

**FISH GELATIN: A RENEWABLE MATERIAL FOR DEVELOPING ACTIVE  
BIODEGRADABLE FILMS**

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## ABSTRACT

Most films used to preserve foodstuffs are made from synthetic plastic materials. However, for environmental reasons, attention has recently turned to biodegradable films. Gelatin has been extensively studied for its film forming capacity and applicability  
5 as an outer covering to protect food against drying, light, and oxygen. Moreover, it is one of the first materials proposed as a carrier of bioactive components. Gelatins from alternatives to mammalian species are gaining prominence, especially gelatins from marine fish species. Because of their good film-forming abilities, fish gelatins may be a good alternative to synthetic plastics for making films to preserve foodstuffs. The  
10 mechanical and barrier properties of these films depend largely on the physical and chemical characteristics of the gelatin, especially the amino acid composition, which is highly species specific, and the molecular weight distribution, which depends mainly on processing conditions. Different film formulations can be developed to extend the films' physical and chemical properties and to add new functional attributes. This paper  
15 reviews the most recent scientific literature dealing with films based on gelatins from different fish species and considers various strategies intended to improve the physical properties of such films by combining fish gelatins with such other biopolymers as soy protein isolate, oils and fatty acids, and certain polysaccharides. The use of plasticizers and cross-linking agents is also discussed. Specific attributes, such as  
20 antimicrobial and antioxidant activities, may be also conferred by blending the gelatin with chitosan, lysozyme, essential oils, plant extracts, or vitamin C to produce an active packaging biomaterial.

**Key words:** biodegradable films, fish gelatin, antioxidant, antimicrobial, physical  
25 properties

## Introduction

Besides serving marketing and consumer information purposes, packaging places a physical barrier between food products and the outside environment, thereby ensuring hygiene and extending the lifetimes of perishable items, especially those susceptible to oxidative and microbiological deterioration. The most common materials used for packaging are paper, fibreboard, plastic, glass, steel, and aluminium. Oil-derived synthetic plastics are commonly used, because they afford various advantages over other packaging materials in terms of sturdiness and low weight. However, they pose a serious global environmental problem by generating large volumes of non-biodegradable waste (Kirwan & Strawbridge, 2003). Moreover, in addition to safety and environmental issues, recycling of plastics is complicated for technical and economic reasons (Aguado & Serrano, 1999).

Thus, new biodegradable films made from edible biopolymers from renewable sources could become an important factor in reducing the environmental impact of plastic waste (Tharanathan, 2003). Proteins, lipids, and polysaccharides are the main biopolymers employed to make edible films and coatings. Which of these components are present in which proportions determines the properties of the material as a barrier to water vapour, oxygen, carbon dioxide, and lipid transfer in food systems. Films composed primarily of proteins or polysaccharides have suitable overall mechanical and optical properties but are highly sensitive to moisture and exhibit poor water vapour barrier properties (Guilbert, Gontard & Gorris, 1996). This may represent a drawback when they are applied to food products with high moisture contents, because the films may swell, dissolve, or disintegrate upon contact with the water. Films composed of lipids are more moisture-resistant, but they are usually opaque, relatively stiff, and more vulnerable to oxidation. For these reasons the current trend in designing biodegradable materials for food packaging is to combine different biopolymers (Bertan, Tanada-Palmu, Siani & Grosso, 2005; Cao, Fu & He, 2007a; Colla, Sobral & Menegalli, 2006;

Jagannath, Nanjappa, Das Gupta & Bawa, 2003; Le Tien et al., 2000; Lee, Shim & Lee, 2004; Li, Kennedy, Jiang & Xie, 2006; Longares, Monahan, O'Riordan & O'Sullivan, 2005; Tapia-Blácido, Mauri, Menegalli, Sobral & Añón, 2007), plasticizers (Arvanitoyannis, Nakayama & Aiba, 1998a; Thomazine, Carvalho & Sobral, 2005; 5 Vanin, Sobral, Menegalli, Carvalho & Habitante, 2005), cross-linking agents (Bigi, Cojazzi, Panzavolta, Rubini & Roveri, 2001; Hernandez-Munoz, Villalobos & Chiralt, 2004; Lai & Chiang, 2006; Tang, Jiang, Wen & Yang, 2005), and even inorganic particles (Sinha Ray & Okamoto, 2003; Sorrentino, Gorrasi & Vittoria, 2007) to fulfil a number of specific functional requirements (moisture barrier and gas barrier features, 10 water or lipid solubility, colour and appearance, as well as mechanical and rheological attributes) so as to obtain properties, as far as possible, similar to those of non-biodegradable plastics.

Furthermore, enriching these films with functional additives allows nutritional and aesthetic quality aspects to be enhanced without affecting the integrity of the food 15 product (Guilbert et al. 1996). In this connection, a number of recent studies have dealt with extending the functional properties of biodegradable films by adding natural substances with antioxidant or antimicrobial activities in order to yield an active packaging biomaterial (Kim, Ko, Lee, Park & Hanna, 2006; Ku & Song, 2007; Oussalah, Caillet, Salmiéri, Saucier & Lacroix, 2004; Seydim & Sarikus, 2006; 20 Zivanovic, Chi & Draughon, 2005).

Gelatin has been extensively studied on account of its film-forming ability and its usefulness as an outer film to protect food from drying and exposure to light and oxygen (Arvanitoyannis, 2002). In addition, gelatin was one of the first materials used 25 as a carrier of bioactive components (Gennadios, McHugh, Weller & Krochta, 1994). Large volumes of gelatin are used annually by the food industry worldwide, and the amount is growing yearly due to its abundance, relatively low cost, and excellent functional properties. Worldwide production of gelatin in 2007 was 326 000 tonnes, of

which 121 800 t were produced in Europe. Annual growth in gelatin production in the past seven years has been put at around 3-4 %. The most abundant sources of gelatin are pig skin (46 %), bovine hide (29.4 %) and pork and cattle bones (23.1 %). Other sources, which include fish gelatin, accounted for around 1.5 % of total gelatin production in 2007. It is worth noting, however, that this percentage has doubled compared with market data for 2002, indicating that the production of gelatin from alternatives to mammalian species is growing in importance (GME, 2007). Skins and bones, consisting primarily of collagen, make up about 30 % of the waste from fish filleting in the seafood industry. The rising interest in putting by-products from the fish industry to good use is one of the reasons why the industrial production of fish gelatin has been growing in recent years (Gómez-Guillén, Turnay, Fernández-Díaz, Ulmo, Lizarbe & Montero, 2002; Muyonga, Cole & Duodu, 2004a). Moreover, socio-culturally, marine gelatins are regarded as an alternative to terrestrial mammalian (bovine and porcine) gelatins, since pork consumption is forbidden by certain religions (Judaism and Islam).

As a rule, the physical properties of gelatin films depend chiefly on the properties of the raw materials extracted from the different animal species and on the processing conditions of gelatin manufacturing. They also depend on the physical parameters used in film processing, such as temperature and drying time (Menegalli, Sobral, Roques & Laurent, 1999), and on formulation ingredients, such as the inclusion of plasticizers (Lukasik & Ludescher, 2006a,b; Vanin et al., 2005) or cross-linkers (Bigi et al., 2001; Cao, Fu & He, 2007b). Sorbitol and glycerol are the plasticizers most commonly used in producing gelatin-based films. Cuq, Gontard, Cuq & Guilbert (1997) reported that hydrophilic, low-molecular-weight molecules like glycerol and sorbitol could easily fit into protein networks and form hydrogen bonds with the reactive groups on amino acid residues, thereby reducing protein-protein interactions. Normally, increasing plasticizer concentration in a film-forming solution produces a film that is

less stiff, less rigid, and more stretchable by reducing the interactions between the biopolymer chains (Arvanitoyannis, 2002). According to Thomazine et al. (2005), gelatin films plasticized with glycerol are quite water sensitive, not as strong, and more stretchable than films that contain sorbitol, while a mixture of glycerol and sorbitol yielded films with intermediate mechanical, viscoelastic, and water vapour barrier properties compared to films plasticized with glycerol or sorbitol alone. Other plasticizers like propylene glycol, diethylene glycol, and ethylene glycol have also been found to be compatible with gelatin, but in terms of the resulting functional properties, they were less efficacious than glycerol and less effective plasticizers as well (Vanin et al., 2005).

Edible films can be formed by two main processes, i.e., casting and extrusion (Hernandez-Izquierdo & Krochta, 2008). The film-formation process most often reported in the scientific literature is the casting method. Briefly, it involves dissolving the biopolymer and blending it with plasticizers and/or additives to obtain a film-forming solution, which is cast onto plates and then dried by driving off the solvent. The extrusion method relies on the thermoplastic behaviour of proteins at low moisture levels. Films can be produced by extrusion followed by heat-pressing at temperatures that are ordinarily higher than 80 °C. This process may affect film properties, but its use would enhance the commercial potential of films by affording a number of advantages over solution-casting, e.g., working in a continuous system with ready control of such process variables as temperature, moisture, size/shape, etc. In a recent study Park, Scott, Whiteside & Cho (2008) compared the properties of pig-skin gelatin films produced either by extrusion/heat-pressing or by casting and found that extruded films had lower tensile strength and higher elongation and water vapour permeability (WVP) values than the corresponding cast films. They explained these differences in terms of the moisture evaporation rate, which was lower in the cast films, and the presence of microvoids in the extruded films.

Most of the scientific literature on edible and/or biodegradable gelatin films has dealt with commercial mammalian gelatins (Arvanitoyannis et al., 1998a; Bertan et al., 2005; Chambi & Grosso, 2006; de Carvalho & Grosso, 2004; Lim, Mine & Tung, 1999; 5 Menegalli et al., 1999; Simon-Lukasik & Ludescher, 2004; Sobral & Habitante, 2001; Vanin et al, 2005). Studies on the production and characterization of films using fish gelatins are quite recent, and all fish gelatins have been observed to exhibit good film-forming properties, yielding transparent, nearly colourless, water soluble, and highly extensible films (Avena-Bustillos et al., 2006; Carvalho, Sobral, Thomazine, Habitante, 10 Giménez, Gómez-Guillén & Montero, 2008; Gómez-Guillén, Ihl, Bifani, Silva & Montero, 2007; Jongjareonrak, Benjakul, Visessanguan, Prodpran & Tanaka, 2006a; Zhang, Wang, Herring & Oh, 2007).

The high hygroscopic nature of gelatin represents the main drawback in gelatin films, 15 because they tend to swell or dissolve in contact with the surface of foodstuffs with high moisture content. To avoid this, the application of edible coatings based on gelatin may constitute an alternative to prolong the shelf life of fresh meat (Antoniewski, Barringer, Knipe, & Zerby, 2007), fish patties (López-Caballero, Gómez-Guillén, Pérez-Mateos & Montero, 2005) or post-harvest avocado (Aguilar-Méndez, San Martín-Martínez, 20 Tomás, Cruz-Orea & Jaime-Fonseca, 2008). Nevertheless, the current trends are more focused on the formulation of gelatin films with improved water resistance.

This paper reviews the most recent scientific literature dealing with fish gelatins and films made from the gelatins of different fish species and considers various strategies 25 for improving the physical properties of these films and conferring specific attributes, such as antioxidant and antimicrobial activity, to produce a renewable, active, packaging biomaterial of marine origin.

## **Fish gelatin**

Gelatin is a protein obtained by hydrolyzing the collagen contained in bones and skin. Source, animal age, collagen type, and manufacturing method all greatly affect the physical and chemical properties of the gelatin (Ledward, 1986). To convert insoluble native collagen into gelatin requires pre-treatment to break down the non-covalent bonds and disorganize the protein structure, allowing swelling and cleavage of intra and intermolecular bonds to solubilize the collagen (Stainsby, 1987). Subsequent heat treatment cleaves the hydrogen and covalent bonds to destabilize the triple helix, resulting in helix-to-coil transition and conversion into soluble gelatin (Djabourov, Lechaire & Gaill, 1993). The degree of conversion of the collagen into gelatin is related to the severity of both the pre-treatment and the extraction process, depending on the pH, temperature, and extraction time (Johnston-Banks, 1990). Two types of gelatin are obtainable, depending on the pre-treatment. These are known commercially as type-A gelatin, obtained in acid pre-treatment conditions, and type-B gelatin, obtained in alkaline pre-treatment conditions. Industrial applications call for one or the other gelatin type, depending on the degree of collagen cross-linking in the raw material, in turn depending on a number of factors, such as collagen type, tissue type, species, animal age, etc.

Collagenous material from fish skins is characterized by a low degree of intra and interchain covalent cross-linking, mainly involving lysine and hydroxylysine (Hyl) residues, along with aldehyde derivatives (Montero, Borderías, Turnay & Leyzarbe, 1990). Accordingly, a mild acid pre-treatment is normally used for type-A gelatin extraction from fish skins (Norland, 1990). In the past 10 years considerable effort has been expended on studying gelatin extraction from different fish species, e.g., cod (Gudmundsson & Hafsteinsson, 1997), tilapia (Jamilah & Harvinder, 2002), megrim (Montero & Gómez-Guillén, 2000), sole and squid (Gómez-Guillén et al. 2002; Giménez, Gómez-Estaca, Alemán, Gómez-Guillén & Montero, 2008), pollock (Zhou &

Regenstein, 2004), Nile perch (Muyonga, Cole & Duodu, 2004b), yellowfin tuna (Cho, Gu & Kim, 2005; Rahman, Al-Saidi & Guizani, 2008), Atlantic salmon (Arnesen & Gildberg, 2007), skipjack tuna (Aewsiri, Benjakul, Visessanguan & Tanaka, 2008); shark (Cho et al., 2004), skate (Cho, Jahncke, Chin & Eun, 2006), grass carp  
5 (Kasankala, Xue, Weilong, Hong & He, 2007), bigeye snapper and brownstripe red snapper (Jongjareonrak, Benjakul, Visessanguan & Tanaka, 2006b) and channel catfish (Yang, Wang, Jiang, Oh, Herring & Zhou, 2007; Zhang et al., 2007). Table 1 summarizes the gel strength and thermal stability (gelling and melting temperatures) of different gelatins extracted from the skins of several fish species and squid, indicating  
10 shortly some details of both pre-treatment and water extracting conditions. Strict comparisons are difficult since methodologies may differ considerably from one work to another. However, for standardizing purposes, measurements are frequently performed at a given gelatin concentration (6.67%) and temperature (around 10°C), which allows in some cases to express the gel strength in the normalized “bloom” value  
15 (Wainwright, 1977). Extraction processes have been modified to improve rheological properties or extraction yields, for instance by using different organic acids for pre-treatment of the skins (Giménez, Turnay, Lizarbe, Montero & Gómez-Guillén, 2005a; Gómez-Guillén & Montero, 2001; Songchotikunpan, Tattiyakul & Supaphol, 2008), different salts for washing the skins (Giménez, Gómez-Guillén & Montero, 2005b),  
20 high-pressure treatment (Gómez-Guillén, Giménez & Montero, 2005), and pepsin-aided digestion (Nalinanon, Benjakul, Visessanguan & Kishimura, 2008; Giménez et al., 2008). The influence of the method used to preserve the fish skins (freezing or drying) on gelatin properties has also been examined. Freezing flounder skins has been reported to affect the molecular composition of the resulting gelatins by  
25 decreasing the amount of high-molecular-weight polymers and  $\beta$  and  $\gamma$  components extracted. This effect decreased gel strength and reduced the subsequent renaturation ability of the corresponding gelatin and grew more severe with frozen storage temperature (-12 °C vs. -20 °C) (Fernández-Díaz, Montero & Gómez-Guillén, 2003).

In contrast, drying Dover sole skins with glycerol, ethanol or dry salt, slightly lowered the viscoelastic properties and the gelling and melting points, but neither the viscoelastic properties nor gel strength appeared to undergo any appreciable changes over the course of storage at room temperature for 160 days (Giménez, Gómez-Guillén & Montero, 2005c).

The physical properties of gelatin depend to a large extent on two factors: (i) the amino acid composition, which is highly species specific (see Table 2), and (ii) the molecular weight distribution, which results mainly from processing conditions (Gómez-Guillén et al., 2002). Marine gelatins have long been known to have worse rheological properties than mammalian gelatins, particularly in the case of gelatins from cold-water fish species, such as cod, salmon or Alaska pollack (Gudmundsson & Hafsteinsson, 1997; Haug, Draget & Smidsrød, 2004; Leuenberger, 1991; Zhou, Mulvaney & Regenstein, 2006). This has mainly been attributed, especially in cold-water fish, to the lower number of Pro+Hyp rich collagen regions that are most likely involved in the formation of nucleation zones conducive to the formation of triple helical structures (Ledward, 1986). Nevertheless, recent studies have indicated that certain fish gelatins (from yellowfin tuna, tilapia, and catfish, for example), while not superior to mammalian gelatins, might afford similar levels of quality, depending on the species from which the gelatin is extracted and on processing conditions (Cho et al., 2005; Choi & Regenstein, 2000; Yang et al., 2007; Zhou et al., 2006). In this respect, warm-water fish species like sole, tilapia, and grass carp are well known to yield gelatins that have better thermostability and rheological properties than the gelatins obtained from such cold-water fish species as cod, salmon, or Alaska pollack (Avena-Bustillos et al., 2006; Gómez-Guillén et al., 2002; Jamilah & Harvinder, 2002; Kasankala et al., 2007) (see Table 1).

### **Fish gelatin-based films**

Data from the scientific literature are not readily comparable because of differences in film preparation, plasticizer type and concentration, gelatin type, measurement methods, etc. Having said this, it seems to be accepted that mammalian gelatins yield stronger films, whereas fish gelatins yield more deformable films (Avena-Bustillos et al., 5 2006; Gómez-Guillén et al., 2007; Sobral & Habitante, 2001; Thomazine et al., 2005). However, as already mentioned above when discussing gelatin properties, there may be appreciable differences depending on the fish species and habitat. For example, films made from gelatin extracted from the skins of the Nile perch, a warm-water fish species, have been reported to exhibit breaking and elongation values similar to those 10 of bovine-bone gelatin (Muyonga et al., 2004b). Similarly, films made from gelatin from channel catfish have also exhibited mechanical and water vapour barrier properties comparable to those of films made from a commercial mammalian gelatin (Zhang et al., 2007).

15 Avena-Bustillos et al. (2006) reported the water vapour permeability (WVP) of cold-water fish gelatin films to be significantly lower than that of films made from warm-water fish gelatin or mammalian gelatin and explained the tendency of fish-gelatin films to exhibit lower WVP values than land animal-gelatin films in terms of the amino acid composition, since fish gelatins, especially cold-water fish gelatins, are known to 20 contain higher amounts of hydrophobic amino acids and lower amounts of hydroxyproline. Similarly, using equivalent procedures and plasticizing conditions (sorbitol or glycerol, 25-30 % of gelatin content), the WVP of halibut-skin gelatin films (Carvalho et al., 2008) and tuna-skin gelatin films (Gómez-Guillén et al., 2007) was also reported to be lower than that of mammalian gelatin films (Sobral & Habitante, 25 2001; Vanin et al., 2005). Plasticizer type and concentration is a critical factor, and due to their hydrophilic -OH groups sorbitol and glycerol molecules are known to increase the water vapour permeability of gelatin films irrespective of gelatin source, and this

effect is directly related to plasticizer concentration (Jongjareonrak, Benjakul, Visessanguan & Tanaka, 2006c; Sobral & Habitante, 2001).

### **Effect of gelatin attributes on film properties**

5 As already mentioned, the molecular weight distribution and amino acid composition are the main factors influencing the physical and structural properties of gelatin, and therefore they are also believed to play a key role in the rheological and barrier properties of the resulting films. Examining the molecular weight distribution, Muyonga et al., (2004a,b) compared the physical and chemical properties of films made from  
10 gelatins extracted from the skins and bones of the Nile perch following mild acid pre-treatment with 0.01 M sulphuric acid and subsequent heating in water at varying temperatures (from 50 to 70 °C). They observed that the gelatin extracted from Nile perch bones, which consisted of a higher proportion of low-molecular-weight fractions as a result of the more severe heating needed for extraction, had considerably lower  
15 tensile strength but higher percentage elongation than the corresponding films made from gelatin extracted from the skins. Since the amino acid compositions were found to be similar, the differences in the functional properties between the skin and bone gelatin films were attributed to differences in the molecular weight distribution of the corresponding gelatins (Muyonga, Cole & Duodu, 2004a,b).

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In a later study, different quality gelatins from catfish skins were prepared using various acidic and/or alkaline pre-treatment methods followed by gelatin extraction in warm water at 50 °C (Zhang et al., 2007). Combined alkaline (0.25 M sodium hydroxide) / acid (0.09 M acetic acid) pre-treatment yielded a gelatin preparation with appreciably  
25 higher molecular weight fractions that displayed two major protein bands, which were ascribed to collagen  $\alpha$  and  $\beta$ -chains. The corresponding films exhibited higher tensile strength and lower elongation values compared to films obtained from gelatins containing more low-molecular-weight fragments.

Recently, Carvalho et al. (2008) reported that adding a concentration step by evaporating at 60 °C before spray drying the gelatin from Atlantic halibut (*Hippoglossus hippoglossus*) skin resulted in sizeable differences in the physical properties of the corresponding films. Differences in the mechanical behaviour of these films were attributed to slight differences in the molecular weight distributions of the two types of gelatin, caused by protein heat degradation during the evaporation step, with the loss of  $\beta$  and  $\gamma$  components and breakdown of the native  $2\alpha_1:\alpha_2$  ratio. As a result, these authors concluded that gelatin having a predominance of lower-molecular-weight fractions underwent greater plasticization by the sorbitol molecules, culminating in greater breaking elongation and lower tensile strength of the resulting films.

The role of the molecular weight distribution in the gelatin in determining the rheological properties of the films may also depend on the presence and concentration of plasticizer in the formulation. All the studies cited above included plasticizers, i.e., glycerol (Muyonga et al, 2004a; Zhang et al., 2007) or sorbitol (Carvalho et al., 2008). Accordingly, for a given concentration of plasticizer, films made using a lower-molecular-weight biopolymer could be plasticized to a higher degree, because the plasticizer:biopolymer molar ratio was higher (Thomazine et al., 2005).

The effect of plasticizer type and concentration on fish-gelatin films have been also examined. In films from both bigeye snapper or brownstripe red snapper skin gelatin, glycerol was found to yield the greatest breaking elongation, while films plasticized using ethylene glycol exhibited the highest tensile strength (Jongjareonrak et al., 2006c). The tensile strength of both these fish-skin gelatin films decreased with the glycerol or sorbitol concentration, whereas the breaking elongation increased as plasticizer concentration increased from 25 to 75 % of protein content. Both gelatins presented a similar imino acid (Pro+Hyp) content, but in terms of the molecular weight

distribution, bigeye snapper-skin gelatin was characterized by lower concentrations of high-molecular-weight fractions, with a concomitant increase in degradation peptides, compared with brownstripe red snapper-skin gelatin (Jongjareonrak, Benjakul, Visessanguan, Prodpran & Tanaka, 2006b). Tensile strength of the films prepared from the bigeye snapper-skin gelatin was lower than that of the brownstripe red snapper-skin gelatin films. As previously described, the predominance of high-molecular-weight fractions in the gelatin was the main factor increasing the strength of the resulting films, although in this study the elongation values for the two gelatins were quite similar.

10 In another study comparing the physical and chemical properties of films made from tuna and halibut-skin gelatins, both following mild acid pre-treatment (0.05M acetic acid) and subsequent extraction by heating in water at 45°C, the lower mean molecular weight of the halibut gelatin was found to be a major factor responsible for the lower breaking strength and appreciably higher deformation value of the corresponding films, 15 both plasticized with 15-25 % sorbitol (Habitante, Montero, Gómez-Guillén, Sobral & Carvalho, 2005).

When gelatins come from different fish species, attention must be paid not only to the molecular weight distribution but also to the amino acid composition, especially that of the most characteristic amino acids in gelatin, Gly, Pro, and Hyp (triplets of these 20 amino acids being clearly predominant in collagen molecules). The pyrrolidine rings of the imino acids may impose conformational constraints, imparting a certain degree of molecular rigidity that can affect film deformability. A recent study compared films made from tuna (*Thunnus thynnus*) skin gelatin and bovine-hide gelatin in identical conditions 25 (Gómez-Estaca, Montero, Fernández-Martín, Alemán & Gómez-Guillén, 2008). Both films were plasticized with a mixture of glycerol and sorbitol and had similar maximum breaking strength values, but the tuna-skin gelatin films had breaking deformation values around 10 times higher than the values for the bovine-hide gelatin films. The

Pro+Hyp content was higher in the bovine-hide gelatin (210/1000 residues) than in the tuna-skin gelatin (185/1000 residues). Both gelatins contained appreciable amounts of high-molecular-weight (>200 kDa) polymers. There were considerably more  $\beta$  components in the fish gelatin, whereas the <100 kDa polypeptide fraction was more abundant in the bovine-hide gelatin. The viscoelastic properties of the film-forming solutions revealed the bovine-hide gelatin's greater gelling capacity, to be expected from the amino acid profile, richer in Pro+Hyp. However, this difference was not enough to impart significantly higher strength to the mammalian-gelatin films, probably because the stabilizing role of water molecules and cold temperature is less important in the films than it is in the hydrogels. In this connection, the triple helical structure content has been shown to decrease upon isothermal dehydration of films (Wetzel, Buder, Hermel & Huttner, 1987). In addition, the larger amounts of  $\beta$  components in fish gelatins could also appreciably strengthen the corresponding films. However, in the case discussed here, the considerably higher deformation values for the tuna-skin gelatin could not be explained in terms of the molecular weight distribution, since the bovine-hide gelatin had larger amounts of hydrolyzed peptide fractions, which readily interact with plasticizer molecules. Thus, the lower imino acid content of the tuna-skin gelatin was put forward as the most likely explanation for the higher film deformability.

All these studies have confirmed that in addition to differences in film-making procedures, plasticizer type and concentration, etc., the physical and chemical properties of the gelatin, especially with regard to the amino acid composition and the molecular weight distribution, play a key role in determining the physical properties of the films.

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### **Blends of fish gelatin and other biopolymers**

The mechanical and barrier properties of fish-gelatin films can be enhanced by producing composite films using such different biopolymers as proteins, lipids, and

polysaccharides. Apart from technical aspects, there may also be economic reasons for contemplating the production of composite films by blending gelatin with other biopolymers.

5 As an animal protein, gelatin is more expensive than other proteins of plant origin, such as soy protein isolate (SPI), which is a mixture of a number of albumins and globulins, including the 7S and 11S fractions, respectively making up about 37 % and 31 % of the total extractable protein (Ziegler & Foegeding, 1990). The physical properties of composite bovine-bone gelatin and soy protein isolate (SPI) films have been described  
10 by Cao et al. (2007a), who found that increasing the proportion of SPI in the film lowered the tensile strength, breaking elongation, and swelling ability of the films. Indeed, SPI films are well known to be rather brittle and to have relatively poor mechanical properties (Rhim, Gennadios, Handa, Weller & Hanna, 2000). In a later study using cod-skin gelatin, composite films were obtained by adding differing  
15 proportions of a laboratory-prepared SPI (0, 25, 50, 75, 100 % w/w) and a glycerol-sorbitol mixture as plasticizer (Denavi, Pérez-Mateos, Añon, Montero, Mauri & Gómez-Guillén, 2007). The formulation comprising 25 % SPI / 75 % cod-skin gelatin was found to have a higher maximum breaking strength ( $\approx 7$  N) compared to the 100-% gelatin and the 100-% SPI films (1.8 and 2.8 times higher, respectively). Moreover, this  
20 formulation afforded the same high percentage deformation values as the 100-% gelatin film ( $\approx 80$  %) and the same relatively low water vapor permeability as the 100-% SPI film ( $2 \times 10^{-8} \text{ g} \cdot \text{mm} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \cdot \text{Pa}^{-1}$ ). Thus, composite films made from gelatin and SPI may benefit from the most advantageous properties of each separate protein component.

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The hydrophilic character of gelatin films can be a drawback in certain applications, hence there is interest in developing blends using such components as oils or waxes to augment the hydrophobic regions in the films and thus reduce the WVP and water

solubility. There are two main methods of adding oil to the film formulation, namely, either by emulsifying the film-forming solutions (Jongjareonrak, Benjakul, Visessanguan & Tanaka, 2006d) or by making bilayer films (Morillon, Debeaufort, Blond, Capelle & Voilley, 2002). Jongjareonrak et al. (2006d) blended bigeye snapper  
5 and brownstripe red snapper-skin gelatins with fatty acids (FAs) (palmitic acid and stearic acid) or the sucrose esters (FASEs) of those same FAs. The resulting composite films underwent an appreciable reduction in the WVP. Adding the FAs lowered the tensile strength, while adding the FASEs progressively increased the tensile strength. A marked increase in breaking elongation was also recorded when  
10 either FAs or FASEs were added to the films in a proportion of 25 %.

Emulsified film-forming solutions made with cod-skin gelatin and increasing proportions of sunflower oil (0, 0.3, 0.6, and 1 % w/w) have been reported to yield white, opaque composite films, also with lowered WVP values (Pérez-Mateos, Montero & Gómez-Guillén, 2009). The maximum breaking strength decreased by 30-60 %, depending on  
15 the amount of oil added. These researchers also reported that the higher the oil concentration in the film, the lower the protein fraction in the water-soluble matter, most likely the outcome of gelatin-oil interactions in the film resulting in protein insolubilization. Lipid-protein interactions (hydrogen bonds, ester formation) as well as  
20 early oil oxidation observed by Fourier transform infrared (FTIR) spectroscopy were related to alterations in the structure of the composite gelatin-oil films. The changes were more pronounced after storage of the film at room temperature for one month and caused a slight decrease in the rheological and water vapour permeability. It is worth noting that cod-skin gelatin films, which were almost completely soluble in water,  
25 became rather insoluble with storage time. High solubility of edible films newly made from cod-skin gelatin was also reported by Piotrowska, Kolodziejka, Januszewska-Jozwiak & Wojtasz-Pajak (2005).

Fish gelatin has also been blended with a number of polysaccharides, such as gellan and *kappa*-carrageenan (Pranoto, Lee & Park, 2007), pectin (Liu, Liu, Fishman & Hicks, 2007) or chitosan (Kołodziejaska, Piotrowska, Bulge & Tylingo, 2006). Adding gellan or *kappa*-carrageenan increased the tensile strength and water vapour barrier  
5 properties of tilapia-skin gelatin films but at the same time made the films slightly darker (Pranoto et al., 2007). These researchers reported a low elongation value (5 %) for the tilapia-gelatin film, lower than that for mammalian-gelatin films. However, it should be noted that they made no mention of the use of any plasticizers, and in any case, elongation increased slightly on adding either gellan or *kappa*-carrageenan.  
10 Thermal (DSC) and FTIR analysis demonstrated effective interaction between the gelatin molecules and the polysaccharides, with the gellan being better at enhancing the films' mechanical and water barrier properties.

Composite films have also been prepared from type-B fish gelatin and citrus pectin,  
15 seeking to improve the physical properties of pectin-based edible films (Liu et al., 2007). Compared with pectin films, the composite films exhibited higher strength and lower water solubility and water transmission rate. The mechanical properties and water resistance were considerably improved by treating the composite films with glutaraldehyde/methanol, which brought about a reduction in the interstitial spaces  
20 among the macromolecules due to extensive chemical cross-linking.

The use of different cross-linking agents has been also reported for fish gelatin films. Yi, Kim, Bae, Whiteside & Park (2006) prepared films using a commercial high-molecular-weight cold-water fish gelatin plasticized with sorbitol by inducing enzymatic  
25 cross-linking with a microbial transglutaminase (MTGase). The tensile strength and oxygen permeability of the MTGase-modified films increased, while elongation decreased. The mechanical and barrier properties of the gelatin films were explainable in terms of the total free volume of the film matrix. Thanks to the triple-helix structures

present in gelatin molecules, the gelatin matrix is usually compact, resulting in low oxygen permeability. The intra and intermolecular covalent bonds formed by MTGase could increase the free volume of the polymer matrix by hindering helical structure formation. The lower number of helical structures could decrease the flexibility of the gelatin matrix, while the higher degree of cross-linking could increase its strength.

The chemical and enzymatic cross-linking induced, respectively, by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and MTGase has been shown to lower the solubility of cod gelatin-chitosan films, with chemically induced cross-linking being more effective than the enzymatic treatment (Kołodziejska et al., 2006; Kołodziejska & Piotrowska, 2007). Furthermore, enzymatic cross-linking increased the brittleness of the films, making the use of plasticizers necessary. In later work these same authors reported that adding glycerol in concentrations of up to 30 % did not change the solubility or WVP of MTGase-modified films but decreased tensile strength sharply while concomitantly increasing elongation (Kołodziejska & Piotrowska, 2007). The hydrophilic nature of the added plasticizers could raise the WVP and mask the effect of the cross-linking induced by the MTGase. These cross-linked gelatin-chitosan films are deemed to be completely biodegradable, since they have been found to be susceptible to degradation by proteinase N from *Bacillus subtilis*, a representative naturally occurring microorganism (Sztuka & Kołodziejska, 2008).

Chitosan has also been put forward as a valuable component for use in producing biodegradable packaging films, since it has been proved to be non-toxic, biodegradable, and biocompatible (Coma, Martial-Gros, Garreau, Copinet, Salin & Deschamps, 2002). This polymer of N-acetyl-D-glucosamine is obtained from natural chitin, one of the most abundant natural polymers in living organisms, present in crustaceans, insects, and fungi. A number of studies combining gelatin and chitosan (normally from crustacean shells) to produce edible films have been carried out

(Arvanitoyannis, Nakayama & Aiba, 1998b; Kołodziejska et al., 2006; Sztuka & Kołodziejska, 2008). Gelatin and chitosan have been found to interact mainly by means of ionic and hydrogen bonds (Taravel & Domard, 1995), thereby affecting the physical properties of the mixtures and giving rise to new potential medical and pharmaceutical applications for developing a new generation of prosthetic implants, wound dressings, artificial skin, contact lenses, controlled release drugs, surgical sutures, and the like (Sionkowska, Wisniewski, Skopinska, Kennedy & Wess, 2004).

Gelatin extracted from tuna skins by the procedure reported by Montero & Gómez-Guillén (2000) was used to produce a composite film with chitosan (95 % deacetylated) obtained from shrimp-shell chitin in a proportion of gelatin:chitosan of 2:1.5. The films were plasticized with glycerol + sorbitol (30 g sorbitol + glycerol per 100 g of gelatin + chitosan) (Gómez-Estaca, 2007). Dynamic analysis of viscoelasticity performed on the film-forming solutions showed appreciable interaction between the polymers, yielding stronger films with the tuna-skin gelatin than with a bovine-hide gelatin used for comparison. This interaction resulted in increased strength and decreased deformability and water solubility of the composite film as compared to a pure-gelatin film, though the WVP remained unaltered.

## 20 **Blends of fish gelatin and antimicrobial or antioxidant compounds**

To date chitosan has been widely used, not only because of its film-forming capabilities but also because of its antioxidant and antimicrobial properties (Coma et al., 2002; Huang, Mendis & Kim, 2005). A pig-skin gelatin and chitosan-based edible film was employed to enhance the storage life of cold-smoked sardine (*Sardina pilchardus*) (Gómez-Estaca, Montero, Giménez & Gómez-Guillén, 2007). Use of the film reduced both aerobic plate counts and H<sub>2</sub>S-producing microorganisms by 2-3 log cycles over the course of storage at 5 °C for 20 days compared with cold-smoked sardine not protected by the film. However, this film did not prevent lipid oxidation as measured by

the TBARs (thiobarbituric acid reactive substances) method and by the peroxide value. Chitosan films have been reported to inhibit both primary and secondary lipid oxidation in herring and Atlantic cod (Jeon, Kamil & Shahidi, 2002), mostly as a result of their good oxygen barrier properties. In addition, Xue, Yu, Hirata, Terao & Lin (1998) 5 reported that the antioxidant mechanism of chitosan could be chelating action of metal ions and/or bonding with lipids. Thus, presumably both these antioxidant mechanisms could be hindered as a result of chitosan-gelatin interactions in composite films.

A coating made from a blend of chitosan and megrim-skin gelatin was applied to chilled 10 cod patties to assess the coating's potential as a preservative (López-Caballero et al., 2005). Under refrigeration, the recommended storage conditions for fish patties, this blend was able to form a strong gel that acted as a thin protective barrier that melted away on cooking. The effect of the coating on rancidity was not conclusively determined due to the low TBARs values recorded in the untreated cod. However, the 15 coating did prevent against spoilage of the cod patties as reflected by a lower total volatile basic nitrogen value and by lower microbial counts, in particular counts of Gram-negative bacteria. The authors concluded that the coating provided good sensory properties and delayed fish spoilage.

20 Essential oils have been included in edible film formulations prepared from various film-forming polymers such as chitosan (Zivanovic et al., 2005) and milk proteins (Oussalah et al., 2004) and have shown promising results as antimicrobials for food packaging. Essential oils have also been included in fish-gelatin films in an attempt to improve the antimicrobial attributes of the films. Gómez-Estaca, López de Lacey, Gómez-Guillén, 25 López-Caballero, & Montero, (2009a) prepared films from a commercial catfish gelatin admixed with chitosan and clove essential oil and achieved good antimicrobial results against *Pseudomonas fluorescens*, *Lactobacillus acidophilus*, *Listeria innocua* and

*Escherichia coli in vitro*. This same formulation also delayed the total bacterial counts by 2 log cycles when used for storing raw sliced salmon chilled at 2 °C for 11 days.

Edible films with antimicrobial properties have been made from cold-water fish-skin  
5 gelatins with added lysozyme, a food-safe antimicrobial enzyme (Bower, Avena-Bustillos, Olsen, McHugh & Bechtel, 2006). The lysozyme-enhanced films appeared to exhibit a slight increase in water vapour permeability compared with control films, and they proved effective against Gram-positive bacteria like *Bacillus subtilis* and *Streptococcus cremoris*. However, these films did not inhibit the growth of *Escherichia*  
10 *coli*, because lysozyme is known not to penetrate the lipopolysaccharide layer of Gram-negative bacteria (Masschalck & Michiels, 2003).

Rancid off-flavors and undesirable chemical compounds in foods result mostly from lipid oxidation, deteriorating quality and shortening shelf life. Because of “clean  
15 labelling” concerns, there is growing interest in using plant extracts as natural sources of polyphenolic compounds for food preservation in place of synthetic antioxidants. Polyphenols readily mix with edible gelatin-based film formulations to make active packaging materials. For instance, the germ plasm of murta (*Ugni molinae* Turcz), a wild shrub growing in southern Chile, has recently been characterized as a source of  
20 polyphenol antioxidants (Rubilar, Pinelo, Ihl, Scheuermann, Sineiro & Nuñez, 2006). Two aqueous extracts from the leaves of different murta ecotypes (Soloyo Grande and Soloyo Chico) were added to tuna-skin gelatin films (Gómez-Guillén et al., 2007). The high phenolic content of these extracts was found to tint the resulting composite films yellow-brown and to enhance their light barrier properties as determined by exposing  
25 the films to light at wavelengths ranging from 690 to 200 nm and measuring absorption. The higher polyphenol content of the Soloyo Chico ecotype increased the antioxidant activity of the film as measured by the FRAP (Ferric Reducing Ability of Plasma)

method but decreased the film's mechanical properties because of greater interaction between the polyphenols and the proteins.

Another study was performed using aqueous extracts of oregano (*Origanum vulgare*)  
5 and rosemary (*Rosmarinus officinalis*) prepared from freeze-dried leaves (Gómez-  
Estaca, Bravo, Gómez-Guillén, Alemán & Montero, 2009b). The films were prepared  
from tuna-skin gelatin, and the oregano and rosemary extracts were added to similar  
phenol concentrations. The extracts substantially increased the antioxidant activity of  
the films as measured by the FRAP method, and the oregano extract produced levels  
10 1.7 times higher than the rosemary extract. Since the total phenol concentrations were  
similar, the difference was attributed to the qualitative composition of the extracts. For  
instance, rosmarinic acid, the most abundant polyphenol in both extracts, was found to  
be more concentrated in the oregano extract than in the rosemary extract. The oregano  
extract also contained appreciable quantities of gallic acid and protocatechuic acid,  
15 whereas the rosemary extract contained chlorogenic acid. These differences affected  
both the antioxidant activity of the extracts themselves as well as the degree of gelatin-  
polyphenol interaction, which, based on determinations of the total quantities of  
phenolics in the films, was higher in the gelatin-rosemary film. In consequence, the  
potential antioxidant activity of the films containing the rosemary extract was lower than  
20 that of the films containing the oregano extract.

To ascertain the effect of gelatin-based films enriched with aqueous extracts of either  
oregano or rosemary on the shelf life of fish, an experiment was performed using cold-  
smoked sardine (*Sardina pilchardus*) under high pressure (300MPa/20°C/15min), alone  
25 or in combination with the active film (Gómez-Estaca et al., 2007). The uncovered fish  
exhibited a certain resistance to oxidation ensuing from the deposition of phenols  
during smoking. The phenol content and the resistance to oxidation of the smoked fish  
increased when the product was covered with the films containing the oregano or

rosemary extract. The effect was more pronounced when the films were used in association with high pressure, probably because of greater migration of antioxidant substances from the films. In conclusion, the edible films with the added plant extracts were found to be effective at reducing lipid oxidation levels in cold-smoked sardine  
5 during chilled storage. It is worth noting that the enrichment of gelatin films with vegetal extracts, especially those from the leaves of aromatic plants, may give certain flavor to the smoked fish, however, from an organoleptic point of view, it could be quiet acceptable for these kind of products.

10 In addition to polyphenolic plant extracts, there has also been growing interest in using other natural antioxidants, such as vitamin E ( $\alpha$ -tocopherol), in food systems. In this connection Jongjareonrak, Benjakul, Visessanguan & Tanaka (2008) characterized films with antioxidant properties made from bigeye snapper and brownstripe red snapper-skin gelatin that incorporated  $\alpha$ -tocopherol or BHT (butylated-hydroxy-  
15 toluene). For identical additive concentrations (200 ppm), the films containing  $\alpha$ -tocopherol displayed appreciably higher radical scavenging capacity (DPPH method) than the films containing BHT. FTIR analysis of the composite films revealed that the interactions between the two types of gelatin and the additives were different, resulting in different alterations in the secondary structure of the protein. In the case of the  $\alpha$ -  
20 tocopherol, these interactions brought about reductions in the mechanical properties and WVP. The films were also used to study their preventive effect on lard oxidation. The fish-skin gelatin films were postulated as functioning as a barrier to oxygen permeability at the lard's surface. In any case, no differences in TBARs values were observed between lard samples covered with gelatin films with and without  
25 antioxidants, which was attributed to low levels of additive release from the films.

## Conclusions

The physical properties of fish-gelatin films are highly dependent on gelatin attributes, which are in turn dependent not only on intrinsic properties related to the fish species used but also on the process employed to manufacture the gelatin. Appreciable differences in mechanical and water vapour barrier properties have been reported for  
5 gelatins made from cold-water (cod, salmon or Alaska Pollack) and warm-water (tilapia, carp or catfish) fish species, largely as a consequence of differing amino acid compositions, in particular the imino acid content (which is higher in warm-water species), which may govern overall film strength and flexibility. To this respect, films based on fish gelatin are usually more deformable than those based on mammalian  
10 gelatin. The molecular weight distribution, greatly affected by the gelatin manufacturing process, is also a key factor in determining mechanical properties. As a rule, the predominance of low-molecular-weight fragments in a given gelatin preparation will yield weaker, more highly deformable films, especially when plasticizers like sorbitol or glycerol are present in the film formulation.

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Film properties can be enhanced by adding a number of substances to the fish gelatin. Various proteins (soy protein isolate), oils (sunflower oil, fatty acids, essential oils), polysaccharides (gellan, *kappa*-carrageenan, pectin, chitosan) and cross-linkers (glutaraldehyde, MTGase, EDC) have been used to improve the rheological properties,  
20 barrier properties, and water resistance of composite fish-gelatin films. Furthermore, adding active compounds (chitosan, clove essential oil, lysozyme, aqueous extracts of murta, oregano or rosemary,  $\alpha$ -tocopherol) may confer specific antioxidant and/or antimicrobial capabilities that can be used to design active biodegradable packaging materials. In such cases, however, special attention needs to be paid to possible  
25 interactions within the film matrix, which may influence the release of active components and in consequence could potentially impair the antioxidant and antimicrobial properties of the resulting film.

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Table 1.- Extracting conditions and some gel properties (at 6.67% concentration) of skin gelatin from several marine species.

Species	Gel strength (g)	Gelling temperature (°C)	Melting temperature (°C)	Pre-treatment Extracting conditions	Reference
Atlantic Cod <i>Gadus morhua</i>	95			0.3% Sulfuric acid/Citric acid 0.7% 45°C - overnight	Gudmundsson & Hafsteinsson, 1997
Cod, Haddock, Pollack (Norland HMW fish gelatin)		4 <sup>1</sup> 10 <sup>1</sup>	13 <sup>1</sup> 16 <sup>1</sup>	Commercial type A gelatin	Haug et al., 2004
Atlantic Cod <i>Gadus morhua</i>	71	10		0.12M Sulfuric acid/0.005M Citric acid 56°C – 2h <i>idem</i>	Arnesen & Gildberg, 2007  <i>idem</i>
Atlantic salmon <i>Salmo salar</i>	108	12			
Alaska Pollack <i>Theragra chalcogramma</i>	98 <sup>2</sup> 217 <sup>2</sup>		21.2 <sup>2</sup> 16.1 <sup>2</sup>	0.1 M Calcium hydroxide/0.05M Acetic acid 50°C – 180 min	Zhou et al., 2006
Alaska Pollack <i>Theragra chalcogramma</i>	186 <sup>3</sup>	4.6		0.2N Sulfuric acid/0.7% citric acid 45°C - overnight	Avena-Bustillos et al., 2006
Alaskan pink salmon <i>Oncorhynchus gorbuscha</i>	216 <sup>3</sup>	5.3		<i>idem</i>	<i>idem</i>
Cod <i>Gadus morhua</i>	72	12	13	0.05M acetic acid 45°C - overnight <i>idem</i>	Gómez-Guillén et al., 2002  <i>idem</i>
Dover sole <i>Solea vulgaris</i>	341	19	21	<i>idem</i>	<i>idem</i>
Megrim <i>Lepidorhombus boscii</i>	353	17	21	<i>idem</i>	<i>idem</i>
Hake <i>Merluccius merluccius</i>	103	11	15	<i>idem</i>	<i>idem</i>
Squid <i>Dosidicus gigas</i>	10	13	19	<i>idem</i>	<i>idem</i>
Squid <i>Dosidicus gigas</i>	147	12	17	Pepsin (1/8000, w/w) + 0.05M acetic acid 60°C – overnight	Giménez et al., 2008

Bigeye snapper <i>Priacanthus macracanthus</i>	106			0.05 M Acetic acid 45°C – 12h	Jongjareonrak et al., 2006c
Brownstripe red snapper <i>Lutjanus vitta</i>	219			<i>idem</i>	<i>idem</i>
Nile perch <i>Lates niloticus</i>	217 <sup>4</sup> 240 <sup>4</sup>			Concentrated (ns) Sulfuric acid 50°C - ns	Muyonga et al., 2004
Black tilapia <i>Oreochromis mossambicus</i>	181	28.9		0.2% Sulfuric acid/1% citric acid 45°C – 12h	Jamilah & Harvinder, 2002
Red tilapia <i>Oreochromis nilotica</i>	128	22.4		<i>idem</i>	<i>idem</i>
Shark (cartilage) <i>Isurus oxyrinchus</i>	112 <sup>5</sup>			1.6N Sodium hydroxide 65°C - 3.4h	Cho et al., 2004
Yellowfin tuna <i>Thunnus albacares</i>	426	18.7	24.3	1.9% sodium hydroxide 58°C – 4.7h	Cho et al., 2005
Skipjack tuna <i>Katsuwonus pelamis</i>	126			0.2M Acetic acid 50°C – 12h	Aewsiri et al., 2008
Skate <i>Raja Kenojei</i>	74	16	19	1.5% Calcium hydroxide 50° - 3h	Cho et al., 2006
Channel catfish <i>Ictalurus punctatus</i>	252 <sup>6</sup>			0.2M sodium hydroxide/0.115 M Acetic acid 55°C – 180min	Yang et al., 2007
Horse mackerel <i>Trachurus trachurus</i>	230	18.8 <sup>7</sup> 15.3 <sup>7</sup> 11.8 <sup>7</sup> 8.1 <sup>7</sup>		0.2% sulfuric acid/0.7% citric acid 45°C - overnight	Badii & Howell, 2006
Grass carp <i>Catenopharyngodon idella</i>	267	19.5	26.8	1.2% HCl 53°C – 5h	Kasankala et al., 2007
Pork	240 <sup>2</sup> 301 <sup>2</sup>		31.2 <sup>2</sup> 30.9 <sup>2</sup>	Commercial	Zhou et al., 2006
Bovine	216	23.8	33.8	Commercial	Cho et al., 2005

- (1) 10% and 30% gelatin solution, respectively  
(2) gels matured at 10°C and 2°C, respectively  
(3) gels matured at 2°C  
(4) gelatin extracted from young and adult fish, respectively  
(5) expressed in kPa  
(6) 3.3% gelatin solution

(7) 10%, 7%, 5% and 3% gelatin solution, respectively  
ns: not specified

Table 2.- Amino acid composition (expressed as No. residues/1000 residues) of several marine and mammalian gelatins

Amino acid	Atlantic Cod	Atlantic salmon	Alaska Pollack	Halibut	Dover sole	Carp	Tilapia	Tuna	Nile perch	Giant Squid	Bigeye snapper	Brownstripe red snapper	Bovine	Pork
Hyp	50	60	59	64	61	73	79	78	8.05	80	91	84	83	91
Asx	52	54	54	51	48	47	48	44	5.91	65	61	56	46	46
Thr	25	23	24	22	20	27	24	21	3.04	24	32	31	33	18
Ser	64	46	65	69	44	43	35	48	3.34	37	38	39	39	35
Glx	78	74	75	96	72	74	69	71	9.85	90	103	105	74	72
Pro	106	106	96	86	113	124	119	107	12.0	95	134	141	127	132
Gly	344	366	365	356	352	317	347	336	22.1	327	193	204	342	330
Ala	96	104	114	108	122	120	123	119	10.1	89	103	108	113	112
Val	18	15	11	19	17	19	15	28	2.35	21	21	17	19	26
Met	17	18	12	7	10	12	9	16	1.58	13	17	15	4	4
Ile	11	9	9	8	8	12	8	7	1.26	18	10	9	11	10
Leu	22	19	19	20	21	25	23	21	2.83	32	27	25	24	24
Tyr	3	3	2	3	3	3	2	3	0.86	6	6	5	4	3
Phe	16	13	10	12	14	14	13	13	2.31	10	21	20	12	14
His	8	13	6	6	8	4.5	6	7	1.10	8	12	9	4	4
Hyl	6	nd	nd	nd	5	4.5	8	6	1.43	15	nd	nd	5	6
Lys	29	24	27	23	27	27	25	25	3.77	13	38	38	25	27
Arg	56	53	51	50	55	53	47	52	8.15	57	92	94	52	49
Reference	(1)	(2)	(3)	(4)	(1)	(5)	(6)	(7)	(8)	(1)	(9)	(9)	(7)	(3)

- (1) Gómez-Guillén et al., 2002
- (2) Arnesen & Gildberg, 2007
- (3) Zhou et al., 2006
- (4) Carvalho et al., 2008
- (5) Norland, 1990
- (6) Sarabia et al., 2000
- (7) Gómez-Estaca et al., 2008
- (8) Muyonga et al., 2004<sup>a</sup> (expressed as g/ 100 g protein)
- (9) Jongjareonrak et al., 2006c

