

Review of Fish Gelatin Extraction, Properties and Packaging Applications

Kaiser Mahmood Lubowa Muhammad *Fazilah Ariffin, Ph.D
Hanisah Kamilah Bt. Abd Razak, Ph.D Syazana Sulaiman

Food Technology Division, School of industrial Technology, University Sains Malaysia, 11800 Minden, Penang

Abstract

Based on physico-functional properties, gelatin is a biopolymer of great interest in food industry. Especially, its rheological and thermal properties diversify its applications. Mammalian gelatin is the main contributor to total gelatin production, but fish gelatin is also a potential alternative. The extraction method, fish type and intensity of the treatment determines the fate of produced gelatin. However, fish gelatin presents some less desirable properties due to the lesser amount of proline and hydroxyproline residues compared to the mammalian gelatins. Nonetheless, it has a good film forming ability and has been suggested as an alternative to the petroleum-based polymers. This review focusses on extraction, physicochemical properties and film forming ability of fish gelatin. Additionally, studies related to possible improvement in film barrier and mechanical properties are also enlisted. Furthermore, a minor description of legislation regarding toxicity issues of the frequently used active additives (plant extract and nanoparticles) in gelatin films is also presented. Fish gelatin applications should be expanded with the growing technological advances in industrial processes.

Keywords: fish gelatin, packaging, nanoparticles, nanocomposite

1. Introduction:

As the global demand for gelatin is continuously on the rise, many potential sources are being sought for combating this growing need. In 2009, the global production of gelatin reached 326 thousand tons; majorly derived from pig skin, bovine hides, bones and others sources contributing 46%, 29.4%, 23.1% and 1.5%, respectively. Due to the fact that half of the production is harvested from porcine source, concerns about Halal or Kosher market strongly dominate. Moreover, in the case of bovine gelatin, the prevalence of spongiform encephalopathy necessitates a look up for possible alternatives (Karim and Bhat, 2009). Thus, fish (skin and bone) and other marine sources, along with insects (melon and sorghum bugs) are being exploited simultaneously. Nevertheless, fish, being in bulk and abundant, accounts more significantly than the insects. A number of studies have addressed the properties of fish skin gelatins, indicating that their properties differ from those of mammalian gelatins and vary among fish species.

Technically, the term gelatin, applies for a series of proteins obtained from collagen after partial hydrolysis, obtained from bones, skin, hides, ligaments and cartilages, etc. (Gómez-Guillén and Montero, 2001). In the conversion process of collagen to gelatin, acid or alkali pretreatment hydrolyze the cross-linking bonds between polypeptides and irreversibly results in gelatin (Yang *et al.*, 2008). The gelatin is water soluble and forms thermo-reversible gels with the melting temperature near to the body temperature (Norziah *et al.*, 2009). The quality of resultant gelatin is determined by its physicochemical behavior that is further based on the species as well as the process of manufacture. Moreover, the specific amino acids and their respective amounts determine physical and functional behavior of gelatin. The higher the level of proline and hydroxyproline, the higher will be the melting point and gel strength (Karim and Bhat, 2009). According to one report (Farris *et al.*, 2009) fish gelatin holds around 20% of proline and hydroxyproline than the bovine or porcine gelatins, which lower the gelling and melting by 5-10°C. Generally, compared to mammalian gelatin, fish gelatins hold lower gelling and melting temperatures, and lower gel strength as well (Norland, 1990).

Gelatin is one of the most commonly used food additive and is an ingredient of many recipes. The proteinaceous nature of gelatin makes it an ideal food ingredient with high digestibility in certain types of diets (Johnston-Banks, 1990). As an additive, it improves water holding capacity, texture, elasticity, consistency and stability of foods (Zhou and Regenstein, 2005). Additionally, it has been used as a stabilizer, emulsifier, clarifying agent and as a protective coating material. Desserts, ice cream, jelled meat, confectionary, dairy and bakery foods are few of the main consumption areas for gelatin. Moreover, in pharmaceuticals, it is used in manufacturing of capsules, tablet coatings, emulsions, ointments and skincare products. Despite the vast applicability of gelatin, theories about structure-function relationship are still under discussion. A 3D model is widely presented using fringed micelle model where microcrystallites are interconnected to amorphous segments of randomly-coiled regions. Some others suggest the presence of quaternary structures that are self-limiting in size, making triple helix or partial triple helix or turn and sheet motifs (Pena *et al.*, 2010).

2. Fish gelatin extraction

Collagen extraction and conversion to gelatin has been diversified for combating the demands of ever-growing

food and packaging industries. It is reported that about 30% of fish processing is the waste containing skins and bones, which is a potential source of collagen (Gómez-Guillén *et al.*, 2002). The extracted gelatin is characterized by the molecular weight, viscosity, gel strength, transparency as well as by the amount of heavy metals. Various researches have proposed different extraction methods; however, use of alkali, acid or enzyme somewhere in the processing, labels the method as alkaline, acidic or enzymatic extraction. Figure 1 depicts different extraction routes for gelatin. Each process's end-products are different in terms of final quality and determines its particular applications.

2.1. Acidic extraction

This is the most widely adopted processing method where mild to harsh acidic treatment is involved. Songchotikunpan *et al.* (2008) reported the acidic extraction method. The fish skin was washed under running tap water for around 1h to remove the superfluous materials. Then the cleaned raw material was soaked in low molarity NaOH (0.4N) for 4h at ambient temperature by maintaining a ratio of 1:7 skin/solution. After the given time of treatment, the solution was neutralized. The treated material was blended with distilled water in 1:2 ratio, and extracted at 70 °C for 1.5h. The extracted material was filtered through double-layered cheese cloth. The filtrate was dried at 50 °C in a hot air oven. Earlier, Gudmundsson and Hafsteinsson (1997) extracted gelatin from skin of shortfin scad (*Decapterus macrosoma*) and sin croaker (*Johnius dussumieri*), the two tropical fish species. The raw material was soaked in 0.2% NaOH for 40 min after cleaning. A 0.2% (w/v) sulfuric acid solution was used to neutralize the digested skins. Further, citric acid (1%) was used for soaking the material again. Acid was removed using running tap water to make the final material neutral. At final extraction step, skin to water ratio (1:3) was maintained at 40-50 °C for 12 h. The extracted material was filtered using Whatman filter paper # 4 and condensed in vacuum and freeze dried.

Similarly, Gómez-Guillén and Montero (2001) used megrim (*Lepidorhombus boscii*) skin using different organic acids; acetic, citric, formic, lactic, malic, propionic and tartaric at different concentrations (0.05-0.5 M). The raw skins were cleaned and mixed with various acid solutions at 20 °C for 16-18 h. In another study, the pressure treatment was also accounted in experimentation by Gómez-Guillén *et al.* (2005). They attempted gelatin extraction from Dover sole (*Solea vulgaris*) where high pressure (250 and 400 MPa) was applied for 10 to 20 min. A mild acid pretreatment with citric acid (50 mM) was applied for 3h to swell the skin. The final extraction was carried out overnight with distilled water at 45 °C. Jamilah and Harvinder (2002) extracted gelatin from red and black tilapia skins (*Oreochromis nilotica* and *O. mossambicus*). The cleaned raw material was soaked in dilute alkali solution (0.2% w/v) for 40 min. Later, the material was soaked in sulfuric acid of the same concentration and followed by 1.0% citric acid treatment. Finally, the extraction was carried out with distilled water at 45 °C for 12 h. Jongjareonrak *et al.* (2006) reported extraction of gelatin from two species, namely brownstripe red snapper (*Lutjanus vitta*) and bigeye snapper (*Priacanthus macracanthus*). The skins were sopped in 0.2 M alkali solution at 1:10 ratio, at 4 °C with continuous stirring. After neutralizing the pH, another soaking in 0.05 M acetic acid was done for 3 h at 25 °C. Final extraction was carried out at 45 °C with continuous stirring for 12 h.

2.2. Alkaline extraction

The main difference in alkaline process of extraction is the use of strong alkali treatment, and the resultant gelatin differs in the isoelectric point (pH of precipitation). This process variations ends up with significant amount of functional and chemical changes in the final product. Jamilah *et al.* (2011) reported gelatin extraction of three freshwater fish, namely striped catfish (*Pangasius sutchi fowler*), walking catfish (*Clarias batrachus*), red tilapia (*Oreochromis nilotica*) by liming process for 2 weeks. Raw skins were cleaned in running water, dried and sopped in saturated solution of Ca(OH)₂ in 1: 2 ratios for 14 days at 20 °C. Excessive lime was removed by washing the material by maintaining the pH ~10. The skins were then soaked in distilled water overnight to solubilize gelatin at 48 °C. The material was filtered using Whatman #4 and the filtrate was passed again through a cation exchange resin to lower the pH~5, followed by freeze drying. In another study, skins and fins of silver carp fish (*Hypophthalmichthys molitrix*) were extracted for gelatin at room temperature. The raw material was soaked in lime solution after reducing the size to 10-30 cm at 15-20 °C. A lime solution (8.25%) at pH~12 was used for 4 weeks. After incubation time, the material was washed with water keeping the pH around 10. Later, the solution was filtered and mixed with 5% solution of hydrochloric acid (HCl) to lower the pH and then freeze dried (Tavakolipour, 2011).

2.3. Enzymatic extraction

In enzymatic extraction, various protein hydrolyzing enzymes have been tried to convert collagen into gelatin. In an attempt, bighead carp (*Aristichthys nobilis*) scales were processed for extraction, where the optimum process parameters were found as: pepsin (547 U/g) at pH 4 and process time and temperature of 1.27 h and 46.98 °C, respectively. It was found that the gelatin obtained in this process has higher gel strength than other methods of

extraction, though the yield was little lower (Tong and Ying, 2013). Similarly, Chinese sturgeon (*Acipenser sturio* Linnaeus) skin was used for gelatin extraction. After washing, the skins were chopped to uniformly small size (0.5-1.0 cm). Further, raw skins were stirred for 24 h with sodium chloride (3.5%) for removal of non-collagenous materials. Then, a solution of 0.5% Na₂CO₃ was used to remove fat soluble material at stirring speed of 200 rpm for two days. The defatted skins were washed to neutrality followed by collagen extraction using pepsin enzyme solution. To remove insoluble material, the viscous solution of gelatin was centrifuged for 20 min at 5000 rpm. The precipitation was done by using ammonium sulfate (2.6 M). A second centrifugation was done to collect the precipitated material and freeze dried. An enzyme concentration of 2.42% with solid to liquid ratio of 1:11.88 and 6.45 h were the most yielding parameters (Feng *et al.*, 2013).

3. Physico-functional properties of fish gelatin

Different extraction methods result into gelatins with different functionality. In general, fish gelatin holds some characteristic properties that make it different from other sources. Conversion of collagen to gelatin modifies its solubility, making it water soluble. It is readily soluble in hot water, swells in cold water, while insoluble in alcohol or non-polar solvents (Jamilah and Harvinder, 2002). It is colorless to yellowish, tasteless, transparent to slightly translucent, powder or flakes or sheets. Mainly, the physicochemical properties are based on the source of collagen and the given treatment of extraction (Johnston-Banks, 1990). However, the intensity of treatment and hydrolysis also impart significant variations in the end product. The yield of skin gelatin varies from 6-19% on wet weight basis (Karim and Bhat, 2009); less than the mammalian sources. Similarly, pH of the final gelatin is also determined by the process of production. In case of moisture, commercial gelatins have 9-14% (Ward and Courts, 1977). At room temperature (25°C) and 46% relative humidity, it maintains its balance of moisture (13%) (Bordignon *et al.*, 2012). For ash, the maximum recommended limit is 2.6% (Muyonga *et al.*, 2004a); however, gelatins from bone have higher ash contents (2.7-3.8%) (Alfaro *et al.*, 2009; Kolia *et al.*, 2012), suggesting the higher mineral contents of bone. Isoelectric points, for acid and alkali processed gelatins are in the pH range of 6.0-9.5 and 4.8-5.2, respectively (Ward and Courts, 1977). Figure 2 presents a sequence of amino acids in gelatin.

Fish gelatin properties are affected by the presence and concentration of amino acids (Gómez-Guillén *et al.*, 2002), molecular weight and particular structural fragments. In general, glycine, proline/hydroxyproline and alanine are the predominant amino acids with respective percentages of 33, 20 and 11% (Sarabia *et al.*, 2000). In a comparative study designed by Muyonga *et al.* (2004b), it was observed that proline and hydroxyproline contents in mammalian, warm-water fish and cold-water fish gelatins are 30%, 23% and 17%, respectively. Table 1 presents a comparative list of amino acids that are found in gelatins obtained from different sources. One of the most important properties of gelatin is its gelling strength, also termed as bloom strength. According to standard method it is defined as; the force required to compress a cylindrical plunger (13 mm diameter) 4 mm into the gelatin gel prepared with 6.7% (w/w), aged for 16-18 h at 10 °C. It determines the grade of functionality of particular gelatin gel. The molecular weight (of α , β chains) in gelatin defines the strength of bloom. Figure 3 presents the hypothetical model of polypeptide chains in collagen. Bloom strength is also correlated to the viscosity of the gel, and is an important criteria for its application in food industry (Johnson-Banks, 1990). Typically, fish gelatin gel bloom varies from 50 to 300. Nonetheless, factors such as temperature, pH, acids, bases, enzymes and bacteria may alter the strength variably (Park *et al.*, 2001). The thermo-reversibility of gels is one of the most interesting inherent property of gelatin that make its use in jellies possible (Gómez-Guillén *et al.*, 2002).

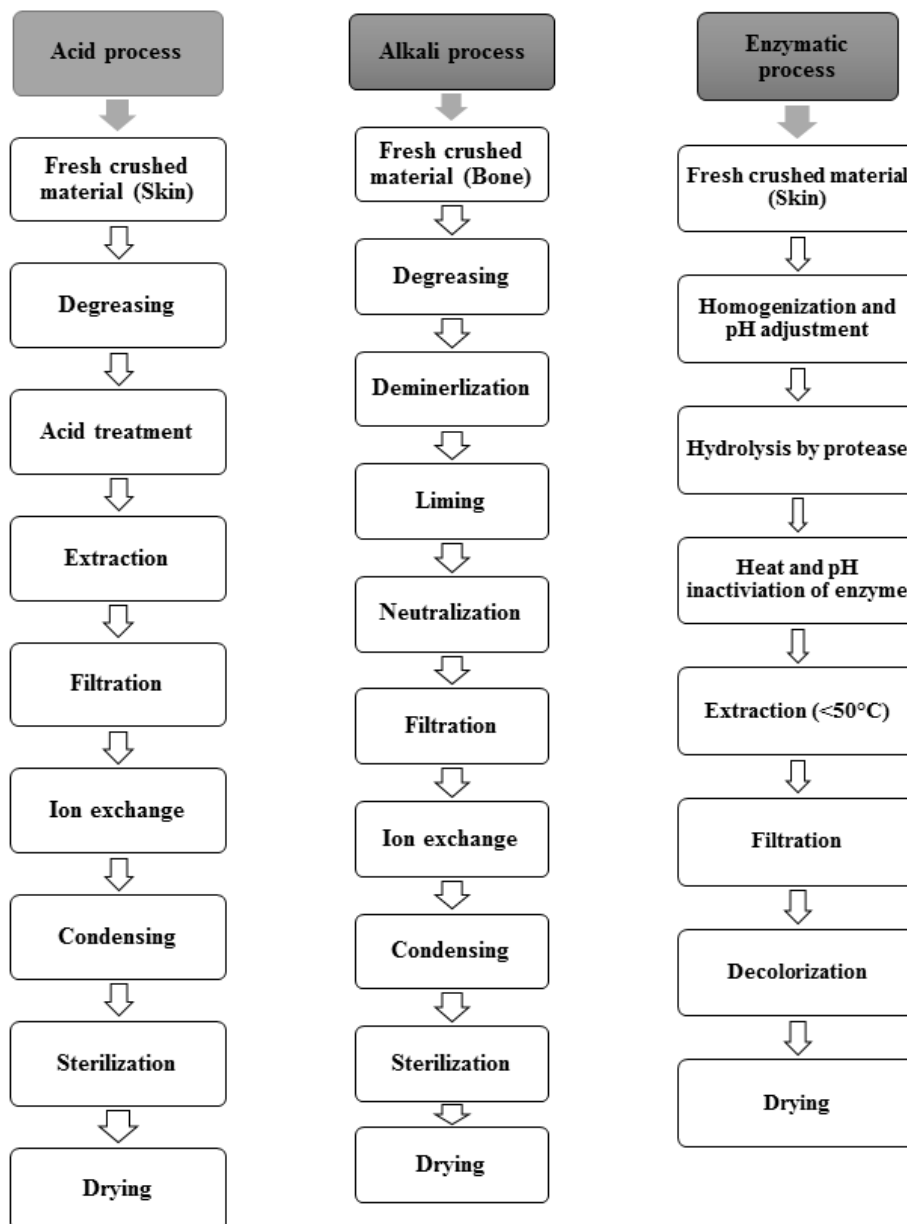


Figure 1. General methods of gelatin extraction from fish

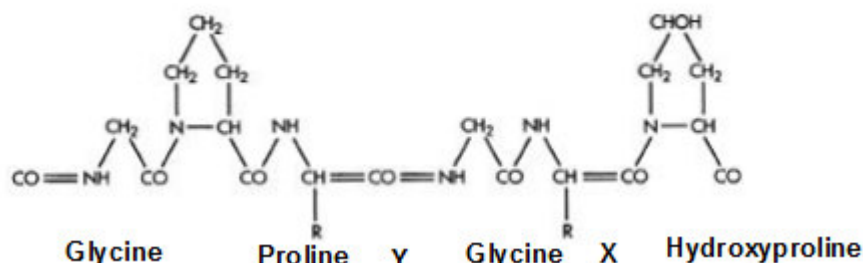


Figure 2: Typical strand chemical structure of gelatin

Another considerable functional property of gelatin is its viscosity, highly related to bloom strength. Various calibrated viscometers or pipettes are used for viscosity measurement, where a standard solution of 6.67% gelatin is used. Based on bloom and molecular weight, commercial gelatins have been reported to present viscosity range of 2.0-7 mPs, while gelatin with viscosity of 13 mPs got special applications (Johnston-Banks *et al.*, 1990). The melting and setting temperatures of gelatin also contribute to possible application of gelatin. Again, the molecular chains and the amino acid composition tend to affect the melting and setting. The melting is determined as the temperature where gelatin gel becomes soft enough to allow carbon tetrachloride drops to fuse in it. The concentration of the gelatin and maturing temperature of gel influence the melting point (Gómez-

Guillén *et al.*, 2002). Conversely, the setting point is the temperature where the softened gel starts hardening. The thermal and mechanical history of gel determines the gelation point. Moreover, gels that are cold slowly present higher setting temperatures compared to rapidly chilled solutions (Simon *et al.*, 2003).

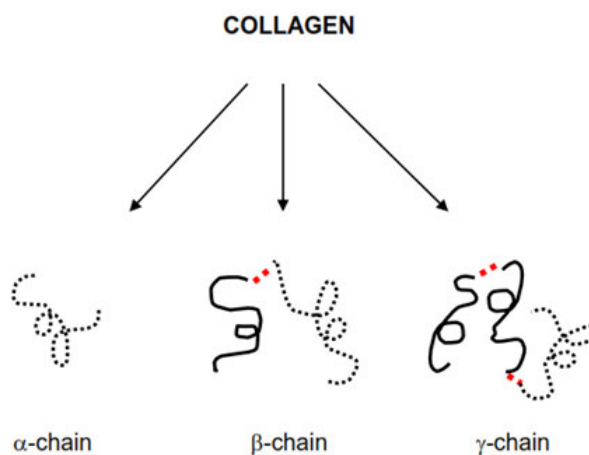


Figure 3: Typical polypeptide strands in fish gelatin

Similar to polysaccharides, gelatin forms micro-structural network upon gelation, but the uniqueness is the thermos-reversible nature of gel. At temperature of 30-40 °C, the gel melts to solution (Morimura *et al.*, 2002). A well accepted theory of gelation is random coil-helix reversion where amino acids rich zones in polypeptides function as junction points. On cooling, the helical polypeptides result into a 3D gel (Nishimoto *et al.*, 2005). It is reported that warm-water fish gelatins possess 119 proline and 70 hydroxyproline residues in a 1000 amino acid segment (See *et al.*, 2010). However, presence of higher number of these residues render the fish gelatin to more mammalian-like; at the same time, shift to higher melting and gelling temperatures (Gudmundsson, 2002).

4. Gelatin film forming properties

Polymer film is generally a thin and continuous structure that finds its application in packaging where it protects the product from the external environment. Films are made more viscoelastic by adding plasticizers that improve gelatin film workability, elongation and uniformity. By scanning of the available literature on mammalian and fish gelatins, it appears that later one yields less stronger and deformable films (Thomazine *et al.*, 2005; Sobral and Habitante, 2001). However, some exceptions are still existing, as Muyonga *et al.* (2004b) reported that gelatin from Nile perch skin represents a similar elongation and breaking values to that of bovine-bone extracted gelatin.

In general, factors affecting the film forming ability of gelatin involves amino acid composition and molecular weight distribution of polypeptide chains. Some processing factors, for instance presence of plasticizers (extenders) and their concentration, their level of physical-chemical interaction with the film forming polymers, and the film formation method can also effect the film properties. In a study, the polymer chain length effect was observed by extracting gelatins from skins and bones of Nile perch (Muyonga *et al.*, 2004a; 2004b). It was observed that the bone gelatin contains more fractions of low molecular weight chains because of severe heating at the time of extraction, and the film presented higher elongation values. Thus, gelatins with similar amino acid composition but different molecular weight chains resulted into films with different characters.

Similarly, Zhang *et al.* (2007) reported that catfish gelatin films prepared with high molecular weight chains presented lower elongation and higher tensile strength (TS). The effect of gelatin concentration and type of plasticizer was assessed for film making properties of brownstripe red snapper or bigeye snapper (Jongjareonrak *et al.*, 2006). The films with addition of glycerol yielded greater elongation, while ethylene glycol resulted into higher tensile strength. However, the higher the concentration of plasticizers, the higher was the elongation-at-break (EAB). In terms of amino acid composition, the gelatins presented similar contents but based on higher molecular weight chains, the TS of film from bigeye gelatin was lower than the brownstripe red snapper's gelatin film. In a study, Gómez-Estaca *et al.* (2009) compared the films made from tuna skin (*Thunnus thynnus*) and bovine-hide gelatin with the same film making conditions. The tuna gelatin film presented 10-fold higher breaking deformation values than the bovine-hide gelatin film. Referral to the change in amino acid composition, bovine hide gelatin hold higher proline/hydroxyproline (210/1000 residues) than the fish gelatin (185/1000 residues) that might have altered the film properties. Thus, higher amount of these amino acids in bovine hide gelatin lowered the deformability of films.

4.1. Gelatin blends for improved film properties

4.1.1. Addition of oil and surfactants

Although gelatins have good film forming ability, in order to accommodate drawbacks (high solubility and hydrophilicity) and designated applications (high strength and sealability) various modifications have been reported. Tongnuanchan *et al.* (2015) attempted to modify the hygroscopic properties of fish gelatin films by adding palm oil. Imbedded oil droplets were seen in the film matrix as observed by scanning electron microscopy (SEM). The films presented higher elongation at break (EAB) but lower elastic modulus (EM) and tensile strength (TS). At the same time, reduced moisture and water vapor permeability (WVP) were seen. The addition of oil improved the hydrophobicity, while lowered the thermal stability. Thus, palm oil addition directly altered the structural integrity and thermal properties of gelatin films. In another study, Ahmad *et al.* (2015) added rice flour in fish gelatin film, and the optical and thermo-mechanical modifications in composite films were observed. It was noticed that by addition of rice flour, higher WVP and lower solubility and transparency were seen. Fourier transform infrared (FTIR) spectra cleared a slight interaction at molecular level between the two components, i.e. gelatin and rice flour. Scanning electron (SEM) micrographs presented surface roughness of composite films and no phase separation was noticed. Thermal analysis presented that the composite films contains microcrystalline/amorphous aggregated zones and the simultaneous existence of two different-order phases. Table 2 summarizes some recent studies related to the diversity of active additives used in fish gelatin films.

Table 1. Amino acid composition of different types of gelatins

Amino Acids (AA)	Mammalian (Type A)	Mammalian (Type B)	Cold water fish gelatin	Warm water fish gelatin
Essential AA				
Histidine	4	4	11	6
Leucine	24	24	21	23
Methionine	4	4	3	9
Threonine	18	18	24	24
Phenylalanine	14	14	13	13
Lysine	27	28	28	25
Isoleucine	10	11	11	8
Hydroxylysine	6	4	5	8
Valine	26	22	18	15
Others				
Proline	132	124	96	119
4-hydroxyproline	91	93	60	79
Serine	35	33	63	35
Tyrosine	3	1	9	2
Asparagine	16			
Aspartic acid	29	}46	}48	}48
Glycine	330	335	347	347
glutamine	48			
Glutamic acid	25	}72	}72	}69

*Haug and Draget (2009)

Recently, Tongnuanchan *et al.* (2016) prepared fish skin gelatin using palm oil and basil essential oil with two surfactants, soy lecithin and Tween-20. Films with oils presented lower EM and TS, while higher elongation (EAB) compared to control. With regard to the surfactants, the films casted with soy lecithin presented lower EAB than those with Tween-20. Differential scanning calorimetry (DSC) analysis depicted that oils incorporation in films reduced the glass transition temperature. Additionally, lower seal strength was observed with films having oils and surfactants. However, in comparison of both surfactants and oils, higher seal efficiency was observed for Tween-20 and palm oil. SEM also authenticated the smoothness of seal, and FTIR results suggested the formation of covalent cross-linkages in emulsified films by heat sealing.

4.1.2. Addition of plant extracts:

The addition of plant extracts in gelatin was tested for possible effect on the film properties as well as on the antioxidant properties. In a study, Tongnuanchan *et al.* (2013) added plant essential oil extracts, such as ginger, plai and turmeric. The films with essential oils presented lower TS values but higher elongation. However, higher WVP of films were seen after addition of plant extracts. At the same time film's transparency values were lowered with increasing concentration of extract. Moreover, film degradation temperature was shifted to a lower temperature. As expected, films with extracts presented good antioxidant activities, as authenticated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) antioxidant assays.

In another study by the same authors, basil and citronella essential oils were added in fish gelatin film and the effect on morphology and thermal properties were observed. The distribution of essential oils was improved using three different surfactants (soy lecithin, Tween-20, Tween-80). However, films presented a bilayer morphology when tween-80 was used. The film surface appeared more homogenous and smoother for soy lecithin. Films with lower glass transition and degradation temperatures were seen after addition of essential oils. FTIR analysis suggested better incorporation of essential oils between protein-protein chains that lowered the glass transition temperatures of films (Tongnuanchan *et al.*, 2014).

Similarly, coconut husk extract was used in tilapia skin gelatin films for possible improvement in antioxidant properties of films (Nagarajan *et al.*, 2015a). The prepared films were used for preserving mackerel meat powder. The powder covered with extract containing gelatin film presented lower moisture than those covered with other films. The powder presented lower oxidation and less thiobarbituric acid reactive species (TBARS). Thus, films with coconut husk extract could be used for extending the shelf life of meat powders.

4.1.3. Gelatin blends with natural polymers:

To improve the strength and functionality of the fish gelatin films, mixing with some other polymers of similar nature is desirable. A careful choice of polymer could develop a synergy between copolymers and signify the structural and mechanical properties of films in a tuned way. In this respect, Pranoto *et al.* (2007) added two polysaccharides, i.e. gellan and kappa-carrageenan in gelatin films and the effect on barrier, mechanical and microstructure were observed. The addition of gellan increased melting point and provide stability to the films. Polysaccharides addition improved the TS and WVP properties, although the transparency of films were reduced. After SEM study, it was concluded that the gellan provided more uniformity and eliminated the cracks in the gelatin films. DSC and FTIR results suggested that there is a significant interaction between gelatin and polysaccharides.

Some polymers of multifunctional nature could enhance the gelatin film functionality, such as mechanical and antimicrobial properties. Likewise, in a study by Jridi *et al.*, (2014), chitosan was added in cuttlefish skin gelatin films, and their antimicrobial properties were assessed. The composite films presented higher TS and lower EAB. DSC and FTIR data indicted a complete interaction between these two polymers. When the chitosan concentration in films was increased, there was a noticeable improvement in thermal stability. The morphology of composite film suggested a compact and uniform structure. Additionally, chitosan addition presented higher antioxidant activities, along with improved antimicrobial properties against some Gram positive (G+) and Gram negative (G-) bacteria.

Hosseini *et al.* (2015) reported the use of chitosan nanoparticles in gelatin films. SEM analysis suggested that chitosan nanoparticles could be well distributed below 8%, but above than this, particle aggregation was seen. After the addition of chitosan nanoparticles, TS and EM were improved but EAB was reduced. A 50% reduction in WVP was noticed at 6% addition of nanoparticles. Besides, good UV-barrier was seen in films with low transparency. FTIR analysis confirmed interaction between fish gelatin and chitosan nanoparticles, suggested a good film with improved properties.

4.1.4. Gelatin blends with synthetic polymers:

Film barrier properties are one of the desirable functionalities for food packaging. Fish gelatin, being hydrophilic in nature presents a lower water barrier. To amend barrier properties, blending with other synthetic polymers of low hydrophilicity have been a good approach. In polymeric composite films, either polymers are blended with each other or laminated as a different layer providing good barrier properties. Polymers like polylactic acid (PLA), polycaprolactones (PCL), low density polyethylene (LDPE) and polyethylene terephthalate (PET) etc. have been reported in conjunction with fish gelatins. Bae *et al.* (2009) developed a three-layer laminated film involving fish gelatin, PET and LDPE. The resultant laminate presented good oxygen barrier properties, and presented a comparable performance to the laminates prepared by ethylene vinyl alcohol at 50% relative humidity. Furthermore, gelatin with nanoclay imparted better barrier properties to the laminates, as tortuosity was developed by nanoparticles. Moreover, the gelatin/nanoclay film presented bond strength similar to other polymers of laminates, i.e. PET and LDPE. A better and reliable performance, in terms of barrier, was observed for the fish gelatin based laminated films that could be employed for food packaging, even at relative humidity around 50%.

Table 2. Summary of some recent studies on improved fish gelatin films.

Fish type	Additives	Findings	Reference
Cold water fish skin	PLA	Lower oxygen and water vapor permeability, improved tensile strength.	Hosseini <i>et al.</i> (2016)
Tilapia skin	Cloisite Na ⁺	Effect of pH (4-8) on film forming; improved water vapor barrier, tensile strength and elastic modulus at pH 6.	Nagarajan <i>et al.</i> (2015)
Tilapia and Squid skin	Coconut husk extract	Lower oxidation of meat powder with good keeping quality.	Nagarajan <i>et al.</i> (2015)
Tilapia skin	Basil essential oil or palm oil	Lower tensile strength, elastic modulus and seal strength; improved elongation at break.	Tongnuanchan <i>et al.</i> (2016)
Fish skin gelatin	Chitosan and curcumin oil	Irradiation at 40 and 60kGy enhanced wettability of the film; unaltered water vapor permeability; delayed release of curcumin.	Benbettaieb <i>et al.</i> (2016)
Commercial fish gelatin	Chitosan	Electron beam (0-60kGy); improved tensile strength and elongation at break; reduced oxygen permeability by electron beam treatment.	BenBettaieb <i>et al.</i> (2015)
Tilapia skin	Citronella and basil essential oil	Smoother and homogeneous film morphology; lower glass transition and degradation temperature.	Tongnuanchan <i>et al.</i> (2014)
Tilapia skin	Palm oil	Lower tensile strength, elastic modulus and thermal stability; higher elongation at break; low water vapor permeability and moisture.	Tongnuanchan <i>et al.</i> (2015)
Tilapia skin(type B)	Rice flour	Decreased elongation at break, low tensile strength; high water vapor permeability; low water solubility; better UV-visible barrier.	Ahmad <i>et al.</i> (2015)
Cold water fish skin	Chitosan Nano-particles	Increased tensile strength, elastic modulus; decreased elongation at break, water vapor permeability and transparency.	Hosseini <i>et al.</i> (2015)
Commercial fish skin gelatin	No additive	Screw speed and temperature effect; high speed improved tensile strength; reduced water vapor permeability, high temperature improved puncture strength and increased solubility; lower water vapor permeability.	Nur Hanani <i>et al.</i> (2012)
Commercial cold water fish skin gelatin	Silver-copper nanoparticles	Increased tensile strength; lower elongation at break, improved UV- barrier and antimicrobial properties; rough surface of films.	Arfat <i>et al.</i> (2017)
Tilapia skin gelatin	Giger, turmeric, plai	Lower tensile strength and water vapor permeability; high elongation at break and better transparency.	Tongnuanchan <i>et al.</i> (2013)
Cuttlefish skin	Chitosan	Improved tensile strength with good antimicrobial activity; Lower elongation at break.	Jridi <i>et al.</i> (2014)
Cold water fish skin	Chitosan	Improved tensile strength and elastic modulus; lowering elongation at break, water vapor permeability and solubility; good UV barrier.	Hosseini <i>et al.</i> (2013)
Tilapia skin gelatin	Cloisite Na ⁺ and Cloisite A	Increased tensile strength and elastic modulus; lower elongation at break, water vapor permeability and transparency; improved heat stability.	Nagarajan <i>et al.</i> (2014)

Similarly, Hosseini *et al.* (2016) developed a multilayer film for improved barrier properties using fish gelatin and PLA. The SEM analysis indicated the PLA layer in closely attached to the fish gelatin and provided a continuous layer. In the multilayered film, oxygen permeability (OP) was reduced about 8-fold than the PLA alone, and for WVP an 11-fold reduction was seen when compared with the fish gelatin film. Moreover, the TS of multilayered film was almost 3.5-fold higher than the fish gelatin control film. Results from thermal analysis proposed compatibility between two polymers. Interestingly, the film optical clarity remained unaltered after lamination of fish gelatin film making it a suitable packaging material.

4.1.5. Gelatin film crosslinking:

Crosslinking of gelatin film is usually done to reduce water solubility and improve stability of the structure. Different methods of polymer crosslinking have been developed and tested for suitability for particular functionality. In physical crosslinking, either irradiation or curing at particular temperature is performed, while

for chemical crosslinking, agents, such as formaldehyde, ferulic acid, citric acid and genipin, etc. are frequently used. Furthermore, enzymatic crosslinking is also adopted to develop a network of polymers. Some classes of crosslinking enzymes are, transglutaminases, tyrosinases, laccases, sulfhydryl oxidases, etc.

In a study, two cold water fish were used for gelatin extraction, namely Alaska pink salmon (*Oncorhynchus gorbuscha*) Alaska pollock (*Theragra chalcogramma*). The gelatin films were crosslinked chemically using formaldehyde (Chiou *et al.*, 2008) and characterized for thermal stability, TS, WVP, OP and biodegradation. The films presented lower TS and EAB than mammalian gelatin films. The crosslinking imparted little effect on melting and tensile properties of films. Nevertheless, crosslinking resulted into Pollock gelatin films with the least WVP and OP, whereas higher permeability values were observed for mammalian gelatin films. Additionally, faster degradation was seen for fish gelatin films compared to mammalian ones. Kolodziejska *et al.*, (2006) also attempted to reduce the water solubility of fish gelatin:chitosan films using transglutaminase (TGase) crosslinking and for comparison, a chemical crosslinking agent (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC) was used. An addition of 0.2 mg/ml of TGase lowered the solubility of film from 65% to 28% at pH 6. After a 15 min heating process at 100 °C, film solubility reduced to 23% at pH 6. Further reduction in solubility was achieved by enzyme in the presence of dithiothreitol (5-10 mM). The composite films prepared in 30 mM (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) were less soluble than the films modified with transglutaminase in the presence of dithiothreitol.

Kolodziejska and Piotrowska (2007) conducted a similar study using Baltic cod skin gelatin and chitosan in 4:1 ratio. The crosslinking of the films were adopted either the TGase or the EDC, while the concentration of plasticizer (glycerol) was varied to a maximum of 30% w/w of composite films. However, the addition of the glycerol did not alter the solubility of crosslinked films at 25 °C for two different pH values; 3 and 6. The TS of modified films was reduced by about 25% and 40% for TGase and EDC, respectively. Interestingly, glycerol addition in TGase and EDC crosslinked films resulted higher elongations of films than non-plasticized composite films, but decline in TS were also seen.

Later, BenBettaieb *et al.* (2015) used commercial gelatin (180 bloom) with chitosan for film preparations and the films were irradiated by electron beam at dose range of 0-60kGy. A successful intermolecular linking was developed as authenticated by electron spin resonance. Gelatin films crosslinked with electron beam were found to have improved TS, but for blend films no significant change was observed. Almost 50% reduction in film elongation was noticed, though 2-fold increase in EM was also observed. The WVP and OP of the composite films were slightly improved after crosslinking treatment. FTIR analysis suggested that some interactions were developed after irradiation.

In another study, crosslinked gelatin:chitosan films were added with antioxidant (coumarin) and the effect of electron beam irradiation on film crosslinking and release of coumarin were studied (BenBettaieb *et al.*, 2016). Electron spin resonance (ESR) study revealed a free radical generation by irradiation that resulted crosslinked films. The gelatin films presented improved thermal stability after irradiation crosslinking. However, the coumarin addition and irradiation of composite films enhanced the surface wettability. Interestingly, at lower relative humidity, neither coumarin nor electron beam made any modifications in barrier properties, while at 80% relative humidity increased WVP values were noticed for all irradiated films. No change in TS values were found for composite films even at the highest dose of irradiation. Interestingly, gelatin films presented improved TS after irradiation treatment. For coumarin release study, irradiation films presented slower release, probably due to the interaction between coumarin and film.

4.1.6. Gelatin nanocomposite films with nanoclays:

Among the nanomaterials, nanoclays (layered silicates, naturally or organically modified) are added in polymers to improve the film barrier properties. In a study, Nagarajan *et al.* (2014) added hydrophilic (Cloisite-Na⁺) and hydrophobic (Cloisite-15A, 20A, 30B) nanoclays in gelatin films. The addition of Cloisite-Na⁺ (0.5-5% w/w) improved the mechanical properties as higher TS were seen. The lowest WVP was found for Cloisite-Na⁺ and Cloisite-20A at 1% loading. Nanocomposite film presented lower lightness after addition of Cloisite-15A at higher level (10%). Similarly, film homogeneity and surface smoothness were reduced by nanoclay as observed by SEM graphs. Nonetheless, thermal stability and rigidity of the films were effected variably, depending on the type of clay used.

In another study, Nagarajan *et al.* (2015b) prepared nanocomposite by adding Cloisite-Na⁺ (hydrophilic clay) in gelatin. The effect of pH (4-8) was noticed on the film forming suspensions. The wide angle X-ray diffraction analysis authenticated the uniform dispersion of clay in films. In terms of mechanical properties, TS and EM were improved until pH 6. Further increase in pH negatively affected the two parameters. At pH 6, the films presented the lowest WVP. The lightness and yellowness of films were enhanced with increasing pH, while a minor impact of pH was seen on the film transparency. SEM graphs presented films with smooth surface and homogeneous nature at pH 6. The thermal stability was haphazardly affected by the pH, suggesting that its direct effect on the film forming properties of nanocomposites.

4.1.7. Gelatin nanocomposite film with metallic nanoparticles:

Due to their functional properties, elemental nanoparticles, such as SiO₂, ZnO, Ag and TiO₂ are widely used in packaging films. Even some commercially developed packaging and containers are added with silver nanoparticles as an antimicrobial agent. Likewise, these nanoparticles have been tested in fish gelatin films to impart antimicrobial and barrier properties to the films. Rouhi *et al.* (2013) prepared gelatin films with nanoparticles of ZnO. With a 5% addition of nanoparticles, 25% and 42% increase in TS and EM of composite films were seen compared to control. Interestingly, UV absorption was maximized by the small addition of nanoparticles, but at the same time the surface roughness of film was increased with increasing the level of addition. Thus, high UV absorbance and good mechanical properties of the film suggest its use in food preservation and packaging.

In improving the film antimicrobial properties, Paul *et al.* (2015) added silver nanoparticles in fish gelatin:chitosan blend films. Bioactivity of the films was successfully assessed against *E. coli* and *Streptococcus*. In another study, the pure effect and mixed effect of different nanoparticles (Ag, Au, Cu, and their blends) were assessed on the gelatin film functional properties (Shankar *et al.*, 2016). The silver nanoparticles and its blends presented strong antimicrobial activity for G(+) and G(-) bacteria. The color and light transmittance was greatly influenced by presence of nanoparticles with stronger UV-scattering. Nonetheless, nanoparticles did not affect the films' mechanical properties significantly ($P < 0.05$). Strong antioxidant activities were noticed for silver and gold nanoparticles alone and in blends.

In a similar study, Arfat *et al.* (2017) developed fish skin gelatin and Ag-Cu nanoparticles composite films. This addition of nanoparticles resulted in enhanced TS of films, while reduced EAB were seen. The film melt-rheology also suggested an improvement in the mechanical properties of composite films. By the addition of nanoparticles, the nanocomposite transparency and UV transmittance were decreased. Moreover, nanoparticles addition improved the thermal stability of the gelatin films. Whereas, the film surface roughness was increased after addition of nanoparticles as observed by SEM. Yet, stronger antibacterial activities were observed against G(+) and G(-) bacteria. In conclusion, nanocomposite films presented good mechanical properties with diverse functionalities.

5. Legislation

Gelatin films for food packaging are being developed using different types of active materials ranging from natural plant extracts to synthetic nanoparticles. However, the ingredients or additives used in packaging should have certain standards and limits pertinent to their use as an active ingredient. At least a 'GRAS' (generally recognized as safe) status should be maintained in preparation of active packing based on fish gelatin. Active packaging is defined as the packaging material that is developed to improve the shelf life or at least maintains the desirable microclimate of the packaged food. Sometimes, this type of packaging is intended for controlled release of active compounds in foods (Arvanitoyannis *et al.*, 2005). Thus, it is direly important to elaborate the safety level and dosage limits of active compounds in packaging. Besides, the GRAS label to fish gelatin and active components, the method of production and processing should be covered under good manufacturing principles and practices. In case of elemental nanoparticles, a strong concern is existing in scientific communities about the possible toxicity and health risk. It is claimed that the release of nanoparticles to food might contaminate the food, especially, when kept under harsh storage conditions like elevated temperature or higher relative humidity. Although FDA has developed limits for various chemical and biological additives, but still gaps are existing for the newly adopted active ingredients, which are frequently used in developing active packaging. Simultaneously, worldwide environmental protection agencies are encouraging to develop green packaging by choosing greener or natural polymers with good biodegradability that could help to reduce burden of toxic residues in the environment.

6. Conclusion

Being unique to its properties, gelatin is overwhelmingly used in different food recipes. Mainly, mammalian gelatin is leading the market, but concerns for Halal or Kosher applications create a need to find some alternatives. In this regard, fish gelatin is getting commercial interests. Fish gelatin is thoroughly studied for its physicochemical and functional properties that allowed to optimize its use in various applications. Moreover, attempts are made to develop biodegradable packaging with improved functionality. Being ecofriendly and easily compostable, fish gelatin could be a good replacement of petroleum-based plastic polymers. Various trends of improving mechanical and barrier properties of fish gelatin films have been discussed. Recently, blending with other polymers, developing laminated films and addition of nanoparticles (nanoclay or nano-metals) are some interesting approaches that impart better barrier and mechanical functionalities to the packaging films. Moreover, addition of active compounds, for instance antimicrobials and antioxidants further elaborate the suitability of fish gelatin films in food packaging. Fish gelatin provides some advantages; 1) no religious reservations and restrictions, 2) fish skin, scales and bone are good in collagen contents, 3) a wide

range of species that can give functionally diverse gelatins, 4) reduction of environment pollution. In near future, a competitive market for fish gelatin as an alternative biopolymer may develop; nonetheless, broadening its utility is based on the technological advancements in industry.

Acknowledgement

Kaiser Mahmood acknowledges and is thankful to the USM-Global Fellowship Scheme, University Sains Malaysia.

Lubowa Muhammad acknowledges and is grateful for the financial support from TWAS-USM Postgraduate Fellowship Programme.

References

- Ahmad, M., Hani, N.H., Nirmal, N.P., Fazial, F.F., Mohtar, N.F., & Romli, S.R. (2015). Optical and thermo-mechanical properties of composite films based on fish gelatin/rice flour fabricated by casting technique. *Progress in Organic Coatings*, 84, 115–127.
- Alfaro, A.T., Fonseca, G.G., Costa, C.S., & Prentice, C. (2009). Effect of extraction parameters on the properties of gelatin from King weakfish (*Macrodon ancylodon*) bones. *Food Science and Technology International*, 15(6), 553–562.
- Arfat, Y.A., Ahmed, J., Hiremath, N., Auras, R., & Joseph, A. (2017). Thermo-mechanical, rheological, structural and antimicrobial properties of bionanocomposite films based on fish skin gelatin and silver-copper nanoparticles. *Food Hydrocolloids*, 62, 191-202.
- Arvanitoyannis, I.S., Choreftaki, S., & Tserkezou, P. (2005). An update of EU legislation (directives and regulations) on food-related issues (safety, hygiene, packaging, technology, GMOs, additives, radiation, labelling): presentation and comments. *International Journal of Food Science & Technology*, 40(10), 1021-1112.
- Bae, H.J., Park, H.J., Darby, D.O., Kimmel, R.M., & Whiteside, W.S. (2009). Development and characterization of PET/Fish Gelatin–nanoclay composite/LDPE laminate. *Packaging technology and Science*, 22 (7), 371–383.
- BenBettaïeb, N., Chambin, O., Assifaoui, A., Al-Assaf, S., Karbowski, T., & Debeaufort, F. (2016). Release of coumarin incorporated into chitosan-gelatin irradiated films. *Food Hydrocolloids*, 56, 266-276.
- BenBettaïeb, N., Karbowski, T., Bornaz, S., & Debeaufort, F. (2015). Spectroscopic analyses of the influence of electron beam irradiation doses on mechanical, transport properties and microstructure of chitosan-fish gelatin blend films. *Food Hydrocolloids*, 46, 37-51.
- Bordignon, C.A., Franco, S.D.R.L., Gasparino, E., Yanjima, M.E., Del Vesco, P.A., Visentainer, V.J., & Mikcha, G.M.J. (2012). Utilization of frozen and salted Nile tilapia skin for batch processed gelatin extraction. *Revista Brasileira de Zootecnia*, 41(3), 473–478.
- Chiou, B-S., Avena-Bustillos, R.J., Bechtel, P.J., Jafri, H., Narayan, R., Imam, S.H., Glenn, G.M., & Orts, W.J. (2008). Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. *European Polymer Journal*, 44, 3748–3753.
- Farris, S., Schaich, K.M., Liu, L.S., Piergiovanni, L., & Yam, K.L. (2009). Development of polyon-complex hydrogels as an alternative approach for the production of biobased polymers for food packaging applications. A review. *Trends in Food Science and Technology*, 20, 316-332.
- Feng, W., Zhao, T., Zhou, Y., Li, F., Zou, Y., Bai, S., Wang, W., Yang, L., & Wu, X. (2013). Optimization of enzyme-assisted extraction and characterization of collagen from Chinese sturgeon (*Acipenser sturio* Linnaeus) skin. *Pharmacognosy Magazine*, 9(1), S32-37.
- Gómez-Estaca, J., Montero, P., Fernández-Martín, F., & Gómez-Guillén, M.C. (2009). Physico chemical and film forming properties of bovine-hide and tuna-skin gelatin: a comparative study. *Journal of Food Engineering*, 90, 480–486.
- Gómez-Guillén, M.C., & Montero, P. (2001). Extraction of gelatin from megrim (*Lepidorhombus boscii*) skins with several organic acids. *Journal of Food Science*, 66(2), 213-216.
- Gómez-Guillén, M.C., Giménez, B., & Montero, P. (2005). Extraction of gelatin from fish skins by high pressure treatment. *Food Hydrocolloids*, 19, 923–928.
- Gómez-Guillén, M.C., Turnay, J., FernándezDíaz, M.D., Ulmo, N., Lizarbe, M.A., & Montero, P. (2002). Structural and Physical Properties of Gelatin Extracted from Different Marine Species: A Comparative Study. *Food Hydrocolloids*, 16, 25-34.
- Gudmundsson, M. (2002). Rheological properties of fish gelatins. *Journal of Food Science*, 67(6), 2172–2176.
- Gudmundsson, M., & Hafsteinnsson, H. (1997). Gelatin from cod skins as affected by chemical treatments. *Journal of Food Science*, 62, 37–47.
- Hosseini, S.F., Rezaei, M., Zandi, M., & Ghavi, F.F. (2013). Preparation and functional properties of fish gelatin–chitosan blend edible films. *Food Chemistry* 136, 1490-1495.

- Hosseini, S.F., Javidi, Z., & Rezaei, M. (2016). Efficient gas barrier properties of multi-layer films based on poly (lactic acid) and fish gelatin. *International Journal of Biological Macromolecules*, 92, 1205–1214.
- Hosseini, S.F., Rezaei, M., Zandi, M., & Farahmandghavi, F. (2015). Fabrication of bio nanocomposite films based on fish gelatin reinforced with chitosan nanoparticles. *Food Hydrocolloids*, 44, 172–182.
- Haug, I.J., & Draget, K.I. (2009). Gelatin. In, “Phillips, G.O., and Williams, P.A. (Eds.). “*Handbook of hydrocolloids*” pp. 67-86. Woodhead Publishing Ltd. Cambridge.
- Jamilah, B., & Harvinder, K.G. (2002). Properties of gelatins from skins of fish – black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). *Food Chemistry*, 77, 81-84.
- Jamilah, B., Tan, K.W., Umi Hartin, M.R., & Azizah, A. (2011). Gelatins from three cultured freshwater fish skins obtained by liming process. *Food Hydrocolloids*, 25(5), 1256–1260.
- Johnston-Banks, F.A. (1990). Gelatin. In, Harris, P. (Ed.). “*Food Gels*”. pp 233–289. Elsevier Applied Science, New York.
- Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2006). Skin gelatin from bigeye snapper and brownstripe red snapper: chemical compositions and effect of microbial transglutaminase on gel properties. *Food Hydrocolloids*, 20 (8), 1216–1222.
- Jridi, M., Hajji, S., Ben Ayed, H., Lassoued, I., Mbarek, A., Kammoun, M., Souissi, N., & Nasri, M. (2014). Physical, structural, antioxidant and antimicrobial properties of gelatin–chitosan composite edible films. *International Journal of Biological Macromolecules*, 67, 373–379.
- Karim, A.A., & Bhat, R. (2009). Fish gelatin: properties, challenges and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids*, 23(3), 563–576.
- Koli, M.J., Basu, S., Nayak, B.B., Patange, B.S., Pagarkar, U.A., & Gudipati, V. (2012) Functional characteristics of gelatin extracted from skin and bone of Tiger-toothed croaker (*Otolithes ruber*) and Pink perch (*Nemipterus japonicus*). *Food and Bioprocess Processing*, 90(3), 555–562.
- Kolodziejaska, I., & Piotrowska, B. (2007). The water vapour permeability, mechanical properties and solubility of fish gelatin–chitosan films modified with transglutaminase or 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide (EDC) and plasticized with glycerol. *Food Chemistry*, 103, 295–300.
- Kolodziejaska, I., Piotrowska, B., Bulge, M., & Tylingo, R. (2006). Effect of transglutaminase and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide on the solubility of fish gelatin–chitosan films. *Carbohydrate Polymers*, 65, 404–409.
- Morimura, S., Nagata, H., Uemura, Y., Fahmi, A., Shigematsu, T., & Kida, K. (2002). Development of an effective process for utilization of collagen from livestock and fish waste. *Process Biochemistry*, 37, 1403-1412.
- Muyonga, J.H., Cole, C.G.B., & Duodu K.G. (2004a). Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). *Food Chemistry*, 85 (1), 81–89.
- Muyonga, J.H., Cole, C.G.B., & Duodu, K.G. (2004b). Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (*Lates niloticus*). *Food Chemistry*, 86 (3), 325–332.
- Nagarajan, M., Benjakul, S., Prodpran, T., & Songtipya, P. (2015b). Effects of pHs on properties of bio-nanocomposite based on tilapia skin gelatin and Cloisite Na⁺. *International Journal of Biological Macromolecules*, 75, 388–397.
- Nagarajan, M., Benjakul, S., Prodpran, T., & Songtipya, P. (2014). Characteristics of bio- nanocomposite films from tilapia skin gelatin incorporated with hydrophilic and hydrophobic nanoclays. *Journal of Food Engineering*, 143, 195–204.
- Nagarajan, M., Benjakul, S., Prodpran, T., & Songtipya, P. (2015a). Effects of bio- nanocomposite films from tilapia and squid skin gelatins incorporated with ethanolic extract from coconut husk on storage stability of mackerel meat powder. *Food Packaging and Shelf Life*, 6, 42–52.
- Nishimoto, M., Sakamoto, R., Mizuta, S., & Yoshinaka, R. (2005). Identification and characterization of molecular species of collagen in ordinary muscle and skin of the Japanese flounder (*Paralichthys olivaceus*). *Journal of Food Chemistry*, 90, 151-156.
- Norland, R.E. (1990). Fish gelatin. In, “*Advances in Fisheries Technology and Biotechnology for Increased Profitability*”. Voight, M. N., and Botta, J. K. (Eds.). pp 325–333. Technomic Publishing Co., Lancaster.
- Norziah, M.H., Al-Hassan, A., Khairulnizam, A. B., Mordi, M. N., & Norita, M. (2009). Characterization of fish gelatin from surimi processing wastes: Thermal analysis and effect of transglutaminase on gel properties. *Food Hydrocolloids*, 23, 1610-1616.
- Nur Hanani, Z.A., Beatty, E., Roos, Y.H., Morris, M.A., & Kerry, J.P. (2012). Manufacture and characterization of gelatin films derived from beef, pork and fish sources using twin screw extrusion. *Journal of Food Engineering*, 113, 606–614.
- Park, S.Y., Lee, B.I., Jung, S.T., & Park, H.J. (2001). Biopolymer composite films based on k-carrageenan and

- chitosan. *Material Research Bulletin*, 36 (3-4), 511-519.
- Paul, S., Jayan, A., & Sasikumar, C.S. (2015). Physical, chemical and biological studies of gelatin/chitosan based transdermal films with embedded silver nanoparticles. *Asian Pacific Journal of Tropical Disease*, 5(12), 975-986.
- Pena, C., Caba, K., Eceiza, A., Ruseckaite, R., & Mondragon, I. (2010). Enhancing water repellence and mechanical properties of gelatin films by tannin addition. *Bioresourse Technology*, 101, 6836-6842.
- Pranoto, Y., Lee, C.M., & Park, H.J. (2007). Characterizations of fish gelatin films added with gellan and kappa-carrageenan. *LWT-Food Science and Technology*, 40, 766-774.
- Rouhi, J., Mahmud, S., Naderi, N., Raymond Ooi, C.H., & Mahmood, M.R. (2013). Physical properties of fish gelatin-based bio-nanocomposite films incorporated with ZnO nanorods. *Nanoscale Research Letters*, 8, 364.
- Sarabia, A.I., Gómez-Guillén, M.C., & Montero, P. (2000). The effect of added salts on the viscoelastic properties of fish skin gelatin. *Food Chemistry*, 70(1), 71-76.
- See, S.F., Hong, P.K., Ng, K.L., Wan Aida, W.M., & Babji, A.S. (2010). Physicochemical properties of gelatins extracted from skins of different freshwater fish species. *International Food Research Journal*, 17, 809-816.
- Shankar, S., Jaiswal, L., Selvakannan, P.R., Hamb, K.S., & Rhim, J.W. (2016). Gelatin-based dissolvable antibacterial films reinforced with metallic nanoparticles. *RSC Advances*, 6, 67340-67352.
- Simon, A., Grohens, Y., Vandanjon, L., Bourseau, P., Balnois, E., & Levesque, G. (2003). A comparative study of the rheological and structural properties of gelatin gels of mammalian and fish origins. *Macromolecule Symposium*, 203, 331-338.
- Sobral, P.J.A., & Habitante, A.M.Q.B. (2001). Phase transitions of pigskin gelatin. *Food Hydrocolloids*, 15 (4-6), 377-382.
- Songchotikunpan, P., Tattiyakul, J., & Supaphol, P. (2008). Extraction and electrospinning of gelatin from fish skin. *International Journal of Biological Macromolecules*, 42, 247-255.
- Tavakolipour, H. (2011). Extraction and Evaluation of Gelatin from Silver Carp Waste. *World Journal of Fish and Marine Sciences*, 3 (1), 10-15.
- Thomazine, M., Carvalho, R.A., & Sobral, P.I.A. (2005). Physical properties of gelatin films plasticized by blends of glycerol and sorbitol. *Journal of Food Science*, 70 (3), 172-176.
- Tong, Y., & Ying, T. (2013). Gelling strength improvement and characterization of a gelatin from scales of bighead carp (*Aristichthys nobilis*). *Journal of Food, Agriculture & Environment*, 11 (1), 146-150.
- Tongnuanchan, P., Benjakul, S., & Prodpran, T. (2013). Physico-chemical properties, morphology and antioxidant activity of film from fish skin gelatin incorporated with root essential oils. *Journal of Food Engineering*, 117, 350-360.
- Tongnuanchan, P., Benjakul, S., and Prodpran, T. (2014). Structural, morphological and thermal behaviour characterisations of fish gelatin film incorporated with basil and citronella essential oils as affected by surfactants. *Food Hydrocolloids*, 41, 33-43.
- Tongnuanchan, P., Benjakul, S., Prodpran, T., & Nilsuwan, K. (2015). Emulsion film based on fish skin gelatin and palm oil: Physical, structural and thermal properties. *Food Hydrocolloids*, 48, 248-259.
- Tongnuanchan, P., Benjakul, S., Prodpran, T., Pisuchpen, S., & Osako, K. (2016). Mechanical, thermal and heat sealing properties of fish skin gelatin film containing palm oil and basil essential oil with different surfactants. *Food Hydrocolloids*, 56, 93-107.
- Ward, A.G., & Courts, A. (1977). The science and technology of gelatin. *Academic Press*, London.
- Yang, H., Wang, Y., Zhou, P., & Regensteind, J.M. (2008). Effects of alkaline and acid pretreatment on the physical properties and nanostructures of the gelatin from channel catfish skins. *Food Hydrocolloids*, 22, 1541-1550.
- Zhang, S., Wang, Y., Herring, J.L., & Oh, J. (2007). Characterization of edible film fabricated with channel catfish (*Ictalurus punctatus*) gelatin extract using selected pretreatment methods. *Journal of Food Science*, 72 (9), 498-503.
- Zhou, P., & Regenstein, J.M. (2005). Effects of alkaline and acid pretreatments on Alaska Pollock skin gelatin extraction. *Journal of Food Science*, 70, C392-C396.