FISH GELATIN FROM SURIMI PROCESSING BY-PRODUCTS: EXTRACTION AND CHARACTERIZATION

MUHAMMAD KHAIRULNIZAM

BIN ABU BAKAR

UNIVERSITI SAINS MALAYSIA

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by

MUHAMMAD KHAIRULNIZAM BIN ABU BAKAR

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LIST OF SYMBOLS/ABBREVIATION

Symbol/ Abbreviation	Caption
α	Alpha
β	beta
δ	delta
γ	gamma
*a**	greenness/redness
ANOVA	one-way analysis of variance
*b**	blueness/yellowness
$Ca(OH)_2$	calcium hydroxide
CCRD	central composite rotatable design
CBG	commercial bovine gelatin
CFG	commercial fish gelatin
CPG	commercial porcine gelatin
EFG	extracted fish gelatin
FMOC	9- fluorenylmethyl chloroformate
FTIR	Fourier transform infrared spectroscopy
G′	dynamic storage/elastic modulus (pronounced "G-prime")
G″	dynamic loss/viscous modulus (pronounced "G-double prime")
HPLC	high performance liquid chromatography
IEP	isoelectric point
*L**	Lightness
OPA	o-phthalaldehyde
RFG	ribbon fish gelatin
SCG	sin croaker gelatin
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrilamide gel electrophoresis
SSG	slender shad gelatin
TBG	threadfin bream gelatin

GELATIN IKAN DARIPADA HASILAN SAMPINGAN PEMPROSESAN SURIMI: PENGEKSTRAKAN DAN PENCIRIAN

ABSTRAK

Gelatin ikan telah diekstrak daripada hasilan sampingan pemprosesan surimi daripada empat spesis ikan yang berbeza iaitu puput (*Elongata ilisha*), gelama (*Johnius dussumieri*), timah (*Trichiurus lepturus*) dan kerisi (*Nemipterus japonicus*). Hasilan sampingan ini terdiri daripada kulit, tulang dan sisik. Dalam kajian awal, hasil gelatin ikan paling banyak diperolehi daripada hasilan sampingan ikan puput dan spesies ini kemudiannya dipilih untuk kajian pengoptimaan kondisi pengekstrakan gelatin. Beberapa faktor yang mempengaruhi proses pengekstrakan gelatin dioptimakan menggunakan pendekatan kaedah respon permukaan (RSM). Eksperimen penskrinan menggunakan rekabentuk faktorial penuh 2-aras menghasilkan persamaan model peringkat pertama yang mencadangkan bahawa tiga faktor iaitu kepekatan asid sitrik, suhu pengekstrakan dan tempoh pengekstrakan mempunyai pengaruh yang signifikan terhadap hasil gelatin. Seterusnya, eksperimen pengoptimaan dijalankan menggunakan rekabentuk komposit pertengahan berputar (CCRD) 5-aras dengan respon yang dikaji ialah hasil gelatin, kekuatan gel dan kelikatan. Kondisi optima yang dicadangkan untuk pengekstrakan gelatin ialah menggunakan kepekatan asid sitrik sebanyak 0.14 M, suhu pengekstrakan pada 63 °C dan tempoh pengekstrakan selama 6 jam. Nilai ramalan bagi respon hasil gelatin, kekuatan gel dan kelikatan pada kondisi optima ini ialah 6.8 %, 163 g dan 3.7 mPa.s, masing-masing. Nilai eksperimen sebenar yang diperolehi daripada eksperimen pengesahan ialah hasil gelatin, 6.6 %; kekuatan gel, 161 g dan kelikatan,

3.4 mPa.s. Sifat fizikokimia, sifat berfungsi, sifat reologi dan sifat terma gelatin ikan yang diekstrak pada kondisi optima ini dikaji dan dibandingkan dengan gelatin ikan, babi dan lembu komersil. Gelatin ikan yang diekstrak berwarna kuning keperangan dan mengandungi: 8.7 % lembapan; 86.3 % protein; 2.2 % abu dan nilai pH 4.7. Kejernihan larutan gelatin ikan yang diekstrak diukur sebagai peratus transmitans ialah 80.7 %. Spektra peralihan Fourier inframerah (FTIR) gelatin ikan yang diekstrak menunjukkan ciri-ciri jalur penyerapan yang sama seperti gelatin komersil. Kandungan asid imino iaitu prolina dan hidroksiprolina gelatin ikan yang diekstrak ialah 112.2 dan 80.1 residu per 1000 residu asid amino, masing-masing. Suhu mengel dan melebur gel gelatin ikan yang diekstrak ialah pada 11.5 ºC and 19.0 ºC, masing-masing diperolehi daripada pengukuran viskoelastik dinamik. Penggunaan hasilan sampingan pemprosesan surimi sebagai bahan mentah untuk pengekstrakan gelatin ikan bukan sahaja menyumbang kepada pengurusan sisa yang lebih efektif malahan untuk penghasilan ingredien makanan bernilai tambah yang boleh menjadi sumber alternatif gelatin halal yang berpotensi untuk dieksploitasi pada masa akan datang.

FISH GELATIN FROM SURIMI PROCESSING BY-PRODUCTS: EXTRACTION AND CHARACTERIZATION

ABSTRACT

Fish gelatin was extracted from surimi processing by-products of four different fish species, namely slender shad (*Elongata ilisha*), sin croaker (*Johnius dussumieri*), ribbon fish (*Trichiurus lepturus*) and threadfin bream (*Nemipterus japonicus*). These by-products consisted of skins, bones and scales. In the initial study, highest yield of fish gelatin was obtained from slender shad fish by-products and this species was then selected for optimization of gelatin extraction condition study. Several factors that could influence the gelatin extraction process were optimized using response surface methodology (RSM) approach. The screening experiment using a 2-level full factorial design resulted in a first-order model equation indicated that three factors, i.e. citric acid concentration, extraction temperature and extraction time had significant effect on gelatin yield. This was followed with optimization experiment consisting of a 5-level central composite rotatable design (CCRD) with responses measured for gelatin yield, gel strength and viscosity. The suggested optimum conditions for gelatin extraction were using 0.14 M for the citric acid concentration, 63 ºC for the extraction temperature and 6 hrs for the extraction time. The predicted values for response gelatin yield, gel strength and viscosity under this optimum condition were 6.8 %, 163 g and 3.7 mPa.s, respectively. The actual experimental values obtained from verification experiment were gelatin yield, 6.6 %; gel strength, 161 g and viscosity, 3.4 mPa.s. Physicochemical, functional, rheological and thermal properties of extracted fish

gelatin under optimum conditions were studied and compared with those of commercial fish, porcine and bovine gelatin preparations. The extracted fish gelatin was brownish yellow in color with contents: moisture, 8.7 %; protein, 86.3 %; ash, 2.2 % ash and pH value, 4.7. The clarity of extracted fish gelatin solution measured as percent transmittance was 80.7 %. The Fourier transform infrared (FTIR) spectra of extracted fish gelatin showed similar absorption band characteristics as the commercial gelatins. The imino acid content i.e. proline and hydroxyproline of extracted fish gelatin were 112.2 and 80.1 residues per 1000 amino acid residues, respectively. The gelling and melting temperatures of extracted fish gelatin gel were found to be at 11.5 °C and 19.0 °C, respectively obtained from dynamic viscoelastic measurement. Utilization of surimi processing by-products as raw material for fish gelatin extraction not only contributes to an effective waste management but also to the production of value-added food ingredient which could serve as a potential alternative source of halal gelatin that could be further exploited in the future.

CHAPTER 1
INTRODUCTION

1.1 Background

Among commercial hydrocolloids, gelatin plays an important role as special ingredient that is widely used in food, pharmaceutical, biomedical, photographic and technical industries. One of the most unique characteristic of gelatin that cannot be exactly substituted with other hydrocolloid is its ability to form thermoreversible gel that has a low melting temperature (below human body temperature) that gives a unique mouth feel sensation and thus makes its use very favorable in the food industry (Choi & Regenstein, 2000). The demand of gelatin for the food and pharmaceutical industry uses has been increasing annually and the total worldwide consumption of gelatin was estimated about 200,000 metric ton/year (Badii & Howell, 2005; Montero & Gómez-Guillén, 2000).

Most of the commercial gelatin is currently derived from mammalian sources, mainly cattle hide, bone and pig skin. According to the Gelatin Manufacturers of Europe report, annual world production of gelatin is about 326,000 metric tons, with pig skin derived gelatin accounting for the highest output (46 %) followed by bovine hide (29.4 %), bone (23.1 %) and other sources (1.5 %) (GME, 2008a). However, porcine gelatin is forbidden for both Jew and Muslim consumption with regards to kosher and halal status while bovine gelatin from cattle that has not been religiously slaughtered is also not permissible for Muslim consumption. In addition, vegetarians and Hindus have objections to the use of gelatin which is derived from animal sources, such as cattle hide and bones (Choi & Regenstein, 2000).

The occurrence of bovine spongiform encephalopathy (BSE) crisis or "mad cow disease" has created much interest in finding alternative sources for gelatin production (Wasswa et al., 2007). Realizing the importance for searching alternative gelatin sources, which could also meet the requirement for halal and kosher by Muslim and Jew community, respectively, there is an increasing attention towards other sources of gelatin such as from poultry and fish. However, poultry skin contains a lot of fat and the concentration of collagen is low and thus makes it less preferable as raw material for gelatin production (Schrieber & Gareis, 2007). In recent years, several studies on gelatin extraction from fish skin, bone and scale of various species have been carried out such as silver carp (*Hypophthalmichthys molitrix*) skin (Boran & Regenstein, 2009), yellowfin tuna (*Thunnus albacores*) skin (Rahman et al., 2008; Cho et al., 2005), nile perch (*Lates niloticus*) skin and bone (Muyonga et al., 2004a) and lizardfish (*Saurida* spp.) scales (Wangtueai & Noomhorm, 2009).

Surimi processing by-products generated during surimi production could be a possible alternative source for gelatin production since they consist of fish skins, bones and scales. It was estimated that 7-8 metric tons of fish by-products are produced from a local surimi processing plant on a daily basis (personal communication with the manager of the plant). These by-products have low market value and were normally discarded or used for fishmeal production. Even though these materials are considered waste, they represent valuable raw materials which are rich in collagen and could be further utilize as a source of value-added food ingredient such as fish gelatin (Batista et al., 2004). This has led to the concerted research efforts to find ways to utilize the by-products obtained from local surimi processing industry. There is also added advantage in utilizing these by-products

which are renewable and abundantly available, for fish gelatin production, as it may help to increase profitability as well as to create an effective waste management system (Choi & Regenstein, 2000; Gudmunsson & Hafteinsson, 1997). To the best of our knowledge, utilization of surimi processing by-products for gelatin production has not yet been done. Thus, this study aims to look into the possibilities of utilizing surimi processing by-products consisting of fish skins, bones and scales as the raw material for the extraction and production of fish gelatin.

1.2 Objectives

The objectives of this study are:

1) To extract fish gelatin from surimi processing by-products from four different fish species namely slender shad (*Elongata ilisha*), ribbon fish (*Trichiurus lepturus*), threadfin bream (*Nemipterus japonicus*) and sin croaker (*Johnius dussumieri*).

2) To optimize the extraction conditions for producing maximum gelatin yield with high gel strength and viscosity using response surface methodology (RSM) approach.

3) To evaluate and to characterize the extracted fish gelatin for its physicochemical, functional, rheological and thermal properties and to make comparisons to several types of commercial gelatins.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction to gelatin

Gelatin is defined as a product obtained by partial hydrolysis of collagen derived from the skin, white connective tissue and bone of animals (United States Pharmacopeia, 1990). The word gelatin is derived from the Latin '*gelata'*, that mean which is frozen, congealed or stiff. The World Health Organization Report No. 48 B recommends identification and purity standards for edible gelatin and the classification of gelatin as a food ingredient rather than food additives. Gelatin is generally recognized as safe (GRAS) for use in human food by Food and Drug Administration (FDA) and the European Commission (EC) (Poppe, 1997).

According to Malaysian Food Act 1983 and Food Regulation 1985 no. 153, specification of edible gelatin is described as: "Edible gelatin shall be the clean, wholesome product obtained by processing the skin, bone or other collagenous material of animals, ordinarily used for human consumption. It shall not contain more than 16 % of water and shall not yield more than 3 % of ash. A 5 % aqueous solution of edible gelatin maintained at 18.5 °C for 2 hours shall form a gel, clear and light to color and free from offensive taste and odor" (Anon, 2007). Gelatin does not exist in nature but is derived from the collagen by hydrolysis process. The precursor of gelatin, collagen is the major structural protein found in the skin and bone of animals. Gelatin differs from other hydrocolloids because most of them are polysaccharides such as carrageenan and pectin whereas gelatin is a digestible protein containing all the essential amino acid except tryptophan (Poppe, 1997).

2.1.1 Structure of gelatin

Gelatin is not a single chemical substance because its mains constituents are large and complex polypeptide molecules of the same amino acid composition as the parent collagen, covering a broad molecular weight distribution range. The total molecular weight of collagen is approximately 330 kDa, it has a 1.5 nm diameter and a length of approximately 300 nm (Hannig & Engel, 1961). Collagen is composed of the basic collagen unit structure namely tropocollagen or polypeptide α-chains that arranged in triple helix structure to form a coil which behaves as a firm rigid rod (Figure 2.1) (Linden & Lorient, 1999b).

Figure 2.1 Triple helix structure of collagen.
(Source: Linden & Lorient, 1999b)

Various distinct types of collagen exist, each with its own genes, which express their characteristic polypeptide chains. The various types of tropocollagen molecules include type I, II, III, IV, V and VI. The best known types present in sufficient quantity to be important to gelatin technology are types I and III. Type I collagen occurs primarily in connective tissue such as skin, bone, cartilage, etc. while type III collagen occurs only in skin. Ossein gelatins which are derived from bone

sources consist solely of collagen type I. Hide and pig skin gelatins are derived from collagens that contain a small portion of type III collagen. Although the main sources of gelatin are limited to bovine and pig skin, the gelatin source has been expanded to vertebrates from aquatic animals such as fish. Gelatin derived from fish consist of type I collagen which is the main component of fish skins (Schrieber & Gareis, 2007; Ashgar & Hendrickson, 1982). Collagen contains at least 18 of the 20 amino acids that are linked together by peptide bonds to form polypeptide chains generally found in protein and is characterized by its high content of glycine, proline and hydroxyproline (Ledward, 1986). In contrast, fish collagen has relatively lower amount of proline and hydroxyproline than those of mammalian collagens (Balian & Bowes, 1977). The source of collagen can influence the composition of gelatin in two ways; firstly the amino acid composition will be similar to that of the parent collagen which varies of types and species, and secondly, different collagen may require variations in the nature or severity of the pretreatment which also result in differences in gelatin composition (Poppe, 1997).

Being a protein, gelatin is composed of a unique sequence of amino acids. The amino acid composition of gelatin is very close to that of its parent collagen and characterized by a repeating typical sequence of Gly-X-Y, where a high proportion of X and Y are the imino acid (proline and hydroxyproline). Proline can be found in either X or Y position, but hydroxyproline is only found in Y position. Several other amino acids are predominantly found in either X or Y positions. Glutamic acid and leucine are associated with the X position whilst arginine favors the Y position in Gly-X-Y triplet. Gelatin contains a large number of glycine (almost 1 in 3 residues, arranged every third residue), proline and hydroxyproline residues as shown in Figure 2.2 (Johnston-Banks, 1990; Veis, 1964).

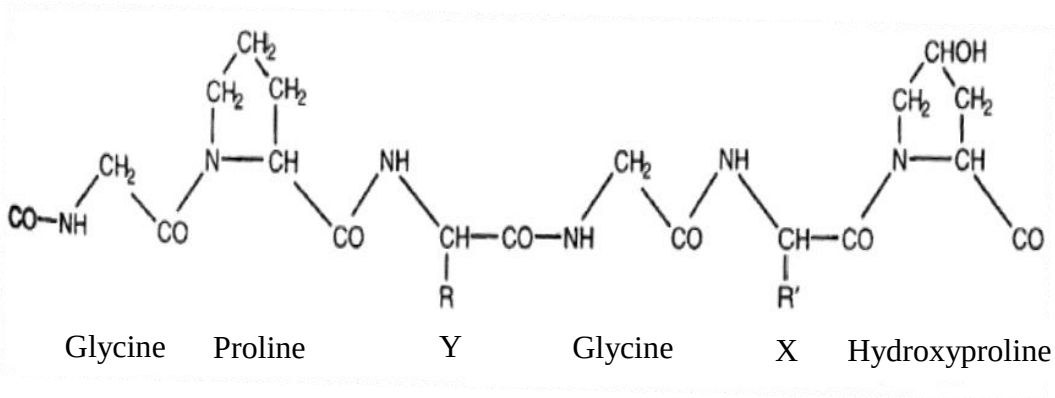

Figure 2.2 Amino acid sequences in gelatin.
(Source: Veis, 1964)

2.1.2 Source and production of gelatin

The conversion of collagen to gelatin is the most essential transformation in gelatin manufacture. The conversion of collagen to gelatin is the process whereby the highly organized fibers of collagen, which is water insoluble is transformed from an infinite asymmetric network of linked tropocollagen units to a more depolymerised system of water-soluble, independent molecules called gelatin. The simplest way to transform collagen to gelatin is to denature the insoluble collagen by heat treatment or hydrolysis which involves the destruction of the tertiary, secondary and to some extent the primary structure of native collagen (Stainsby, 1977a).

The hydrolysis can achieve the following three results: (1) the formation of three α-chains; (2) the formation of a β-chain (two α-chains linked by one or more covalent bonds) and an independent α-chain; and (3) the formation of a γ-chain (three chains linked by covalent bonds (Figure 2.3). The alpha (α), beta (β), and gamma (γ) forms of gelatin is differ mainly in their molecular weight. The molecular weight varies from 80,000 to 125,000 for the α form; from 160,000 to 250,000 for the β form; and from 240,000 to 375,000 for the γ form (Poppe, 1997; Veis, 1964).

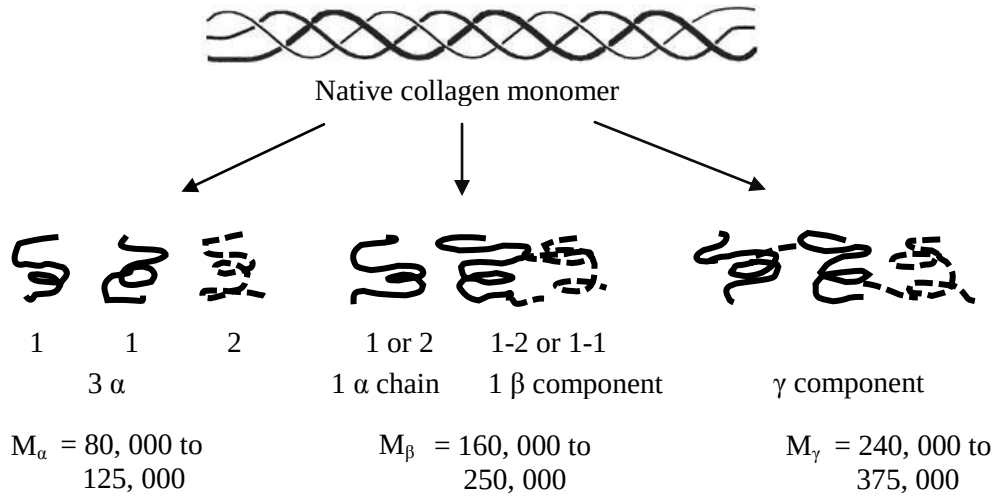

Figure 2.3 Possible paths of collagen conversion to gelatin. (Source: Veis, 1964)

The most important sources of raw materials used for the production of commercial gelatin are from healthy animals including pig skin, cattle hide and demineralized cattle bone or ossein that have been approved for human consumption and abundantly available throughout the year (Poppe, 1997). The raw material availability both in quantity and at a reasonable cost is very critical to the gelatin manufacturer. There are 2 types of gelatin: Type A, derived from acid processed materials, primarily pork skin; and Type B, derived from alkaline or lime processed materials, primarily cattle or calf hide and ossein. The main purpose in gelatin extraction is to convert the insoluble collagenous material into a maximum quantity of soluble and highly purified gelatin with good physicochemical properties such as high gel strength, high viscosity and high clarity (Hinterwaldner, 1977). In general mammalian gelatin production, the process involved several steps such as pretreatment, extraction, filtration, drying and grinding process as summarized in Figure 2.4.

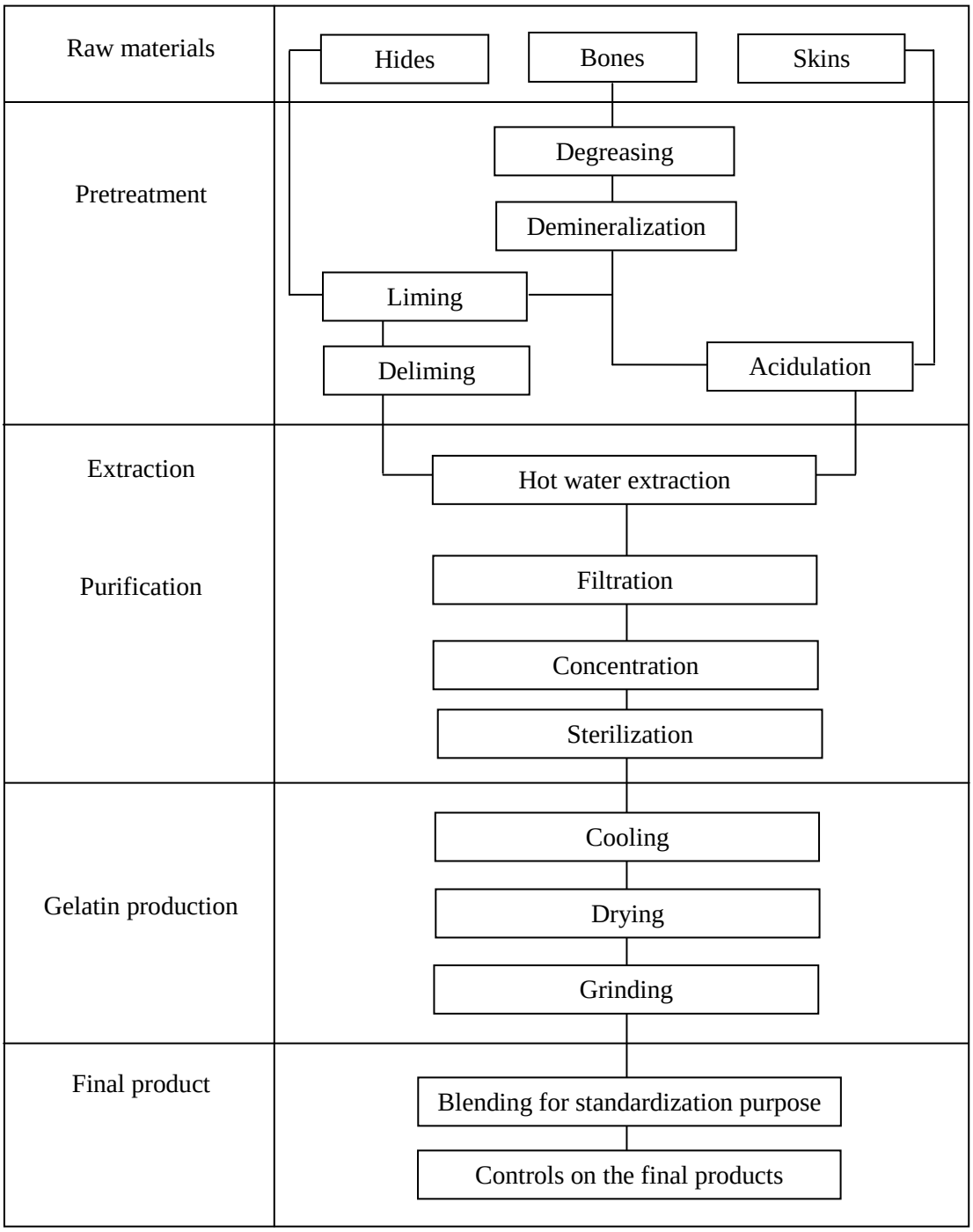

Figure 2.4 Gelatin manufacturing process.
(Source: Poppe, 1997)

The initial step includes washing the starting raw materials to remove impurities and also degreasing and demineralization of bones to produce ossein before the pretreatment process. The pretreatment process is design to convert collagen into a form suitable for extraction. To achieve this, a sufficient number of covalent cross-links in the collagen must be broken in order to enable the release of the free α-chains. The process is also designed to remove other naturally organic substances such as blood, sugar, etc. Pretreatments are optimized by each manufacturer to give the required physical and chemical properties that are produced. To convert the insoluble collagen into soluble gelatin, two processes are in current use: acid pretreatments leading to acid process type A gelatin and alkaline or basic pretreatments leading to alkaline process type B gelatin. Acid pretreatment processes are applied to pig skin and ossein whereas alkaline pretreatment processes are normally applied to bovine hide.

The extraction process is designed to obtain the maximum yield by optimizing the balance between temperature and the extraction time. In practice, gelatin is obtained from the raw material in three or four separate extractions, each at an increasing temperature. Typical temperatures are 55 °C for the first extraction, 60 °C for the second extraction, 70 °C for the third extraction and 80-90 °C for the final extraction, each giving gelatins of decreasing gel strength, viscosity, and increasing color. Following the extraction process, gelatin liquors are filtered to remove insoluble suspended such as fat or unextracted collagen fibers. The extracted gelatin was further purified by deionization which removes inorganic salts left from pretreatment. The final stage is evaporation, sterilization, drying and grinding process. Then, this gelatin is subjected to laboratory testing for their physical and microbiological characteristics (Poppe, 1997; Harris et al., 2003).

2.1.3 Composition, characteristic and properties of gelatin

Gelatin is commonly produced in the form of sheet, granule or powder with slightly white to yellow in color and it is rather tasteless and odorless. Gelatin typically contains 8-12 % moisture, 2-4 % mineral salts and the remainder being protein (85-90 %). It contains neither fat nor carbohydrate and is free from preservative or other additive. Gelatin consists of different amounts of 18 amino acids, where glycine (26-34 %), proline (10-18 %) and hydroxyproline (7-15 %) are the most abundant. However, it is not a complete protein in that it is lacking in the essential amino acid, tryptophan. Gelatins from different sources may exhibit small variations in amino acid composition. An edible gelatin should contain less than 1 ppm arsenic, less than 50 ppm heavy metals, less than 200 ppm sulphur dioxide, less than 100 ppm peroxides (as H_2O_2) and be essentially free of phenolic preservatives. An edible gelatin also must comply fully with bacteriological standard which are; 1 g of gelatin must be free of *Escherichia coli* and 10 g of gelatin be free of salmonella and must have a total viable aerobic count of less than 10^3 microorganism per gram, determined by plate count (Ledward, 2000; Poppe, 1997; Glicksman, 1969).

Gelatin is relatively insoluble in cold water, however, swell when immersed in water and completely soluble in warm water. Factors such as temperature, concentration and particle size affected the rate of solubility. Gelatin is insoluble in alcohol and other organic solvents such as carbon tetrachloride and petroleum ether, but it is soluble in polyhydric alcohols such as sorbitol, mannitol and glycerol with the presence of water. Gelatin in solution form should be crystal-clear and its clarity depends mainly on the extraction and post extraction condition. In general, the first extraction contains the highest quality of gelatin with a very good clarity while the later extraction, in contrast, can be turbid and contains more intense

color. The quality of gelatin in the later extractions can be improved by the clarification and filtering process. The gelatin solution also should be colorless to light amber or faint yellow, but lower grade gelatin will have an orange-brown color (Ledward, 2000; Ockerman & Hansen, 2000; Poppe, 1997; Glicksman, 1969).

The pH value of commercial gelatin has been reported ranging from 4-7 (Johnson-Banks, 1990). The different pH value of gelatin may be due to the different type and strength of acid and/or alkaline used during the pretreatment process (Jamilah & Harvinder, 2002). Gelatin in solution is amphoteric, capable of acting either as an acid or as a base, depending on the pH value. In an acidic solution, gelatin is positively charged and migrates as a cation while in alkaline solution gelatin is negatively charged and migrates as an anion. The pH of intermediate point, where the net charge is zero, is known as isoelectric pH or point (IEP). Type A gelatin, produced by acid pretreated raw material has a broad isoelectric region between pH 7-9 whereas type B gelatin, produced by alkaline pretreated raw material has IEP values between pH 4.8-5.5 (Foegeding et al., 1996).

The physicochemical and functional properties of gelatin depend on the raw material, pretreatment method and extraction condition (Johnston-Banks, 1990). The unique property of gelatin is its ability to form cold-setting thermo-reversible gel which melts to a liquid when heated and solidifies when cooled again. It is unusual among the proteins, in this ability to transform liquid into material which is solid like, retain their shape and have elastic properties. When a gelatin solution is cooled, the viscosity increases progressively and at the same time, the liquid changes into a gel if the concentration is sufficiently great and the temperature is low enough. The gelling temperature of a gelatin solution is dependent on its thermal and mechanical history (Stainsby, 1977a; Glicksman, 1969).

The well accepted gelling mechanism of gelatin can be explained by the formation of a three dimensional network from random coil helix reversion. The transformation of a gelatin sol to a gel can be explained as a three stage process as described in Figure 2.5. The first stage involves the intramolecular rearrangement of imino acid-rich chain segments of single-chain gelatin molecules or called as collagen fold. The imino acid-rich regions (Gly-Pro-Hyp) of different polypeptide chains act as a potential junction zones which upon cooling they partially reform a helical conformation similar to that proposed for native collagen. In the second stage, a three dimensional network is formed by the association of separate chains of collagen fold forms with other non-helical regions of the gelatin chains. In the third stage, this structure is stabilized by lateral inter-chain hydrogen bonding within the helical region (Haug & Draget, 2009; Djabourov, 1989).

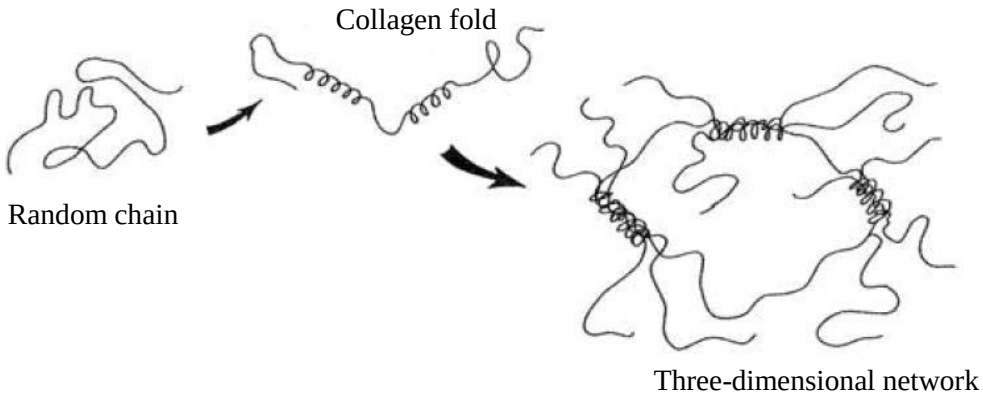

Figure 2.5 Model of gel formation (from sol to gel upon cooling).
(Source: Haug & Draget, 2009)

During heating, gelatin gel melts and the three-dimensional network starts to lose the triple helices conformation resulting in the coil chains being able to move more freely (Figure 2.6). The melting temperature is the temperature at which a gelatin gel softens sufficiently and flow as a liquid. Factors such as maturing

temperature and concentration of the gelatin gel tend to affect its melting temperature. The melting temperature of gelatin gel that is below human body temperature (27-34 °C) is one of the most important properties of gelatin that gives melt-in-mouth characteristic. The gel with lower melting temperature dissolves faster in the mouth, releasing the flavors more quickly for an instant taste sensation. This is a desirable property in ready to eat food product such as marshmallow and dessert jellies (Haug & Draget, 2009; Choi & Regenstein, 2000; Poppe, 1997).

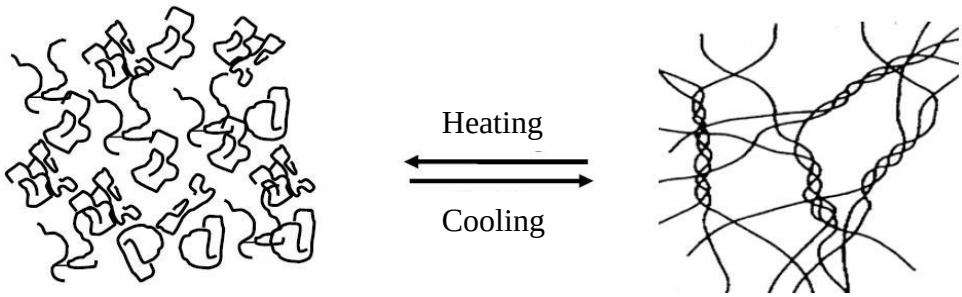

Figure 2.6 Gelling and melting mechanism of gelatin gel.
(Source: Haug & Draget, 2009)

Unlike most hydrocolloids of polysaccharide origin, gelatin gel formation is independent of the pH and does not require the presence of other reagent such as sucrose, salts, cations, etc. (Linden & Lorient, 1999a). The need to define and evaluate the characteristic of gelatin gel has resulted in the concept of gel strength or Bloom strength which represent as one of the most important physical properties of gelatin. It is associated with the rigidity of gelatin gel which is used to assess the grade and quality of gelatin. The gel strength or Bloom strength is determined according to international standard and methodology such as described by British Standards Institute (BSI, 1975) and Gelatin Manufacturers Institute of America (GMIA, 2006a).

The gel strength or Bloom strength is determined by measuring the weight in grams that is required for 12.7 mm flat bottomed cylindrical plunger to depress the surface of a 6.67 % (w/v) gelatin gel (prepared in standardized Bloom jar and matured at 10 °C for (16-18 hrs) to a depth of 4 mm. The rigidity of gelatin gel increases with time as the gel matures reaching equilibrium, approximately after 18 hrs of maturation. The strength of gelatin gel depends on the concentration, thermal history, the presence and concentration of electrolytes and intrinsic strength of the gelatin which is function of both structure and molecular weight. The gel strength increases with increasing concentration, but varies inversely with increasing temperature (Linden & Lorient, 1999a).

The gel strength of commercial gelatin is expressed using Bloom strength and quality of gelatin is generally graded by the gel strength; low (<150), medium (150-220), and high Bloom (220-300) (Johnston-Banks, 1990). According to Holzer (1996), the gel strength of most commercial gelatins has been reported to vary from less than 100 to more than 300 Bloom, however gelatin with Bloom strength of 250-260 is most desired and has been used for making desserts and confectionery such as marshmallow, gummy candies and ice cream for the food industry as well as for making hard capsules for the pharmaceutical industry.

Gelatin produces a viscosity in solution which at most temperatures and concentrations displays Newtonian rheological properties in nature. Viscosity is the second most important physical property of gelatin. The viscosity of a gelatin solution depends mainly on the hydrodynamic interactions between gelatin molecules; the contribution from the solvent and from the individual gelatin molecules. The viscosity of gelatin solution also depends on temperature (viscosity decreases exponentially with rising temperature); on pH (viscosity is minimum at the

isoelectric point); and on concentration (viscosity increases with increasing concentration) (Poppe, 1997; Stainsby; 1977b). Molecular weight distribution of the gelatin molecules also seems to be much more important in its effect on viscosity than on gel strength. Gelatin of higher gel strength may give lower viscosity than gelatin of lower gel strength, which showed that gel strength and viscosity are not directly related (Glicksman, 1969). Viscosity of commercial gelatin has been reported to vary from 2-7 mPa.s and up to 13 mPa.s for specialized ones (Johnston-Banks, 1990). However, the viscosity of gelatin has only a small effect on the viscosity of the finished product, which depends primarily on gelatin concentration and total solids concentration. Gelatin solution of low viscosity usually results in a short and brittle gel texture, while gelatin solution of high viscosity provides tough and extensible gel texture. In food applications, gelatin of high viscosity gives more 'tailing' when pouring pastille and results in chewier jelly than gelatin of low viscosity (Glicksman, 1969). According to Badii & Howell (2006), gelatin of high viscosity is preferred for many commercial applications and fetches a higher commercial price.

The ability of gelatin to stabilize emulsion and foam is the result of its protective colloidal action, which allows it to form a semisolid interfacial film around the dispersed globules. An emulsion is a dispersion of one substance in a bulk of another substance, for example, liquid system (emulsion) and gas system (foam). The strong gelatin film around the air bubbles or water droplets would stabilizes the systems. This stability is probably due to the gelatin groupings within the molecular structure of the system which permits it to orient its micelles both toward water-seeking and water repelling substances (Glicksman, 1969).

2.2 Applications of gelatin

2.2.1 Food industry uses

Food industry is the largest user of gelatin and it is used because of its unique physical properties rather than for its nutritional value as a protein (Poppe, 1997). Gelatin displays multiple functional roles in food processing and formulations as summarized in Table 2.1. The functional properties of gelatin can be divided into two groups; the first group are associated with gelling for example, gel strength, gelling time, setting and melting temperatures, viscosity, thickening, texturising, and water binding, and the second group relates to the surface behaviour of the gelatin for example, emulsion formation and stabilization, foam formation and stabilization, protective colloid function, film formation, and adhesion/cohesion (Schrieber & Gareis, 2007).

Gelatin is used as gelling agent in confectionery and jelly dessert because it gives the melt in mouth characteristic and produces more elastic and rubbery jelly, fruit pastilles and gummy bear. Gelatin is also used as whipping agent to gives chewier nougats and marshmallows. In dairy products, gelatin is used as texturising agent to improve the texture of yoghurt and as stabilizer to control the ice and sucrose crystallization in ice cream products. In spite of that, gelatin also has been used as thickener in flavoring syrups, canned soup and gravy while in salad dressing and whipped cream, gelatin acts as emulsifier. To maintain the body of fondant, gelatin is used as a binder and to reduce fat content, provide creaminess and mouth feel, gelatin is used as fat replacer. The meat industry used gelatin in the canning of cooked hams to provide water binding properties. The gelatin is added to gel the juices which exudes from the meat products during cooking or pasteurization to gives the meat an attractive appearance as well as improving its slicing properties.

The amphoteric nature of gelatin enables it to be used in the clarification of wines, beers and fruit juices in order to remove the presence of unstable and undesired levels of polyphenolic compounds which cause cloudiness during the storage and an unacceptable astringency in taste (Poppe, 1997).

Table 2.1 The multifunctionality of gelatin in the production of foodstuffs.

Application	Gelatin type	Concentration	Principal function	Secondary function
Desserts	200-260 Bloom	1.5-3.0 %	Gel formation	Texture, transparency, brilliance
Fruit gummies	200-280 Bloom	6.0-10.0 %	Gel formation	Texture, elasticity, transparency, brilliance
Marshmallows	160-260 Bloom	1.0-3.0 %	Foam formation	Foam stabilizer, gel formation
Nougat	180-220 Bloom	1.5-3.0 %	Foam formation	Foam stabilizer, gel formation
Pastilles	160-220 Bloom	1.0-2.0 %	Binding agent	Texture, improvement of melting properties in the mouth, prevents disintegration
Caramels	140-200 Bloom	0.5-2.5 %	Emulsifier, foam stabilizer	Chewability
Yogurt	220-260 Bloom	0.2-1.0 %	Syneresis stabilizer	Texture, creaminess
Foamed milk dessert	180-240 Bloom	0.3-3.0 %	Foam formation	Texture, stabilization
Jellied milk dessert	180-240 Bloom	1.0-2.0 %	Gel formation	Texture, creaminess
Meat and sausages	220-260 Bloom	0.5-2.0 %	Emulsion stabilizer	Water binder
Broths and canned meats	220-260 Bloom	0.5-2.0 %	Binding agent	Texture, sliceability

Source: Schrieber and Gareis (2007)

2.2.2 Pharmaceutical and biomedical uses

In pharmaceutical industry, gelatin is commonly used for manufacturing soft and hard capsules for medicines, dietary and health supplements because it is highly digestible, and serve as a natural protective coating for medications from light and oxidation which make them more palatable, easy to swallow and permitting smooth oral drug delivery (Ledward 2000). Gelatin capsule may be manufactured in many shapes and sizes. Various plasticizing and hardening agents are added into gelatin solution to produce capsule and aldehyde is used with gelatin to cross-link and stiffen the hard capsules (Ledward, 2000; Wood, 1977). The manufacture of hard capsule which is used for powder filling involved the dipping of stainless steel mould pins into a gelatin solution, drying, stripping from the pins, trimming of the caps and bodies, and joining together for shipment. The strength, flexibility, and the clarity of gelatin provides unique characteristics that allow the manufacture of various sizes, colors, and designs for assuring snap closure after filling (GME, 2008b; Eaton, 1989).

Soft capsule or soft gel which is mainly used for liquid filling is hermetically sealed to enclose a liquid or semi liquid. Soft gelatin capsule is manufacture-formed, filled and sealed in one continuous operation (Stringer, 1989). Glycerol is a common plasticizer used to make soft gel capsule while other plasticizers used with or instead of glycerol includes propylene glycol, mannitol, and sorbitol. In the production of tablets and gelatin-coated tablets (caplets), gelatin helps to bind the active pharmaceutical agents, reduce dusting, mask unpleasant taste, extend their shelf life and allow for printing and color coating for product identification (GMIA, 2001).

Gelatin can also be used to microencapsulate oxygen-sensitive and light-sensitive ingredients such as fish oil, vitamin A, and E to retain its value, increase the shelf life and easy to handle because microencapsulated form behaves as a dry powder. The traditional method of encapsulation is known as coacervation in which the dispersed oil is encapsulated by gelatin at the interface between the aqueous phase and the non-aqueous phases. The size and formation of the spherical microcapsule can be controlled by various methods and the typical size of microcapsule ranged from 5 to 500 micron (GMIA, 2001).

Gelatin also plays an important role in biomedical industry. In emergency treatment, plasma expander (blood volume replacement) of gelatin based is often used to replace lost blood, hence restoring the patient's blood volume balance. High Bloom gelatin is used in the manufacture of surgical sponge which is use to improve wound healing and control the bleeding during surgery. Glycerinated gelatin finds another use as base for suppositories where it is superior to other materials. The criteria for any suppository formulation are the base should be non-toxic and non-irritating to mucous membrane, compatible with a variety of drugs, the base melts or dissolves in body fluids, and the base should be stable on storage (GMIA, 2001).

2.2.3 Photographic uses

Gelatin is used as a main component in the preparation of the photographic developer (silver emulsions) during the processing of the exposed film material. It is suited as the primary vehicle for the photographic material because its physical properties are essential for the coating of the discretely layered film material. Gelatin acts as a protective colloid during the precipitation of the silver halides by controlling

the size of the silver halides grains and protects the halides grains in the reducing action, which reduces silver halides grains rapidly to metallic silver in proportion to their exposure to light when the reaction is catalyzed by the latent image formed during exposure, and slowly when the silver halides has not been exposed (GMIA, 2001). The production of photographic materials involves four processes in which different properties of gelatin are important; firstly, the formation of the emulsion, that is the precipitation and growth of microcrystal of silver halide; secondly the washing of the emulsion to remove soluble salts; thirdly a chemical sensitization process during which the light sensitivity of the emulsion crystals is greatly increased; and fourthly the coating and drying of the emulsion on a base which may be glass, paper or plastic (Kragh, 1977).

2.2.4 Other applications

Technical gelatin differs from edible and pharmaceutical gelatins principally in that it is not essential to meet the rigid specifications for human consumption set forth by the various municipal, state and federal governments to protect the health of the general public. In paper manufacturing, gelatin is either used alone or with other adhesive material for surface sizing and for coating papers such as posters, playing cards, wallpapers and glossy magazine pages. The gelatin coating creates a smooth surface by filling up the small surface imperfection thereby ensuring improved printing reproduction. The technical application for gelatin based on microencapsulation includes the microencapsulation of dyes which is used in carbonless copy paper (GMIA, 2001).

2.3 Fish gelatin

Fish gelatin has been produced commercially since 1960 and the most important producers of fish gelatin worldwide are Norland Products Inc. (US) and Croda Colloids (UK) which produce significant amount of fish gelatin for pharmaceutical industry (Schrieber & Gareis, 2007; Norland, 1990). Recently, several studies on gelatin extraction from the skin of various fish species have been published as listed in Table 2.2.

The common methods used for fish gelatin extraction has been described by Zhou and Regenstein (2005), Sarabia et al. (2000) and Gudmundsson and Hafteinsson (1997). Grossman and Bergman (1992) and Holzer (1996) have patented the extraction process for the production of gelatin from fish skins. The procedure used for preparing fish gelatin typically involved mild acid and/or alkaline pretreatment of the fish skins prior to gelatin extraction at mild extraction temperature. Calcium hydroxide (Liu et al., 2008a; Cho et al., 2006) and citric acid (Zhou and Regenstein 2005; Gómez-Guillén & Montero, 2001) are generally used in the pretreatment of fish skin gelatin due to its mild hydrolysis of the raw material.

The yields of fish gelatin obtained from fish skin has been reported for bigeye snapper skin gelatin (4.0 %) by Binsi et al. (2009), bigeye snapper and brown stripe red snapper skin gelatins (6.5 % and 9.4 %, respectively) by Jongjareonrak et al. (2006a), sin croaker and shortfin scad skin gelatins (14.3 % and 7.3 %, respectively) by (Cheow et al., 2006), red tilapia and black tilapia skin gelatins (7.8 % and 5.4 %, respectively) by Jamilah and Harvinder (2002) and sole, megrim, cod, and hake skin gelatins (8.3 %, 7.4 %, 7.2 % and 6.5 %, respectively) by Gómez-Guillén et al. (2002).

Table 2.2 Examples of available reports on extraction and characterization of fish gelatin.

(Common name)	(Scientific name)	Reference
Alaska pollock	*Theragra chalcogramma*	Zhou & Regenstein (2005) Zhou et al. (2006)
Atlantic cod	*Gadus morhua*	Gudmundsson & Hafsteinsson (1997)
Alaska pink salmon	*Oncorhynchus gorbuscha*	Chiou et al. (2006)
Atlantic salmon	*Salmo salar*	Arnesen & Gildberg (2007)
Bigeye snapper	*Priacanthus macracanthus*	Nalinanon et al. (2008) Jongjareonrak et al. (2006a)
Black tilapia	*Oreochromis mossambicus*	Jamilah & Harvinder (2002)
Blue shark	*Prionace glauca*	Yoshimura, et al. (2000)
Brownstripe red snapper.	*Lutjanus vita*	Jongjareonrak et al. (2006a)
Channel catfish	*Ictalurus punctatus*	Yang et al. (2007) Yang et al. (2008) Liu et al. (2008b)
Common carp	*Cyprinus carpio*	Ninan et al. (2010)
Dover sole	*Solea vulgaris*	Gómez-Guillén et al. (2005)
Grass carp	*Ctenopharyngodon idella*	Kasankala et al. (2007)
Hake	*Merluccius merluccius*	Montero et al. (1990) Montero et al. (1999)
Horse mackerel	*Trachurus trachurus*	Badii & Howell (2006)
Megrim	*Lepidorhombrus boscii*	Montero & Gómez-Guillén (2000)
Nile perch	*Lates niloticus*	Muyonga et al. (2004a)
Nile tilapia	*Oreochromis niloticus*	Songchotikunpan et al. (2008)
Red and black tilapia	*Oreochromis nilotica, mossambicus*	Jamilah & Harvinder (2002)
Rohu	*Labeo rohita*	Ninan et al. (2010)
Shark	*Isurus oxyrinchus*	Cho et al. (2004)
Shortfin scad	*Decapterus macrosoma*	Cheow et al. (2007)
Sin croaker	*Johnius dussumieri*	Cheow et al. (2007)
Silver carp	*Hypophthalmichthys molitrix*	Boran & Regenstein (2009)
Skate	*Raja kenojei*	Cho et al. (2006)
Yellowfin tuna	*Thunnus albacares*	Cho et al. (2005) Rahman et al. (2008)

The type of fish gelatin varies depending on the fish species particularly with respect to imino acids, proline and hydroxyproline content. Exposure to a wide range of environment conditions, particularly the temperature and production process can also influence type of gelatin produced. From the point of view of gelatin composition, fishes can be divided into four groups namely: elasmobranchs (sharks, dogfishes, and rays); cold-water fish (cod, halibut, and plaice); warm-water fish (sturgeons, carp, and threadfin); and hot-water fish (lungfishes). The gelatins from these groups cover a wider range of composition than mammalian gelatins (Eastoe & Leach, 1977).

Fish gelatin differs from mammalian gelatin in behavior and characteristic which can be classified as gelling and non-gelling types. The gelling and melting temperature of fish gelatin is lower than those of mammalian gelatin and the gel strength of fish gelatin is also lower than that of mammalian gelatin due to its lower content of the imino acids, proline and hydroxyproline (Gómez-Guillén et al., 2002).

Cold-water fish gelatin is a non-gelling type and do not have Bloom strength. Cold-water fish gelatin can be used in application which do not require gelling, in which the function of gelatin lies in its other abilities such as prevention of syneresis and texturization such as in frozen or refrigerated products which are consumed quickly following removal from the fridge or defrosting. Cold-water fish gelatin of higher molecular weight grade have the same range of amino acids as bovine and porcine gelatins but do not gel at room temperature due to the lower content of proline and hydroxyproline. These amino acids are believed to be responsible for hydrogen bonding and the gelling characteristic (Karim & Bhat, 2009).

Warm-water fish gelatin is a gelling type and has gel strength or Bloom strength. Tilapia skin has been reported as an excellent source of warm-water fish gelatin due to high yield and superior physical and functional properties (Grossman & Bergman, 1992). Warm-water fish gelatin which has Bloom strength in the range of 100-250 g and melting point of 25-27 °C can therefore readily compete in the traditional gelatin market (Karim & Bhat, 2009).

2.4 Future of fish gelatin

The future of fish gelatin looks very bright. Fish gelatin might become a niche product offering unique and competitive properties to other biopolymers. The research on properties, challenges and prospects of fish gelatin as an alternative to mammalian gelatin has been well reviewed and discussed by Karim and Bhat (2009). Fish gelatin broadens the application of gelatin as a food ingredient due to its unique properties, which is unlike mammalian gelatin.

Several studies on the applications of fish gelatin have been published recently. A sensory study conducted on gelatin gel dessert by Choi and Regenstein (2000) suggested that fish gelatin gel had better release of aroma and offered stronger flavor due to its lower melting temperature. Surh et al. (2006) reported the use of fish gelatin as food emulsifier while Cheng et al. (2007) found that combination of fish gelatin to pectin at low ratio might increase the bulk density, firmness, compressibility, adhesiveness, elasticity, and meltability of a low-fat spread. As a protein, gelatin is low in calories and the melt in mouth characteristic gives excellent sensory properties resembling fat, making it ideal for use in low-fat products.