In: Natural Polymers: Derivatives, Blends and Composites Vol. I ISBN: 978-1 -63485-831-1
Editors: Saiqa Ikram and Shakeel Ahmed

Chapter 10

GELATIN: A COMPREHENSIVE REPORT COVERING ITS INDISPENSABLE ASPECTS

Wahid Ul Rehman*, Aasim Majeed, Richa Mehra, Satej Bhushan, Pooja Rani, Khem Chand Saini and Felix Bast
Molecular Genetics Laboratory, Central University of Punjab, Bathinda, Punjab, India

ABSTRACT

Gelatin is a collagen derived product, obtained by incomplete hydrolysis of collagen procured from skin, bones and connective tissues of animals and exhibits flavourlessness, colourlessness and translucency. It is commonly utilised as a gelling agent and also as additive in food, drugs, cosmetics, paints, matches, photographic films and foam stabilizer. The overall amino acid composition and proportion of gelatin varies according to the source of raw material, however glycine, proline and hyrdoxyproline constitute almost 60% of the total amino acid residues while cysteine is absent. Besides being used in food industries, gelatin based composites and blends are used in pharmacy for manufacturing biocompatible gelatin scraps, tissue engineering films and controlled drug delivery systems. This chapter focuses on the physio-chemical properties of gelatin, its extraction, composites and blends.

Keywords: gelatin, collagen, food industries, hydrogels, composites

1. INTRODUCTION

Gelatin (Latin: *gelatos* meaning "stiff") is a translucent, dismal, flavourless and colourless biopolymer obtained from collagenous animal products. It is commonly utilised as a gelling agent and is also used as additive in food, drugs, cosmetics, paints, matches, photographic films and foam stabilizer. Gelatin gels have a lower melting temperature (below 35°C) than those obtained from starch, alginate, pectin and agar, thus, making it suitable for

* Corresponding Author address: Molecular Genetics Laboratory, Central University of Punjab, Bathinda-151001, Punjab, India; Email: wahidulrehman@gmail.com.

food industry where flavour release is desirable (Djagny et al., 2001). The process of extraction of gelatin from collagen has been developing for over 150 years and has taken advantage in food technology and food engineering (Gómez-guillœn and Montero, 2001; Mariod and Adam, 2013). It is obtained by incomplete hydrolysis of collagen procured from skin, bones and connective tissues of animals. As it is derived from a complex molecule, its degradation is totally arbitrary and generates a variety of peptide chain species. Hence, most gelatin arrangements are non-homogenous leading to varied molecular weight s (Liu et al., 2015). Gelatin has a very wide range of functional properties which promotes its utility as a major ingredient in many food items (Gibbs, 1999; Karim and Bhat, 2009). Gelatin is used in desserts, ice creams, yoghurt, confectionary juices, jellies and marshmallow. There have been various important new product coming into the market that are developed from the gelatin like low fat products, spreads, micro particulation of proteins, microencapsulation of proteins, fining of wines, fruit containing beverages (Gibbs, 1999; Gómez-Guillén et al., 2007). Global gelatin demand has been increasing over the years recently and so are the research initiatives for gelatin manufacturing. Some hydrolyzed gelatins are non-gelling proteins. Now a days instant gelatins, which are soluble in cold water, are gaining attention in food industry. In all of these applications the chemistry and functionality of gelatin is a key factor (Karim and Bhat, 2009). So a better understanding of structure and chemistry gelatin is required to understand its functionality and its utilization in various industrial products.

2. PROPERTIES OF GELATIN

2.1. Gelatin Structure

Gelatin is very similar to synthetic high polymers, shows a rather wide molecular weight distribution (Ward, 1954). It is interesting to note that gelatin can form a large variety of structures ranging from the simplest globular structure which is typical of amorphous polymers, to supermolecular structures and well-developed fibrillary structure with various intermediate states as well. The differences in supermolecular structures is reflected in the physicomechanical properties of the gelatin materials (Courts, 1959).

The Structural diversity of gelatin chains consequently affects its properties. Among the unique features of gelatin, the presence of acidic and basic functional groups in the macromolecules configuration, its ability to form a triple-stranded helical structure not observed in other synthetic polymers, the rate of which depends on many factors such as the presence of iminoacids, gelatin molecular weight, the presence of covalent cross-bonds, and the gelatin concentration in the solution (Harrington and Von Hippel, 1961; Pchelin, Izmailova, and Merzlov, 1964) and its interaction with water which is different to that observed with synthetic hydrophilic polymers.

Gelatin is manufactured by denaturation of native structure of collagen. The collagen conformation is distorted on heating and partially recovered during cooling process. Thus, the amino acid composition of gelatin remains largely close to collagen with a few changes due to manufacturing process. For instance, alkaline pre-treatment process deaminates glutamine to glutamic acid and asparagine to aspartic acid; consequently the proportion of glutamic acid and aspartic acid is more in Type B gelatin (Boran and Regenstein, 2010). Upon gelation,

water gets trapped in the mesh of helix fibres and the structure of gelatin changes. Helix chains undergo different space rearrangements and interactions depending upon the state of gel. For example, in a second order reaction, one double stranded structure can be formed either by two α-chains or by single α-chain forming loop. Likewise, in a third order reaction, a triple stranded structure can be formed either by three different α-chains or by two α-chain with one forming loop or by single α-chain forming two loops (Duconseille et al., 2015).

2.2. Amino Acid Composition

Though definite amino acid composition of gelatin is not clearly known, it is claimed that glycine, proline and hyrdoxyproline constitute almost 60% of the total amino acid residues in collagen as well as gelatin. The basic amino acid sequence of collagen as well as gelatin is "Gly-X-Y" where X is proline and Y is hyrdoxyproline. Cysteine is absent in gelatin. However, the overall amino acid composition and proportion varies depending upon the source of raw material (Eastoe, 1955).

2.3. Molecular Weight

The molecular weight and other physical properties of gelatin not only depend on its amino acid composition but also on the relative number of α, β, γ, high and low molecular weight fragments. Large quantity of β and γ chains have been reported to exert negative effects on the functional properties of the gelatin viz. lowering melting and setting points and lowering viscosity (Olijve et al., 2001). The severity of extraction treatment affects the molecular weight profile of gelatin. Gelatin obtained from high temperature treatment exhibits lower molecular weight profiles than that obtained from lower temperature. Thus, the molecular weight profile of gelatin primarily depends on the extraction process.

2.4. Chemical Interactions

Glycine molecules are mainly stabilized by hydrogen bonds, covalent bonds, hydrophobic interactions and electrostatic interactions. The double stranded or triple helices are stabilized by hydrogen bonding between glycine residues occurring after every third amino acid residue in the α-chains. The H-atoms of glycine are situated inside the triple helix and form a weak interaction with the O-atom of the carboxyl group. Water molecules are also known to be involved in hydrogen bonding of the gelatin network (Vaca Chávez et al., 2006). It has been reported that gelatin gels in deuterium oxide are stabilized by both hydrogen bonds of –NH group of one amino gelatin chain with –CO group of other chain and hydrogen bonds of water molecules with gelatin chains (Oakenfull and Scott, 2003). Likewise, hyrdoxyproline also forms hydrogen bonds with water molecules. However, the exact number and types of hydrogen bonds is still not clearly defined.

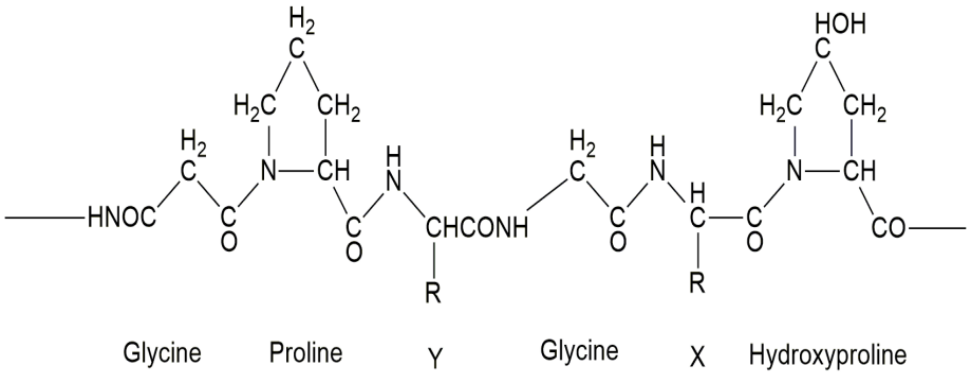

Figure 1. Chemical (configuration) interactions of gelatin Adopted from (Ofori R. A. 1999).

Despite the thermal and chemical treatments, covalent bonds still remain within gelatin molecules and impart it mechanical properties. These cross-links are favoured by high temperature, humidity, UV-light and some chemical compounds like formaldehyde and reducing sugars (Bhat and Karim, 2009; Singh et al., 2002). The types of cross links reported in gelatin include pentosidine, desmosine, methlylene, aminal and aminoglycoside bonds (Haug et al., 2009). Hydrophobic interactions have an insignificant role in triple helix structures, but they are proposed to have a major impact in β-sheet structures. Xu et al., (2012) demonstrated using UV analysis that hydrophobic interactions have a positive relation with increasing gelatin concentration and outcompete hydrogen bonds (Xu et al., 2012). Similarly electrostatic interactions between cationic and anionic groups of protein molecules contribute in gelatin stability. These interactions are, however, influenced by pH and salt concentrations. A study revealed that degree of swelling of gelatin gels was influenced by the degree of ionization of the solution which was attributed to the generation of ion pairs between counter ions and network charges (Miyawaki et al., 2003).

Other types of cross links have also been found and characterized in the gelatin. For example, free amine group of any lysine residue may react with an aldehyde group forming a hydroxymethylamine that yields a water molecule to generate a secondary aldimine. This imine group further reacts with another lysine residue and generates dimethylene ether which undergoes rearrangements and links two lysine residues with methylene bond (Haug et al., 2009). Thus, the cross links in gelatin involves multiple interactions which occur intra-molecular or inter-molecular regions of the helices. However, there are a few cross links which are still under discussion viz. disulphide linkages and pyridinoline.

2.5. Gelatin Derived Peptides

An extensive enzymatic hydrolysis of gelatin leads to the formation of gelatin hydrolysates. It is commonly used to improve the nutritional and functional properties of food proteins. Commercial proteases like pepsin, alcalase, trypsin , papain, α-chmyotrypsin, neutrase, properase E, savinase, protamex, NS37005 and endogenous fish proteases have been utilized for the production of fish gelatin hydrolysates (Himaya et al., 2012; Zhang et al., 2012). The molecular weights of gelatin hydrolysates also varies with gelatin sources, enzymes used and hydrolysis conditions. A few gelatin derived peptides have been identified

and sequenced. For example, Alaska Pollock skin gelatin derived peptide contains glycine at every third position (Gly-Glu-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly) like that from gelatin (Kim et al., 2001). Another peptide derived from hoki skin gelatin contains typical Gly-X-Y repeat unit where X is mostly Proline and Y is leucine or histidine (Mendis et al., 2005). A few peptides without the Gly-X-Y repeats were also identified viz. Japanese flounder skins (Gly-Gly-Phe-Asp-Met-Gly), Pacific cod skins (Thr-Gly-Gly-Gly-Asn-Val) and Alaska Pollock skins (Ser-Cys-His) (Himaya et al., 2012; Kim et al., 2001). However, there are chances that some of these peptides were actually impurities which were not properly removed during extraction.

3. SOURCES OF GELATIN

Gelatin can be obtained from any collagen containing tissue. It was first time extracted from the pig skin and is still being used widely at commercial scale. The most commonly used raw materials for gelatin are skins, bones and cartilages of mammals like porcine and bovine, however, alternative sources include marine sources, especially, fishes and fowls. Around 45% of global gelatin production is procured from pork skin, followed by bovine hides (30%) and bones of bovine and porcine (23%) (Baziwane and He, 2003). The two types of mammalian gelatins i.e., type A (from bovine) and type B (from porcine) contain different components with molecular weight varying from 10 to 400 kDa. A strong correlation between average molecular weight and gel strength is also suggested. Despite being the major contributor in gelatin industry, mammalian gelatin has been facing scepticism amongst consumers due to health-related and socio-cultural concerns. Gelatin market crippled after the outbreaks of bovine spongiform encephalopathy (BSE) and foot mouth diseases in light of public health concerns (Mariod, 2016).

Recently, fish gelatin gained great interest as an alternative to mammalian gelatin, eliminating the risk of bovine and porcine diseases. Another advantage of fish gelatin is that it is acceptable in Islam and faces minimal restrictions in Hinduism and Judaism. Furthermore, it can be obtained from the by-products of fishing industries and reduces the pollution burden. Post-filleting waste from fish industries accounts for almost 75% of the catch weight, 30% of which comprises skin and bones that can be utilized for gelatin production (Boran and Regenstein, 2010). In light of sustainable development, the productive use of fish waste is necessary and is expected to increase in near future (Karim and Bhat, 2009). Many fish species like Atlantic salmon, Cod, Hake, Sin Croaker, Lumpfish, Megrium, Tilapia, Allaska pollock, Yellow fish tuna, Nile perch, Rainbow trout, Black and red tilapia have been investigated for gelatin extraction and many more are still being investigated (Feng et al., 2014; Niu et al., 2013; Wu et al., 2013).

Insect gelatin is yet another alternative for mammalian gelatin. The watermelon bug, *Coridius viduatus* (formerly *Aspongopus viduatus*) and sorghum bug, *Agonoscelis versicoloratus* (formerly *Agnoscelis pubescens*), are commonly used as a source of edible oil and medicinal products in Sudan. The biochemical analyses revealed that crude protein content of these bugs is 27.0 and 28.2%, respectively and both contained 16 essential amino acids (Mariod and Fadul, 2014). However, as per the recommended amino acid profiles by FAO and WHO, these proteins are of medium quality. SDS-PAGE patterns of both the insect

gelatins contained 40 kDa protein as the main component. Scanning electron microscope images showed that gelatin network of melon bug is finest with smaller voids than sorghum bug. Apart from gelatin, the edible oils obtained from these bugs can also be used to prepare biodiesel with H_2SO_4 catalysed reactions (Mariod, 2013).

4. EXTRACTION OF GELATIN

Gelatin is obtained from the hydrolysis of collagen. There are several processes for the gelatin extraction depending up on the source and type of tissue. The principle of gelatin production is to remove interfering moieties and to convert water-insoluble collagen to soluble gelatin form. The net yield of gelatin depends on the parameters of extraction protocol like pH, temperature, pressure and treatment time. The general layout is discussed below:

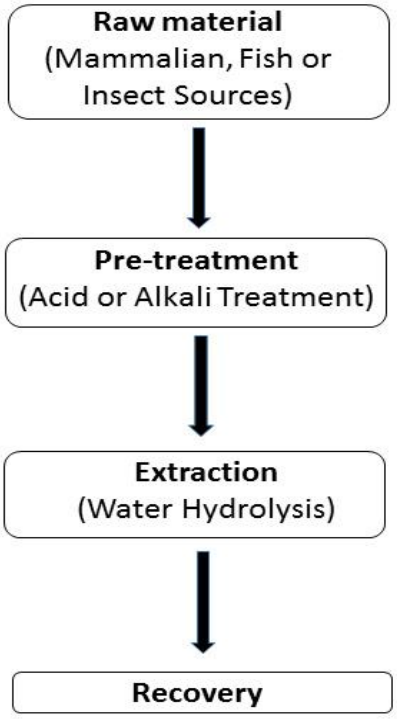

Figure 2. General layout of gelatin production.

4.1. Pre-Treatment

Raw materials are thoroughly washed to remove surface contaminants. Bones are processed differently as after washing, grinding and rewashing, crushed bone chips are subjected to acid solutions (4-7% HCl) for atleast two days. The samples are, later, treated with either acid or alkali to weaken the collagen framework by breaking intramolecular cross-linkages. Skins are preferably treated with acid while bones are treated with alkali. Gelatin produced by acid treatment leads to Type-A gelatin and that by alkali treatment becomes Type-B gelatin. In acid treatment, clean and hydrated raw material is sopped in cold dilute

acid solution (pH 1.5-3.0) for 18-24 hrs as per the sample size and thickness. The commonly preferred choice of acid is citric acid, as it does not impart objectionable colour and odour to gelatin unlike acetic acid. After acid treatment, samples are washed under running water and neutralised (Boran and Regenstein, 2010; Haug et al., 2009). While in alkali treatment, bones are first demineralised with a mild acid to get rid of non-collagenous components and placed in liming pits or vats and sopped in hydrated lime solution for at least 20 days that can extend up to 60 months depending on the sample properties. For hides and skins caustic soda solution is more suitable for shorter time period. After the treatment samples are thoroughly washed under running water and neutralised for subsequent steps. Isoelectric point of Type- A gelatin (9.4) is higher as compared to Type-B (4.8) as mild acid treatments do not remove amide nitrogen of asparagine and glutamine (Mariod and Adam, 2013).

4.2. Extraction

The extraction procedure for both acid and alkali treated samples is similar and involves a series of steps of water extraction at controlled temperatures. It is the most important step in gelatin production and determines the yield of gelatin. Mostly, multiple extractions are done using water with progressively increasing temperatures (5-10° rise) ranging from 50-100°C. Gelatin fractions obtained from the low temperature extraction have minimal degradation while subsequent fractions have more variable degradation products with varying molecular weights. Different combinations of pre-treatments and extractions result in final gelatin product which is a mixture of polypeptide chains of different compositions and molecular weights (Mariod, 2016). Good quality gelatin with standard average molecular weight and gel strength is obtained from low temperature extractions. High temperature extractions generate more coloured product containing depolymerised gelatin. This coloured product is due to the 'Maillard reaction' between α-amino groups of amino acids of gelatin and carbohydrate residues in the sample (Hoque et al., 2010).

4.3. Recovery and Refinement

The gelatin solution obtained after extraction is clarified using lamellar clarifier, an inclined plate clarifier to remove particulate matter, and filtered. The filtrate is then deionized using ion exchangers and concentrated to attain standard viscosity. The concentrate is sterilized by plate heat exchangers and steam sterilization. This sterilized gelatin is then cooled to form a gel which is later extruded to produce 'gelatin noodles' followed by drying and crushing. The final powdered gelatin is, thus, obtained with moisture content varying from 8-12% (Haug et al., 2009).

Several research groups have investigated design of experiments to optimise gelatin production and quality, including high pressure treatment, radiation exposure , different organic acids, protease inhibitors, pre-treatment time, extraction temperatures and water/sample ratio (Bhat and Karim, 2009; Chen et al., 2014; Khiari et al., 2015; Nalinanon et al., 2008).

5. TYPES OF GELATIN

There are mainly two types of gelatin, Gelatin A and gelatin B, which differ in the way of preparation. Gelatin A is processed by an acidic pretreatment before thermal denaturation, while gelatin B is processed by an alkaline pretreatment. The alkaline pretreatment is thought to convert amide residues of glutamine and asparagines into glutamic and aspartic acid, which leads to a 25% higher carboxylic acid content for gelatin B than for gelatin A. These gelatin gels were chemically crosslinked with *N*,*N*-(3-(dimethylamino)propyl)-*N*¢-ethyl carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). The chemical cross-linking of gelatin gels resulted in chemically cross-linked physical gelatin networks that probably forms network structure and called chemical gelatin gels (Fernandez- Dıaz et al., 2001; Kuijpers et al., 1999). It was shown that the properties of these chemical gelatin A and gelatin B gels differed with respect to initial lysozyme uptake from solution by the gels and the total release time. These differences are caused either by differences in lysozyme interaction with gelatin A or B or by differences in the network structures of both gelatin types (Ross-Murphy, 1992).

6. GELATIN BASED COMPOSITES AND BLENDS

Gelatin, a collagen derivative renewable resource, forms a key raw material in several industries. It can be used as such or in the form of hydrogels, blends and composites. Different manufacturing industries in the field of pharmacy, cosmetic, food etc. generate substantial quantity of gelatin scraps whose disposal is an environmental concern. Being high in carbon and nitrogen and due to their high swelling property in water, they create greater oxygen demand after reaching the drainage systems or treatment plants which imposes labor intensive and more expensive disposal management (Chiellini et al. 2001). Such waste gelatin can be used to form blends and composites for further use. The cast films produced from the blends and composites of the poly vinyl alcohol and sugarcane bagasse with pharmaceutical waste gelatin possess good mechanical and thermal characteristics thereby making them favorable to be used as biodegradable mulching films (Chiellini et al. 2001). The edible films generated at low temperature from chitosan and gelatin possess enhanced tensile strength and low rate of transmission for water vapors and gas (Arvanitoyannis et al. 1998a). Similar results were obtained by using hydroxypropyl starch and gelatin (Arvanitoyannis et al. 1998b). Using electrospinning technique and fluorinated alcohol of 2,2,2-trifluoroethanol (TFE) as a solvent, the superfine gelatin fibers generated showed varying morphology depending upon gelatin concentration. Further 10% v/w of gelatin/TFE solution after co-electro spun with 10% poly (ϵ caprolactone) resulted in gelatin poly (ϵ caprolactone) composite membranes which possess enhanced mechanical characteristics and wettability . Preliminary tests of these membranes in bone marrow stromal cell cultures showed that they have excellent scaffolding properties where the cells not only attach to their surface but also migrate through it. Hence they act as a good candidate to be be used in tissue engineering applications (Zhang et al. 2005). Nanobiocomposites generated from gelatin plus bimetallic silver gold nanoparticles exhibited biodegradable properties plus enhanced tensile strength and Young's modulus. Further these nanocomposites showed positive results for fibroblast cell tissue culture and are claimed to be used as scaffold in tissue engineering experiments

(Mandal and Sastry 2014). Bone replacement therapy is in practice nowadays. For a material to be used in such applications, it should be biocompatible and dissolved or reabsorbed during the growth of the bone. Gelatin being less antigenic than collagen, biocompatible and biodegradable is also used as a scaffold for bone replacing experiments (Chen et al. 2011). Composites of gelatin and Cu_2S and CdS nanoparticles show sensitivity to different gasses and can be used as gas sensors and detectors (Murdov et al. 2007). Gelatin based composites are also used in drug delivery systems. Genipin crosslinked gelatin composite gel loaded with indomethacin, a hydrophobic anti-inflammatory drug, showed controlled release of the drug and biocompatibility with tissue culture experiments (Thakur et al. 2011). Chitosan/gelatin composite films are claimed to suitable for contact lenses because they possess greater transparency, flexibility, biocompatibility, and increased solute and oxygen permeability (Xin-Yuan et al. 2004).

7. GELATIN BASED HYDROGEL

Gelatin hydrogels are formed by physical crosslinking in water above a certain concentration (around 2% w/v), and below 30-35 ^{0}C. Gelatin molecules during the process undergo aggregate, and conformational changes from a random coil to form a triple helix, and intermolecular hydrogen bonds form between the large fractions of gelatin chains. The non-covalent associations are broken easily at higher temperatures more than 30-35 ^{0}C, thereby destroying the physical network (Zhao et al. 2006; Bode et al. 2011; Peña et al. 2010). The gelatin hydrogels have poor mechanical strength, low elasticity, and low shape stability (Dash et al. 2013), which limit their biomedical applications at physiological temperatures (37 ^{0}C). To increases its stability and mechanical properties, the gelatin gel is covalently crosslinked by small chemicals such as glutaraldehyde, carbodiimides, and formaldehyde, which couple the carboxyl groups with amino groups, and formed stable amide bonds (Kuijpers et al. 1999). The cell attachment was observed by culturing human mesenchymal stem cells, which are obtained adult stem cells that show extensive and significant use in biomedical applications (Xing et al. 2010).

CONCLUSION

Although gelatin has wide range of applications in cosmetic and food industries, being used in in desserts, ice creams, yoghurt, confectionary juices, jellies and marshmallow, low fat products, spreads, micro particulation of proteins, microencapsulation of proteins, fining of wines, fruit containing beverages, yet it raises certain ethical concerns in some sections of the society. Being obtained from the animal origin products, it raises concern in vegetarian sect of the society and when obtained from pig products, it certainly a concern in the Muslim sect.

REFERENCES

Arvanitoyannis, Ioannis S., Atsuyoshi Nakayama, and Sei-ichi Aiba. 1998. "Chitosan and gelatin based edible films: state diagrams, mechanical and permeation properties." *Carbohydrate polymers* 37 (May): 371-382.

Arvanitoyannis, Ioannis, Atsuyoshi Nakayama, and Sei-ichi Aiba. 1998. "Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water." *Carbohydrate Polymers* 36 (July): 105-119.

Baziwane, David, and Qian He. 2003. "Gelatin: the paramount food additive." *Food Reviews International* 19 (4): 423-435.

Bhat, Rajeev, and A. A. Karim. 2009."Ultraviolet irradiation improves gel strength of fish gelatin." *Food chemistry* 113 (4): 1160-1164.

Buice, Jr, Robert G., Thomas B. Gold, Robert A. Lodder, and George A. Digenis. 1995. "Determination of moisture in intact gelatin capsules by near-infrared spectrophotometry." *Pharmaceutical research* 12 (1): 161-163.

Chen, Kuo-Yu and Yao, Chun-Hsu. 2011. "Repair of bone defects with gelatin based composites: a review." *Biomedicine* 1 (1): 29-32.

Chen, Liqing, Liang Ma, Mengrou Zhou, Yi Liu, and Yuhao Zhang. 2014. "Effects of pressure on gelatinization of collagen and properties of extracted gelatins." *Food Hydrocolloids* 36 (May): 316-322.

Chiellini, Emo, Patrizia Cinelli, Elizabeth Grillo Fernandes, El-Refaie S. Kenawy, and Andrea Lazzeri. 2001. "Gelatin-based blends and composites. Morphological and thermal mechanical characterization." *Biomacromolecules* 2 (3): 806-811.

Cole, C. G. B., and A. E. J. McGill. 1988. "Technical Note: Effect of animal age and conditioning method on the conversion of bovine hide into gelatine." *International Journal of Food Science and Technology* 23 (5): 525-529.

Courts, A. 1959. "The N-terminal amino acid residues of gelatin. 4. Chain weight and rigidity relationship in fractionated gelatins." *Biochemical Journal* 73 (4): 596.

Dickinson, E., and G. Lopez. 2001. "Comparison of the emulsifying properties of fish gelatin and commercial milk proteins." *Journal of food science* 66 (1): 118-123.

Djabourov, Madeline, Jean Maquet, Hélène Theveneau, Jacques Leblond, and Pierre Papon. 1985. "Kinetics of gelation of aqueous gelatin solutions." *British polymer journal* 17 (2): 169-174.

Djagny, Kodjo Boady, Zhang Wang, and Shiying Xu. 2001. "Gelatin: a valuable protein for food and pharmaceutical industries: review." *Critical Reviews in Food Science and Nutrition* 41 (6): 481-492.

F. Gibbs, Selim Kermasha, Inteaz Alli, Catherine N. Mulligan, Bernard. 1999."Encapsulation in the food industry: a review. " *International journal of food sciences and nutrition* 50 (3): 213-224.

Feng, Xiao, Shaojuan Lai, Hongshun Yang, Charles C. Muscoplat, Carla Carlson, S. Mundi, and Rotimi E. Aluko et al. 2014. "Sustainable seafood processing: utilisation of fish gelatin."*Austin Journal of Nutrition and Food Sciences* (2): 2.

Fernandez-Dıaz, M. D., P. Montero, and M. C. Gomez-Guillen. 2001. "Gel properties of collagens from skins of cod (Gadus morhua) and hake (Merluccius merluccius) and their modification by the coenhancers magnesium sulphate, glycerol and transglutaminase." *Food Chemistry* 74 (2): 161-167.

Gold, Thomas B., Robert G. Buice Jr, Robert A. Lodder, and George A. Digenis. 1997. "Determination of extent of formaldehyde-induced crosslinking in hard gelatin capsules by near-infrared spectrophotometry." *Pharmaceutical research* 14 (8): 1046-1050.

Gómez-Guillén, M. C., M. Ihl, V. Bifani, A. Silva, and P. Montero. 2007. "Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (Ugni molinae Turcz)." *Food Hydrocolloids* 21 (7): 1133-1143.

Gómez-Guillén, M. C., J. Turnay, M. D. Fernández-Dıaz, N. Ulmo, M. A. Lizarbe, and P. Montero. 2002. "Structural and physical properties of gelatin extracted from different marine species: a comparative study." *Food Hydrocolloids* 16 (1): 25-34.

Gómez-guillœn, M. C., and Pilar Montero. 2001. "Extraction of gelatin from megrim (Lepidorhombus boscii) skins with several organic acids." *Journal of Food Science* 66 (2): 213-216.

Grossman, Shlomo, and Margalit Bergman. 1992. "Process for the production of gelatin from fish skins." U.S. Patent 5,093,474, issued March 3.

Harrington, William F., and Peter H. Von Hippel. 1961. "The structure of collagen and gelatin." *Adv Protein Chem* 16 (Jan): 1-138.

Harris, P. 1990. "Gelatin in Food Gels." 233-289.

Haug, Ingvild J., Kurt I. Draget, and Olav Smidsrød. 2004. "Physical and rheological properties of fish gelatin compared to mammalian gelatin." *Food hydrocolloids* 18 (2): 203-213.

Haug, I. J., K. I. Draget, G. O. Phillips, and P. A. Williams. 2009. "Gelatin." *Handbook of hydrocolloids* ISBN 978-1-84569-414-2: 142-163.

Hoque, Md Sazedul, Soottawat Benjakul, and Thummanoon Prodpran. 2010. "Effect of heat treatment of film-forming solution on the properties of film from cuttlefish (Sepia pharaonis) skin gelatin." *Journal of Food Engineering* 96 (1): 66-73.

Hudson, Christopher B. 1994."Gelatine—Relating Structure and Chemistry to Functionality." *Food Hydrocolloids* 347-354. Springer US.

Jonas, Richard A., Gerhard Ziemer, Frederick J. Schoen, Lewis Britton, and Aldo R. Castaneda. 1988. "A new sealant for knitted Dacron prostheses: minimally cross-linked gelatin." *Journal of vascular surgery* 7 (3): 414-419.

Karim, A. A., and Rajeev Bhat. 2009. "Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins." *Food hydrocolloids* 23 (3): 563-576.

Karim, A. A., and Rajeev Bhat. 2008. "Gelatin alternatives for the food industry: recent developments, challenges and prospects." *Trends in food science and technology* 19 (12): 644-656.

Karim, A. A., and Rajeev Bhat. 2009. "Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins." *Food hydrocolloids* 23 (3): 563-576.

Khiari, Zied, Daniel Rico, Ana Belen Martin-Diana, and Catherine Barry-Ryan. 2015. "Characterization of Blue Whiting Skin Gelatines Extracted After Pretreatment with Different Organic Acids." *Journal of Aquatic Food Product Technology* 24 (6): 546-555.

Koli, Jayappa M., Subrata Basu, Binay B. Nayak, Surendra B. Patange, Ashif U. Pagarkar, and Venkateshwarlu Gudipati. 2012. "Functional characteristics of gelatin extracted from skin and bone of Tiger-toothed croaker (Otolithes ruber) and Pink perch (Nemipterus japonicus)." *Food and Bioproducts Processing* 90 (3): 555-562.

Kopp, J., Madeleine Bonnet, and J. P. Renou. 1990. "Effect of collagen crosslinking on collagen-water interactions (a DSC investigation)." *Matrix* 9 (6): 443-450.

Kozlov, P. V., and G. I. Burdygina. 1983. "The structure and properties of solid gelatin and the principles of their modification." *Polymer* 24 (6): 651-666.

Kuijpers, A. J., G. H. M. Engbers, J. Feijen, S. C. De Smedt, T. K. L. Meyvis, Jo Demeester, J. Krijgsveld, S. A. J. Zaat, and J. Dankert. 1999. "Characterization of the network structure of carbodiimide cross-linked gelatin gels." *Macromolecules* 32 (10): 3325-3333.

Lim, Ying Pei, and Abdul Wahab Mohammad. 2011. "Physicochemical properties of mammalian gelatin in relation to membrane process requirement." *Food and Bioprocess Technology* 4(2): 304-311.

Lou, Yan, and Michael J. Groves. 1995. "The Use of Gelatin Microparticles to Delay the Release of Readily Water-soluble Materials." *Journal of pharmacy and pharmacology* 47 (2): 97-102.

Mandal, A and Sastry, TP. 2014. "Fabrication of gelatin based composites containing bimetallic Ag-Au nanoparticles for biomedical applications." *International Journal of Innovative Research in Science Engineering and Technology.* 3 (5): 12463-12473.

Maquet, J., H. Theveneau, M. Djabourov, J. Leblond, and P. Papon. 1986. "State of water in gelatin solutions and gels: An 1H nmr investigation." *Polymer* 27 (7): 1103-1110.

Mariod, Abdalbasit Adam, and Hadia Fadol Adam. 2013. "Review: gelatin, source, extraction and industrial applications." *Acta Sci. Pol., Technol. Aliment* 12 (2): 135-147.

Mariod, Abdalbasit Adam, and Hadia Fadul. 2014. "Extraction and characterization of gelatin from two edible Sudanese insects and its applications in ice cream making." *Food Science and Technology International*: doi: 10.1177/1082013214541137.

Mariod, Abdalbasit Adam. 2016. "Extraction, Purification, and Modification of Natural Polymers." In *Natural Polymers*: 63-91.

Mariod, Abdalbasit Adam. 2013. "Insect oil and protein: Biochemistry, food and other uses: Review." *Agricultural Sciences* 4 (9B): 76.

McCormick, R. "Exploiting the novel properties of pectin and gelatin gels. 1987. " *Prepared Foods* 5: 204-205.

Montero, P., and M. C. Gómez-Guillén. 2000. "Extracting conditions for megrim (Lepidorhombus boscii) skin collagen affect functional properties of the resulting gelatin." *Journal of Food Science* 65 (3): 434-438.

Morrison, N. A., R. C. Clark, Y. L. Chen, T. Talashek, and G. Sworn. 1999. "Gelatin alternatives for the food industry." In *Physical chemistry and industrial application of gellan gum* 127-131. Springer Berlin Heidelberg.

Muradov, M. B., M. A. Nuriev, and G. M. Eivazova. 2007. "Sensitivity of composites based on gelatin and nanoparticles Cu2S and CdS to vapors of some organic compounds." *Surface Engineering and Applied Electrochemistry* 43 (6): 512-515.

Nalinanon, Sitthipong, Soottawat Benjakul, Wonnop Visessanguan, and Hideki Kishimura. 2008. "Improvement of gelatin extraction from bigeye snapper skin using pepsin-aided process in combination with protease inhibitor." *Food Hydrocolloids* 22 (4): 615-622.

Niu, Lihong, Xin Zhou, Chuqiao Yuan, Yun Bai, Keqiang Lai, Fuxin Yang, and Yiqun Huang. 2013. "Characterization of tilapia (Oreochromis niloticus) skin gelatin extracted with alkaline and different acid pretreatments." *Food Hydrocolloids* 33 (2): 336-341.

Ofner III, Clyde M., and William A. Bubnis. 1996. "Chemical and swelling evaluations of amino group crosslinking in gelatin and modified gelatin matrices." *Pharmaceutical research* 13 (12): 1821-1827.

Ofori, Rosemary Anima. 1999. *Preparation of gelatin from fish skin by an enzyme aided process*.

Pchelin, V. A., V. N. Izmailova, and V. P. Merzlov. 1964. "Mutarotation, the conformation of the polypeptide chains, and structure-formation in gelatin solutions." *Polymer Science USSR* 5 (3): 526-533.

Pezron, I., M. Djabourov, and J. Leblond. 1991. "Conformation of gelatin chains in aqueous solutions: 1. A light and small-angle neutron scattering study." *Polymer* 32 (17): 3201-3210.

Poppe, J. 1997. "Gelatin." In *Thickening and gelling agents for food* 144-168. Springer US.

Rbii, Khalid, Olivier Surel, Noëlle Brambati, Anne-Marie Buchert, and Frédéric Violleau. 2011. "Study of gelatin renaturation in aqueous solution by AFlFFF–MALS: Influence of a thermal pre-treatment applied on gelatin." *Food Hydrocolloids* 25 (3): 511-514.

Regenstein, J. M., and G. Boran. 2010. "Fish gelatin." *Advances in Food and Nutrition Research* 60: 119-143.

Ross-Murphy, Simon B. 1992. "Structure and rheology of gelatin gels: recent progress." *Polymer* 33 (12): 2622-2627.

Schrieber, Reinhard, and Herbert Gareis. 2007. *Gelatine handbook: theory and industrial practice*. John Wiley and Sons.

Shyni, K., G. S. Hema, G. Ninan, S. Mathew, C. G. Joshy, and P. T. Lakshmanan. 2014. "Isolation and characterization of gelatin from the skins of skipjack tuna (Katsuwonus pelamis), dog shark (Scoliodon sorrakowah), and rohu (Labeo rohita)." *Food Hydrocolloids* 39: 68-76.

Tabata, Yasuhiko, Shigeki Hijikata, and Yoshito Ikada. 1994. "Enhanced vascularization and tissue granulation by basic fibroblast growth factor impregnated in gelatin hydrogels ." *Journal of Controlled Release* 31 (2): 189-199.

Thakur, Goutam, Analava Mitra, and Amit Basak. 2011. "Genipin Crosslinked Drug–Gelatin Composite for Drug Transport and Cytocompatibility." *Biomedical Engineering: Applications, Basis and Communications* 23 (2): 113-118.

Tsereteli, G. I., and O. I. Smirnova. 1991. "Calometric study of the melting of gelatin gels." *Polymer Science USSR* 33 (10): 2112-2118.

Ulubayram, Kezban, Inci Eroglu, and Nesrin Hasirci. 2002. "Gelatin microspheres and sponges for delivery of macromolecules." *Journal of biomaterials applications* 16 (3): 227-241.

Ulubayram, Kezban, Ebru Aksu, S. Ismet Deliloglu Gurhan, Kemal Serbetci, and Nesrin Hasirci. 2002. "Cytotoxicity evaluation of gelatin sponges prepared with different cross-linking agents." *Journal of Biomaterials Science, Polymer Edition* 13 (11): 1203-1219.

Veis, Arthur. 1964. "The macromolecular chemistry of gelatin." 433.

Ward, A. G. 1954"The physical properties of gelatin solutions and gels." *British Journal of Applied Physics* 5 (3): 85.

Wu, Jiulin, Shanfei Chen, Shangying Ge, Jing Miao, Jianhua Li, and Qiqing Zhang. 2013."Preparation, properties and antioxidant activity of an active film from silver carp (Hypophthalmichthys molitrix) skin gelatin incorporated with green tea extract." *Food Hydrocolloids* 32(1): 42-51.

Xin-Yuan, Shi, and Tan Tian-Wei. 2004. “New contact lens based on chitosan/gelatin composites.” *Journal of Bioactive and Compatible polymers* 19 (6): 467-479.

Zhang, Yanzhong, Hongwei Ouyang, Chwee Teck Lim, Seeram Ramakrishna, and Zheng-Ming Huang. 2005. “Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds.” *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 72 (1): 156-165.