, Processing and Assembly'', (J. Brinckmann, H. Notbohm, and P. K. Müller, Eds), pp. 208–225. Springer, New York.

Fernandez-Diaz, M. D., Montero, P., and Gómez-Guillén, M. C. (2003). Effect of freezing fish skins on molecular and rheological properties of extracted gelatin. *Food Hydrocolloid.* **17**, 281–286.

Gilsenan, P. M. and Ross-Murphy, S. B. (2000). Rheological characterization of gelatins from mammalian and marine sources. *Food Hydrocolloid.* **14**, 191–195.

Gómez-Guillén, M. C., Turnay, J., Fernández-Díaz, M. D., Ulmo, N., Lizarbe, M. A., and Montero, P. (2002). Structural and physical properties of gelatin extracted from different marine species: A comparative study. *Food Hydrocolloid.* **16**, 25–34.

Gudmundsson, M. (2002). Rheological properties of fish gelatins. *J. Food Sci.* **67**(6), 2172–2176.

Gudmundsson, M. and Hafsteinsson, H. (1997). Gelatin from cod skins as affected by chemical treatments. *J. Food Sci.* **62**(1), 37–39. See also p. 47.

Harada, O., Kuwata, M., and Yamamoto, T. (2007). Extraction of gelatin from sardine scales by pressurized hot water. *Nippon Shokuhin Kagaku Kogaku Kaishi* **54**, 261–265.

Hinterwaldner, R. (1977). Technology of gelatin manufacture. *In* ''The Science and Technology of Gelatin'', (A. G. Ward and A. Courts, Eds), pp. 315–361. Academic Press, New York.
Holzer, D. (1996). Gelatin Production. United States Patent and Trademark Office (USPTO), Alexandria, VA, USA. United States Patent No: 5,484,888.
Hulmes, D. J. S. (2008). Collagen diversity, synthesis, and assembly. *In* ''Collagen, Structure and Mechanics'', (P. Fratzl, Ed.), pp. 16–22. Springer, New York.
Imeson, A. (1997). Thickening and Gelling Agents for Food. Springer, New York, p. 146.
Jamilah, B. and Harvinder, K. G. (2002). Properties of gelatins from skins of fish: Black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). *Food Chem.* **77**, 81–84.
Johns, P. and Courts, A. (1977). Relationship between collagen and gelatin. *In* ''The Science and Technology of Gelatin'', (A. G. Ward and A. Courts, Eds), pp. 138–168. Academic Press, New York.
Jones, N. R. (1977). Uses of gelatin in edible products. *In* ''The Science and Technology of Gelatin'', (A. G. Ward and A. Courts, Eds), pp. 366–392. Academic Press, New York.
Jongjareonrak, A., Benjakul, S., Visessanguan, W., and Tanaka, M. (2006). Skin gelatin from big eye snapper and brown stripe red snapper: Chemical compositions and effect of microbial transglutaminase on gel properties. *Food Hydrocolloid.* **20**, 1216–1222.
Karim, A. A. and Bahat, R. (2009). Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloid.* **23**(3), 563–576.
Kasankala, L. M., Xue, Y., Weilong, Y., Hong, S. D., and He, Q. (2007). Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. *Bioresour. Technol.* **98**(17), 3338–3343.
Kragh, A. M. (1977). Swelling, adsorption and the photographic uses of gelatin. *In* ''The Science and Technology of Gelatin'', (A. G. Ward and A. Courts, Eds), pp. 439–474. Academic Press, New York.
Muyonga, J. H., Cole, C. G. B., and Duodu, K. G. (2004). Extraction and physico-chemical characterization of Nile perch (*Lates niloticus*) skin and bone gelatin. *Food Hydrocolloid.* **18**, 581–592.
Nelson, D. L. and Cox, M. M. (2005). Lehninger's Principles of Biochemistry. 4th edn. WH Freeman and Company, New York, pp. 127–129.
Petersen, B. R. and Yates, J. R. (1977). Gelatin Extraction. United States Patent No: 4,064,008.
Regenstein, J. M. (2004). Total utilization of fish. *Food Tech.* **58**, 28–30.
Sadowska, M., Kolodziejska, I., and Niecikowska, C. (2003). Isolation of collagen from the skins of Baltic cod (*Gadus morhua*). *Food Chem.* **81**, 257–262.
Shahidi, F. (1995). Seafood processing by-product. *In* ''Seafood: Chemistry, Processing Technology and Quality'', (F. Shahidi and J. R. Botta, Eds), pp. 320–334. Kluwer Academic Publishers, New York.
Stainsby, G. (1977). The gelatin gel and the sol–gel transformation. *In* ''The Science and Technology of Gelatin'', (A. G. Ward and A. Courts, Eds), pp. 179–206. Academic Press, New York.
Wainewright, F. W. (1977). Physical tests for gelatin and gelatin products. *In* ''The Science and Technology of Gelatin'', (A. G. Ward and A. Courts, Eds), pp. 529. Academic Press, New York.
Wang, Y. and Regenstein, J. M. (2009). Effect of EDTA, HCl, and citric acid on Ca salt removal from Asian (silver) carp scales prior to gelatin extraction. *J. Food Sci.* **74**(6), C426–C431.
Wangtueai, S. and Noomhorm, A. (2009). Processing optimization and characterization of gelatin from lizardfish (*Saurida* spp.) scales. *LWT-Food Sci. Technol.* **42**, 825–834.
Ward, A. G. and Courts, A. (1977). The Science and Technology of Gelatin. Academic Press, New York, pp. 564.
P. A. Williams, A. (Ed.) (2007). Handbook of Industrial Water Soluble Polymers Wiley-Blackwell, New York, pp. 75–76.

Wood, P. D. (1977). Technical and pharmaceutical uses of gelatin. *In* ''The Science and Technology of Gelatin'', (A. G. Ward and A. Courts, Eds), pp. 413–437. Academic Press, New York.

Yang, H., Wang, Y., Jiang, M., Oh, J. H., Herring, J., and Zhou, P. (2007). 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. *J. Food Sci.* **72**(4), C188–C195.

Young, G. E. and Lorimer, J. W. (1960). The acid-soluble collagen of cod skin. *Arc. Biochem. Biophys.* **88**, 373–381.

Zhang, S., Wang, Y., Herring, J. L., and Oh, J. H. (2007). Characterization of edible film fabricated with channel catfish (*Ictalurus punctatus*) gelatin extract using selected pretreatment methods. *J. Food Sci.* **72**(9), C498–C503.

Zhou, P. and Regenstein, J. M. (2004). Optimization of extraction conditions for pollock skin gelatin. *J. Food Sci.* **69**(5), 393–398.

Zhou, P. and Regenstein, J. M. (2005). Effects of alkaline and acid pretreatments on Alaska pollock skin gelatin extraction. *J. Food Sci.* **70**(6), 392–396.

Zhou, P. and Regenstein, J. M. (2006). Determination of total protein content in gelatin solutions with the Lowry or Biuret assay. *J. Food Sci.* **71**(8), 474–479.

Zhou, P., Mulvaney, S. J., and Regenstein, J. M. (2006). Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. *J. Food Sci.* **71**(6), 313–321.

Zhou, P. and Regenstein, J. M. (2007). Comparison of water gel desserts from fish skin and pork gelatins using instrumental measurements. *J. Food Sci.* **72**(4), C196–C201.