DUCK FEET GELATIN – CHARACTERIZATION AND THE EFFECTS OF SUGARS AND SALTS ON ITS GELATION, FOAMING AND EMULSIFYING PROPERTIES

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DUCK FEET GELATIN – CHARACTERIZATION AND THE EFFECTS OF SUGARS AND SALTS ON ITS GELATION, FOAMING AND EMULSIFYING PROPERTIES

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LIST OF ABBREVIATIONS

Abbreviation	Caption
–C=O	Carbonyl group
–OH	Hydroxyl group
ANOVA	Analysis of Variance
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
BG	Bovine gelatin
BSA	Bovine Serum Albumin
BSE	Bovine spongiform encephalopathy
DFG	Duck feet gelatin
DLVO Theory	Theory named after Derjaguin, Landau, Verwery, and Overbeek
DST	Dynamic surface tension
e-OH	Equatorial hydroxyl group
et al.	Latin *et* (“and”) + *alii* (“others”)
etc.	Latin *et cetera* (“and other things” or “and so forth”)
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
Gln	Glutamine
Glu	Glutamic acid
GME	Gelatin Manufacturers of Europe
GMIA	Gelatin Manufacturers Institute of America

His	Histidine
HPLC	High Performance Liquid Chromatography
i.e.	Latin *id est* (“that is”)
ICUMSA	International Commission for Uniform Methods of Sugars Analysis
IFIC	International Food Information Council
Ile	Isoleucine
LVR	Linear Viscoelastic Region
Lys	Lysine
NMR	Nuclear Magnetic Resonance
O/W	Oil-in-water
Phe	Phenylalanine
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
W/O	Water-in-oil

LIST OF SYMBOLS

Symbol	Caption
–C=O	Carbonyl group
–OH	Hydroxyl group
a_m	Mean surface-to-surface distance
c_0	Bulk concentration
c_i	Concentration of ion
c_s	Subsurface concentration
N_A	Avogadro's number
W_{dl}	Electrostatic double layer interaction
z_i	Valency of ion
Γ_{eq}	Surface/interfacial concentration equilibrium
$\dot{\gamma}$	Shear rate
η_∞	Viscosity at high shear rate
η_0	Viscosity at low shear rate
η_1	Viscosity of the continuous phase
ϕ_{eff}	Effective volume fraction
$(CH_3)_4N^+$	Tetramethylammonium ion
°C	Degree Celsius
°F	Degree Fahrenheit
±	Plus-minus sign
<	Less than
>	More than

a	Average droplet radius
Br^-	Bromide ion
Ca^{2+}	Calcium
$CaCl_2$	Calcium chloride
Cl^-	Chloride ion
COO^-	Carboxyl group
cP	Centipoise
D	Diffusion coefficient
$D[4,3]$	Volume mean diameter
Da	Dalton
F^-	Fluoride ion
g	Gram
G'	Storage or elastic modulus
G''	Viscous of loss modulus
h	Hour or hours
h	Film thickness between droplets
HCl	Hydrochloric acid
Hz	Hertz
I^-	Iodide ion
k	Kilo
K	Kelvin
k	Flow consistency index
K^+	Potassium ion
KBr	Potassium bromide
kg	Kilogram

k_{gel}	Gelation rate constant
L	Litre
Li^{+}	Lithium ion
M	Molarity
Mg^{2+}	Magnesium ion
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
min	Minute or minutes
mm	Millimeter
N	Normality
n	Flow behaviour index
N	Collision frequency
Na^{+}	Sodium ion
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NH_3^{+}	Amine
Nm	Nanometer
NO_3^{-}	Nitrate ion
pH	Potential of hydrogen
pI	Isoelectric point
R	Radius
Rb^{+}	Rubidium ion
s	Second or seconds
SCN^{-}	Thiocyanate ion
SO_4^{2-}	Sulphate ion

t	Time
T_g	Gelation temperature
t_{gel}	Gelation time
T_m	Melting temperature
Zn^{2+}	Zinc ion
α	Lower case alpha
β	Lowercase beta
β	Lower case beta
γ	Lowercase gamma
γ	Surface tension
Γ	Surface/interfacial concentration
γ_{eq}	Equilibrium surface tension
η	Apparent viscosity
κ	Debye-Hückel parameter
λ	Long time gradient
μm	Micrometer
σ	Shear stress
ϕ	Volume fraction of dispersed phase

GELATIN KAKI ITIK – PENCIRIAN DAN KESAN GULA DAN GARAM TERHADAP SIFAT PENGGELAN, PEMBUSAAN DAN PENGEMULSIAN

ABSTRAK

Industri makanan adalah pengguna utama gelatin bagi pelbagai applikasi disebabkan oleh kemampuan yang unggul dalam penggelan, pembusaan dan pengemulsian. Sumber-sumber umum bagi pengekstrakan gelatin adalah terdiri daripada bovin dan porsin, akan tetapi ianya telah diharamkan di sesetengah negara kerana isu-isu ugama dan penyakit berjangkit. Oleh itu, suatu sumber alternatif gelatin daripada avian – gelatin kaki itik (DFG) telah dipersembahkan dalam tesis ini. Tesis ini membentangkan perbandingan sifat-sifat fisikokimia dan fungsional bagi DFG dengan gelatin bovin (BG), serta kaitan struktur–fungsi (iaitu, penggelan, pembusaan, dan pengemulsian) DFG ditambahkan dengan bahan-bahan makanan umum (iaitu, gula – sukrosa dan laktosa; dan garam – NaCl dan $MgCl_2$) pada 5 tahap kepekatan [0, 5, 10, 20 dan 40% bagi sukrosa; 0, 5, 10, 20 dan 21.6% (tepu pada 25 °C) bagi laktosa; serta 0, 1, 3, 5, 10% bagi NaCl dan $MgCl_2$). Bagi perbandingan DFG dengan BG, keputusan yang didapati daripada komposisi kimia, komposisi asid amino, serta sifat-sifat struktur dan fungsional bagi DFG adalah sebanding dengan BG komersil, menunjukkan potensi DFG untuk digunakan sebagai alternatif bagi BG.Kehadiran gula dalam gel DFG (6.67% w/w) menurunkan pemalar kadar penggelan (k_{gel}) dan kekuatan gel pada kepekatan gula yang rendah, disebabkan oleh halangan rantaian gelatin untuk membentuk rangkaian gel; akan tetapi meningkatkan k_{gel} dan kekuatan gel pada kepekatan gula yang tinggi (40% bagi sukrosa dan 20% bagi laktosa), disebabkan oleh peningkatan kelikatan yang diakibatkan oleh

penyepuhlindapan dan penghabluran molekul-molekul gula. Penambahan gula juga meningkatkan kestabilan termodinamik struktur gel, disebabkan oleh peningkatan suhu penggelan dan suhu lebur (P < 0.05). Dalam sistem pembusaan DFG, kehadiran gula menurunkan sifat-sifat pembusaan (P < 0.05) dan membentukkan buih yang lebih besar pada kepekatan gula yang rendah, disebabkan oleh kenaikan nilai kecerunan masa yang lama (λ) yang menunjukkan penurunan penyebaran dan penjerapan molekul-molekul DFG pada antara muka udara–air untuk tujuan penstabilan. Walau bagaimanapun, pada kepekatan gula yang tinggi, sifat-sifat penambahbaikkan pembusaan (P < 0.05), pembentukkan buih yang lebih kecil, dan penurunan nilai λ telah diperhatikan, disebabkan oleh kesan penstabilan daripada peningkatan kelikatan pukal. Dalam sistem pengemulsian DFG, kehadiran gula menambahbaikkan sifat-sifat pengemulsian (P < 0.05) dan membentukkan titisan minyak yang lebih kecil (P < 0.05), disebabkan oleh penstabilan titisan minyak akibat daripada peningkatan kelikatan dalam fasa berterusan (η_1). Lebih-lebih lagi, pengurangan penipisan ricih telah diperhatikan apabila kepekatan gula meningkat, iaitu menunjukkan pengurangan pemecahan struktur emulsi semasa perincihan. Secara keseluruhannya, sukrosa mempunyai kesan yang lebih ketara dibandingkan dengan laktosa. Kehadiran garam pula menurunkan k_{gel}, suhu penggelan dan suhu lebur (P < 0.05). DFG dengan peningkatan kepekatan garam, disebabkan oleh penurunan kelarutan protein akibat daripada penggaraman-keluar. Walau bagaimanapun, peningkatan k_{gel} telah diperhatikan pada kepekatan garam yang rendah (1% $MgCl_2$), disebabkan oleh penggaraman-laut akibat daripada pengikatan ion. Maka, penambahbaikkan kekuatan gel telah diperhatikan pada kepekatan garam yang rendah (1% NaCl and $MgCl_2$) bagi sebab yang sama. Dalam sistem pembusaan DFG, penurunan sifat-sifat pembusaan (P < 0.05) telah diperhatikan disebabkan oleh

penurunan penyebaran dan penjerapan molekul-molekul DFG pada antara muka udara–air akibat daripada peningkatan nilai λ apabila kepekatan garam meningkat. Walau bagaimanapun, kepekatan garam yang rendah (1% $MgCl_2$) dapat menambahbaikkan sifat-sifat pembusaan ($P < 0.05$). Dalam sistem pengemulsian DFG, penurunan sifat-sifat pengemulsian ($P < 0.05$), pembentukan titisan minyak yang lebih besar ($P < 0.05$), dan penipisan ricih yang lebih ketara pada reologi emulsi telah diperhatikan apabila kepekatan garam meningkat. Keputusan-keputusan tersebut menunjukkan penambahan garam meningkatkan pemecahan struktur emulsi, akibat daripada peningkatan kecenderungan berlakunya penyatuan dan pengelompokan. Secara keseluruhannya, NaCl mempunyai kesan yang lebih ketara dibandingkan dengan $MgCl_2$. Kesimpulannya, keputusan-keputusan yang didapati menunjukkan bahawa gula dan garam boleh mengubah sifat-sifat struktur DFG secara langsung dan tidak langsung, sehubungan itu menyumbang kepada variasi yang diperhatikan pada sifat-sifat penggelan, pembusaan dan pengemulsian. Oleh itu, dengan kawalan yang teliti terhadap keadaan yang dikehendaki, iaitu, jenis gula dan garam serta kepekatan yang dikehendaki, DFG berpotensi untuk digunakan sebagai ejen penggelan, pembusaan dan pengemulsian yang baharu dalam pelbagai produk-produk makanan.

DUCK FEET GELATIN – CHARACTERIZATION AND THE EFFECTS OF SUGARS AND SALTS ON ITS GELATION, FOAMING AND EMULSIFYING PROPERTIES

ABSTRACT

The food industry is a major user of gelatins for numerous applications due to its superior gelling, foaming and emulsifying abilities. The common sources for gelatin extraction are bovine and porcine, but they are banned in some countries due to religious and human transmitting disease reasons. Thus, an alternative source of avian gelatin – duck feet gelatin (DFG) was presented in this thesis. This thesis addressed the physicochemical and functional properties of DFG in comparison with bovine gelatin (BG), as well as the structure–function relationship (i.e., gelling, foaming, and emulsifying properties) of DFG added with common food ingredients (i.e., sugars – sucrose and lactose; and salts – NaCl and $MgCl_2$) at 5 levels of concentrations [0, 5, 10, 20 and 40% for sucrose; 0, 5, 10, 20 and 21.6% (saturated at 25 °C) for lactose; and 0, 1, 3, 5, 10% for NaCl and $MgCl_2$]. For the comparison of DFG and BG, the results obtained from the physical, chemical and functional properties of DFG were comparable with that of commercially available BG, indicating the potential of DFG to be utilized as an alternative to BG. The presence of sugars in DFG gels (6.67% w/w) reduced the gelation rate constant (k_{gel}) and gel strength at lower sugars concentrations, due to the prevention on the formation of junction zones; but increased the k_{gel} and gel strength at high sugars concentration (40% for sucrose and 20% for lactose), due to more enhanced viscosity resulted from the annealing and crystallization of sugars molecules. The addition of sugars also

increased the thermodynamic stability of the gel structure, resulting in an increased gelling and melting temperatures ($P < 0.05$). In DFG foam systems, the presence of sugars reduced the foaming properties ($P < 0.05$) and formed larger air bubbles at low sugars concentrations, attributed to the increment in long time gradient (λ) values which indicates reduced diffusivity and adsorption of DFG molecules into the air–water interface for stabilization. However, at high sugars concentrations, improved foaming properties ($P < 0.05$), smaller air bubbles, and reduced λ values were observed, resulted from the stabilization effect attributed to the increased bulk viscosity. In DFG emulsion systems, the presence of sugars improved the emulsifying properties ($P < 0.05$) and formed smaller oil droplets ($P < 0.05$), resulted from the stabilization of oil droplets attributed to the increased viscosity in the continuous phase (η_1). Moreover, lesser shear-thinning effect was observed upon increasing sugars concentrations, indicating lesser disruption of the emulsion structure during shearing. Overall, sucrose had more pronounced effect than that with lactose. The presence of salts lowered the k_{gel}, gelling and melting temperatures ($P < 0.05$) of DFG gels with increasing salts concentration, resulted from the reduced protein solubility attributed to the salting-out event. However, an increased k_{gel} was observed at low salt concentration (1% $MgCl_2$), resulted from salting-in event due to ion binding. Consequently, improved gel strength was observed at low salts concentrations (1% of NaCl and 3% of $MgCl_2$) due to the same reason. In DFG foam systems, reduced foaming properties ($P < 0.05$) were observed due to the reduced diffusivity and adsorption of DFG into the air–water interface, resulted from an increased in λ values upon increasing salts concentrations. However, low salt concentration (1% $MgCl_2$) could improve foaming properties ($P < 0.05$). In DFG emulsion systems, decreased in emulsion properties ($P < 0.05$), formation of larger

oil droplets ($P < 0.05$) and more pronounced shear-thinning effect on the rheology of emulsions were observed upon increasing salts concentrations. These results indicate that the addition of salts enhanced the disruption of the emulsions structure, resulted from the increased tendency of coalescence and flocculation. In overall, NaCl had more pronounced effect than that with $MgCl_2$. In conclusion, these results indicate that sugars and salts could directly or indirectly alter the structural properties of DFG, thus contributing to the variations observed in gelling, foaming, and emulsifying properties. Therefore, with careful control on the desirable conditions, i.e., types of sugars and salts as well as desirable concentrations, DFG can be potentially used as a novel type of gelling, foaming and emulsifying agent in many food products.

CHAPTER 1

INTRODUCTION

1.1 Background

Gelatin is a denatured product of collagen and has been widely used in various products. To date, gelatin has been applied in food, confectionery, pharmaceutical and cosmetic products. In the food industry, gelatin is mainly used as gelling, foaming and emulsifying agents, colloid stabilizer, biodegradable film-forming material, and microencapsulating agent (Gómez-Guillén *et al*., 2011). Because of the unique rheological properties and melt-in-the-mouth-texture, gelatin is particularly very useful to the food industry (Haug & Draget, 2011). It is important to note that there are no plant sources of gelatin, and there is no chemical relationship between gelatin and materials referred to as vegetable gelatin (i.e., seaweed extracts) (Mariod & Adam, 2013). The most abundant sources for global gelatin production are derived from porcine skin (45.8%), bovine hide (28.4%), and cattle bones (24.2%) (Haug & Draget, 2011). However, because of the outbreak of bovine spongiform encephalopathy (BSE) and the ethnical/religious issues on the usage of pork products, the demand to search for alternative sources for porcine and bovine gelatin replacement is increasing (GME, 2016). Gelatin can be produced from cold and warm water fish species, but the low availability of raw materials for manufacturing limits the production (Karim & Bhat, 2009; Haug & Draget, 2011). Therefore, gelatin extracted from avian sources could be a potential replacement for mammalian gelatin.

Asia dominates world duck annual production for nearly 3.7 million tones from a total production of almost 4.4 million tones (FAO, 2015). This means

that Asia accounts for more than 80% of the total world duck production. Among those Asia duck producers, Malaysia is the third main producer ranked after China and Thailand, with an industry growth rate at 3.6% per year (FAO, 2015). Along with the increasing duck meat production, wastes or by-products from duck meat processing, such as skins, feet and bones are generated (Almeida & Lannes, 2013; Nik Aisyah *et al*., 2014; Tan *et al*., 2014). Utilization of these wastes could yield ample amount of gelatin and also minimize wastage from the poultry industry (Nik Aisyah *et al*., 2014). Besides, production of gelatin from these by-products is free from the BSE and religion concerns, unless they are slaughtered according to Jewish and Islamic laws (Almeida & Lannes, 2013). Furthermore, a group of researchers from Universiti Sains Malaysia have reported that duck feet contain a very high amount of extractable collagen based on their preliminary study (Huda *et al*.,2013). Poultry feet are more favorable than skins because skins contain high amount of fat content with low collagen concentration (Almeida & Lannes, 2013). Based on these reasons, it is possible that duck feet could be utilized as a novel source for mammalian gelatin replacement and therefore was selected as a subject of study in this research.

In food processing, sugars and salts are examples of food ingredients that commonly formulated in various gelatin food systems. Apart from contributing certain sensory characteristics, the addition of sugars and salts could modify or interact with gelatin and ultimately influence the product functionalities (i.e., gelling, foaming and emulsifying) (Haug & Draget, 2011). However, the knowledge on the effects of sugars and salts on gelatin remains incomplete today, because the structure–function of gelatin upon the addition of these ingredients is hardly predicted (Harper, 2009). Therefore, approaches on studying the structure–function

relationship of sugars and salts in gelatin gels, foams and emulsions were suggested herein. Investigating the gelation kinetics on sugars and salts in gelatin gel systems could advance the knowledge on the gelation behavior in real food formulation, such as jellies, gummies and candies (Fonkwe, Narsimhan, & Cha, 2003). In aerated products such as ice creams, cakes and marshmallow, the information on the stability and/or interfacial properties of these products could be investigated by studying the adsorption kinetics of gelatin at the interface (Foegeding & Davis, 2011). By employing dynamic surface tension (DST) modelling using asymptotic equation, the adsorption kinetics of gelatin upon the addition of sugars and salts can be quantified (Eastoe & Dalton, 2000). In gelatin-based food emulsions such as yogurt, sauces, cream cheese and margarine, the understanding on the bulk rheology could provide useful fundamental information on the structural organization and interactions of the components within emulsions as well as the flow behavior of emulsions during processing (McClements, 2009; Pal, 2011).

In a nutshell, in order to fully expand the utilization of duck feet gelatin (DFG) in various food products, the understanding of the structure–function relationship within DFG and common food ingredients (sugars and salts) on its gelling, foaming and emulsifying properties is crucial. Furthermore, quantitative data on the study of sugars and salts in gelatin gel, foam and emulsion systems are lacking. By employing well-developed theories and mathematical models in studying the gelling, foaming and emulsifying properties of DFG, the structure–function of this novel source of gelatin with common food ingredients can be quantified and predicted. It is envisaged that the study on the effects of sugars and salts in these simplified systems could provide reproducible basis of knowledge for future studies in real food systems.

1.2 Research Objectives

The main objective of this entire research was to investigate the possibility of DFG to be used as a novel source of gelatin for mammalian gelatin replacement and its structure-function relationship with common food ingredients (i.e., sugars and salts). The effects of sugars and salts on DFG gels, foams and emulsions were studied to provide a basis for further research into the potential application in the food industry. The specific objectives were:

1. To investigate and compare the physicochemical and functional properties of DFG with commercial BG in relation to the chemical composition, amino acid composition, secondary structure, molecular weight distribution, protein solubility, gel strength, viscosity, color, clarity, rheological properties, emulsifying properties, and foaming properties.
2. To investigate the effects of sugars (sucrose and lactose) and salts (NaCl and $MgCl_2$) on the secondary structure, gelation kinetics, thermal and textural properties of DFG.
3. To investigate the effects of sugars (sucrose and lactose) and salts (NaCl and $MgCl_2$) on the interfacial and functional properties of DFG foams with respect to the dynamic surface tension, modelling of the adsorption kinetics at air–water interface, foaming properties and foam morphology.
4. To investigate the effects of sugars (sucrose and lactose) and salts (NaCl and $MgCl_2$) on the rheological and functional properties of DFG emulsions in relation to the bulk rheology of emulsions, oil droplet size distribution and emulsifying properties.

1.3 Thesis Outline

The attempt to make DFG as a novel source of replacement for mammalian gelatin was presented in this thesis. In order to further expand the utilization of DFG, the effects of common food ingredients (i.e., sugars and salts) on DFG gels, foams, and emulsions were also presented. The main body of this dissertation comprises of a general introduction and background, literature reviews, results and discussion, overall conclusions as well as recommendations for future study. Results and discussion on the effects of sugars and salts were presented within each food system (i.e., gels, foams, and emulsions, respectively) in each separate chapter to ease reading.

CHAPTER 1 comprises a general introduction on the background and rationales of this entire research, in which the current progress and challenges of gelatin utilization encountered by food industry were presented. The alternative source of gelatin from avian was then proposed. Moreover, the objectives of this research were also presented in this chapter.

CHAPTER 2 illustrates the general literature review on the basic properties of gelatin and the common food ingredients used in this research, as well as some general knowledge on the adsorption kinetics of foams and rheology of emulsions. The theories and the mathematical models used to study the interfacial properties of foams and the bulk rheology of emulsions were expressed as well.

CHAPTER 3 demonstrates the comparison of physicochemical and functional properties of duck feet and bovine gelatins in order to explore the possibility on the utilization of DFG as a commercial BG alternative. The methods employed, results and discussion as well as a brief conclusion were presented.

CHAPTER 4 entails the effects of sugars and salts on the secondary structure, gelation kinetics, thermal properties and texture of DFG gels. The methods and mathematical model used, results and discussion as well as a brief conclusion were elaborated as well.

CHAPTER 5 illustrates the effects of sugars and salts on the interfacial properties and foaming properties of DFG foams. The adsorption kinetics of DFG on foams containing sugars and salts were quantified using theories and mathematical model. A brief conclusion was drawn as well.

CHAPTER 6 comprises of the effects of sugars and salts on the bulk rheology, oil droplet size distribution and emulsifying properties of DFG emulsions. Well-developed theories and model equations were used to explain the variations observed.

The last chapter (CHAPTER 7) consists of overall conclusions on the entire research and several recommendations for the future study on the utilization of this novel type of gelatin.

CHAPTER 2

LITERATURE REVIEW

2.1 Gelatin

2.1.1 Introduction

Gelatin is a proteinaceous material obtained from the denaturation of animal connective tissue (collagen) via hydrolysis in acidic (Type A) or basic (Type B) condition followed by hot water extraction that destroys the quaternary, tertiary and secondary structure of collagen (Fernandez-Diaz, Montero, & Gomez-Guillen, 2001; Stevens, 2010). In food industry, gelatin is sold commercially according to its gel strength, or so called "Bloom" strength, which is varied depending on the raw materials used and manufacturing processes applied (Stevens, 2010). It is available in sizes ranging from a fine to coarse powder, or in the form of sheets or flakes. Gelatin can readily hydrate in warm or hot water to provide low-viscosity solutions for whipping and foaming purposes. On the other hand, concentrated gelatin solutions can provide good gelling properties for use in confectionery (Harris, Normand, & Norton, 2003; Stevens, 2010). Gelatin is widely sold in the food industry more than any other gelling agents because it is relatively cheap to produce in bulk and there is abundance of raw materials to be utilized (Harris, Normand, & Norton, 2003). Owing to these reasons, gelatin is used in a wide range of food, pharmaceutical and photography industries. Some examples of food products that contain gelatin are jelly candies, marshmallow, ice cream, yogurt, dairy desserts, creams, low-fat spreads, canned meat products and water desert gels (Stevens, 2010). This section reviewed the chemical structure, sources as well as the physicochemical and functional properties in relation with its food applications.

2.1.2 Raw Material Sources

Commercially, the hides, skins and bones from mammalian sources such as porcine and bovine are preferred for gelatin production (Haug & Draget, 2011). Gelatins are also produced from skins of cold and warm water fish species but only in minor quantities (Stevens, 2010). These raw materials are normally collected from animals approved for human consumption and have gone through ante- and post-mortem veterinary examination (GME, 2016). The distributions of raw materials used for gelatin production worldwide and in Europe are shown in Figure 2.1. Based on Figure 2.1, it can be observed that Europe prefers gelatin extracted from porcine sources due to the outbreak of BSE. However, in other parts of the world such as Asia and Africa, gelatins extracted from bovine sources are preferred due to ethnical or religious reasons (Stevens, 2010). Some other sources that account for about 2% in the pie charts include fish and poultry gelatins. Gelatin extracted from cold-water fish species has sub-optimal physical properties compared to mammalian gelatins and this limits its application. Warm water fish gelatins may replace mammalian gelatin but the low availability of raw materials for production limits the yield (Haug & Draget, 2011). Therefore, over the last decades, the demand for exploration on alternative sources for gelatin production is increasing. Table 2.1 summarizes some reports on possible alternative gelatin sources for food application.

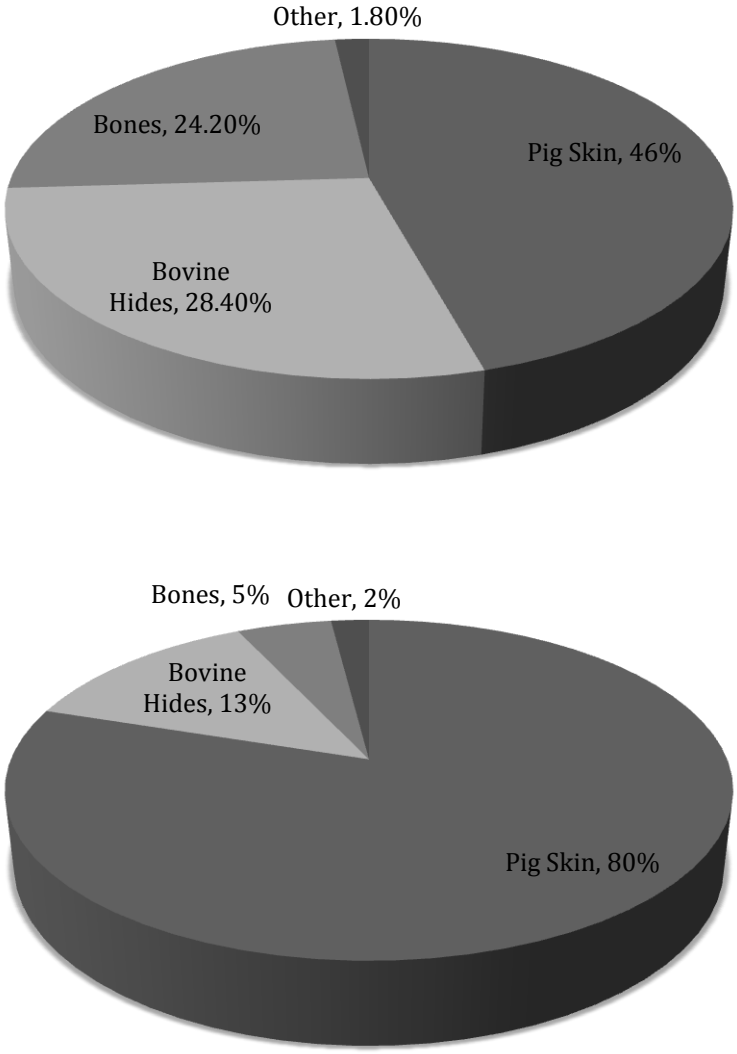

Figure 2.1 The raw material sources for world (top) and Europe (bottom) gelatin production in 2016. (adapted from: GME, 2016)

Among those alternatives sources listed in Table 2.1, despite being inferior to mammalian gelatins, gelatin extracted from aquatic sources such as scales, skins, bones and swim bladders from fish species have received considerable attention for its potential use as an ingredient in food industry over the last decades. Examples of extracting procedures and gelling properties of gelatins extracted from different typical cold-water species, such as cod, Atlantic salmon, haddock, Alaska Pollock or hake; tropical or sub-tropical species, such as black or red tilapia, Nile

perch, channel catfish, yellowfin tuna, sin croaker, shortfin scad, skate or grass carp; flat species, such as megrim, Dover sole; as well as cephalopods, such as giant squid have been extensively reviewed by Gómez-Guillén *et al*. (2009) and Karim and Bhat (2009). The major drawbacks and challenges in utilizing fish gelatins are the dark color, strong fishy odor, and poor gel and film forming properties of the fish gelatins (Montero & Gómez-Guillén, 2000). Apart from these, insect gelatins may serve as an alternative source that is acceptable for Muslims products. In some areas in Sudan, the crude oil was extracted from melon bug (*Aspongopus viduatus*) and sorghum bug (*Agonoscelis pubescens*) for cooking and some medicinal purposes. Mariod and Adam (2013) reviewed some properties of gelatin extracted from these two insect species.

Recently, gelatin extracted from avian sources has gained perceivable attention, e.g., gelatin extracted from by-products of chicken meat processing and gelatin extracted from turkey meat (see Table 2.1). Moreover, a group of researches from Universiti Sains Malaysia has recently reported than duck feet contains a highly extractable collagen content that is potential for gelatin extraction based on their preliminary study (Huda *et al*., 2013). As previously indicated that the global production on duck breeds has grown tremendously in recent years, with Malaysia is the third main producer ranked after China and Thailand. Duck breeds are commonly used for meats and eggs production (FAO, 2015). This is because duck meats and eggs contain optimal amino acids as well as favorable composition of fatty acids which are highly nutritious for human diet. Among those duck breeds mentioned, Pekin, Muscovy, Khaki Campbell, India Runner and mule are widely used for meat and/or egg production in Asia countries (Adzitey & Adzitey, 2011). It is reported that the modern domestic white Pekin duck (*Anas domesticus*) performs better than

that with modern broiler chicken in terms of weight gain and feed efficiency to the same live weight due to genetic improvement (Zhou, 2011). In conjuction with the increasing duck meat production, wastes, such as skins, feet and bones are generated enourmously. Therefore, utilization of these wastes could gain ample amount of gelatin and also minimize wastage from the poultry industry (Almeida & Lannes, 2013; Nik Aisyah, Nurul, Azhar, & Fazilah, 2014).

Table 2.1 Some reports on possible alternative gelatins extracted from (a) aquatic, (b) insects, and (c) avian sources for food application

Gelatin Sources	Results	References
(a) Aquatic		
Shark skin	The elastic modulus G' of shark skin gelatin was lower than that of commercial pig skin gelatin, but the melting enthalpy of shark skin gelatin gel was greater than that of pig skin gelatin.	(Yoshimura*et al*., 2000)
Tilapia skin	The bloom strength of gelatin extracted from black tilapia skin (180.8 g) was higher than that from red tilapia skin (128.1 g). The black tilapia skin gelatin was also higher in viscosity, melting point and amino acid content.	(Jamilah & Harvinder, 2002)
Sin croaker and shortfin scad skin gelatin	Shortfin scad skin gelatin could have better thermal and rheological properties with salt addition than sin croaker skin gelatin. The melting temperatures for both fish skin gelatins were higher than that of bovine gelatin.	(Sarbon, Cheow, Kyaw, & Howell, 2014)
Thornback ray skin	The molecular weight and gel strength for thornback ray skin gelatin was lower than that with commercial bovine gelatin. Thornback ray skin gelatin also showed stronger ability to clarify apple juice than bovine gelatin.	(Lassoued*et al*., 2014)
Yellow tuna skin	Yellow tuna skin gelatin extracted with pretreatment at 0.2 M NaOH solution for 30 min followed by 0.1 M acetic acid solution for 1 h and subsequently subjected to extraction at 55 °C for 1 h could provide 11.02% yield with higher gel strength (260.1 g).	(Karayannakidis, Chatziantoniou, & Zotos, 2014)
Dover sole skin	Lactic acid (25 mM) proved to be an excellent substitute for acetic acid during	(Giménez *et al*., 2005)

(*continued from previous page*)	pretreatment, with identical properties obtained to that prepared by using 50 mM acetic acid.	
Catfish skin	The catfish skin gelatin exhibited higher molecular weight fractions than commercial mammalian gelatin. The film forming properties of catfish skin gelatin determined by tensile strength, elongation, and water permeability were comparable with those of commercial mammalian gelatin.	(Zhang, Wang, Herring, & Oh, 2007)
	The optimum conditions for catfish skin gelatin preparation were 0.2 M NaOH for 84 min, followed by 0.115 M acetic acid extraction at 55 °C.	(Yang, Wang, Jiang, Oh, Herring, & Zhou, 2007)
Silver carp skin	The properties of silver carp skin gelatin were similar with those of fish gelatins currently being exploited commercially. The optimum conditions for silver carp skin extraction were 0.1 N HCl for 45 min at 50 °C.	(Boran & Regenstein, 2009)
Alaska pollock skin	The combination of an alkaline pretreatment followed by an acid pretreatment removed the non-collagenous protein and also provided the proper pH condition for extraction.	(Zhou & Regenstein, 2005)
Catla catla swim bladders	The melting and gelling temperatures of gelatin extracted from the swim bladder were found to be 23.3 °C and 13.7 °C respectively. The bloom strength obtained was 264.6 g and was categorized as high bloom gel.	(Chandra & Shamasundar, 2015)
Zebra blenny skin	A protease-aided process using commercial pepsin improved gelatin extracted from zebra blenny skin. The extracted gelatin illustrated relatively high gel strength and desirable solubility, foaming and emulsifying properties.	(Ktari, Jridi, Nasri, Lassoued, Barkia, & Nasri, 2014)
Bigeye snapper	The bloom strength obtained from bigeye snapper gelatin (227.73 ~ 254.10 g) was slightly lower than that of commercial bovine gelatin (293.22 g).	(Benjakul *et al*., 2009)
Red snapper and grouper	The results on physical and functional properties for gelatin extracted from fish bones suggested that their qualities were identical to mammalian gelatin.	(Shakila, Jeevithan, Varatharajakumar, Jeyasekaran, & Sukumar, 2012)
(b) Insect		
Sorghum bug Melon bug	Alkaline and acid pretreatment during insect gelatin extraction removed non-	(Mariod *et al*., 2011)

(*continued from previous page*)	collagenous proteins with minimum collagen loss. Both gelatins showed distinctive different on secondary structure as identified by FTIR.	
(c) Avian		
Chicken feet	A gelatin with high bloom (294.78 g) was obtained from gelatin extracted from skin and tendon of chicken feet, indicating that the extracted gelatin could be used in food products that require high bloom.	(Almeida & Lannes, 2013)
	Physicochemical and functional properties of chicken skin and feet gelatin showed similar characteristic with that of bovine gelatin but better than fish gelatins.	(Sarbon, Badii, & Howell, 2013; Widyasari & Rawdkuen, 2014)
Turkey meat	Gelatin extracted from turkey meat via collagen biomass illustrated higher bloom value (353.2 g) compared with that of thermally extracted gelatin. Gelatin extracted at 60 °C possessed higher foaming and emulsifying properties than those extracted from 50 °C.	(Du, Keplová, Khiari, & Betti, 2014)

2.1.3 Chemical Structure

It is well known that gelatin is obtained from thermal denaturation of collagen, the important component of skin, bones and cartilage. Therefore, it can be understood that the primary structure and composition of gelatin resembles the parent collagen (Haug & Draget, 2011; Mariod & Adam, 2013). The collagen molecule is about 300 nm long, 1.5 nm in diameter and with a molecular weights around 280 – 300 kDa (Ziegler & Foegeding, 1990). The basic collagen unit (tropocollagen) is a triple helical rod made up of three parallel α-chains stabilized by hydrogen bonding, with each chain containing 1000 amino acids (see Figure 2.2). The amino acid composition of these chains is quite unique with that the three α-chains involved in the formation of a collagen molecule may differ slightly in their amino acid composition (Johnston-Banks, 1990). Generally, the amino acid sequence in α-chain is formed by glycine–X–Y, where X is mostly proline and Y is

mostly hydroxyproline (Asghar & Henrickson, 1982). The total content of proline and hydroxyproline is termed imino acids, which provide the stabilizing effect in the triple helical structure (Harris, Normand, & Norton, 2003). Approximately one-fourth of the collagen molecule is proline or hydroxyproline. On the other hand, glycine accounts for about one-third of all the residues that serves as the backbone for the molecule of collagen and gelatin (Ziegler & Foegeding, 1990). The amino acid compositions of several examples of gelatins and collagen are depicted in Table 2.2. Based on Table 2.2, a striking difference between gelatins extracted from different sources can be observed, i.e., the total imino acids varies markedly between species. Warm water fish gelatin has higher content of imino acids content than cold water fish gelatin as reported by Sarabia, Gómez-Guillén, and Montero (2000).

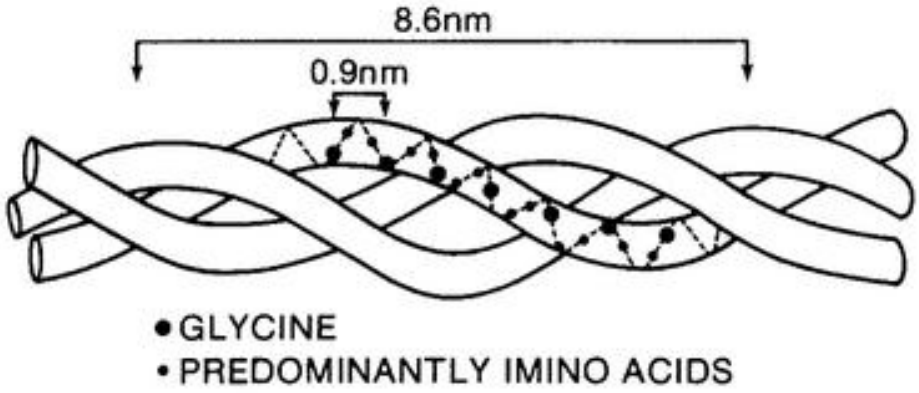

Figure 2.2 The collagen triple helix. Individual α-chains are left-handed helices with approximately three residues per turn. (adapted from: Ziegler & Foegeding, 1990)

Table 2.2 Amino acid compositions for several examples of collagen and four different gelatins

Amino acid	Number of residues/1000*				
	Type I collagen (bovine)[a]	**Type A gelatin[a]**	**Type B gelatin[a]**	**Cold water fish gelatin[b]**	**Warm water fish gelatin[c]**
Nonpolar					
Glycine	332	330	335	347	347
Alanine	114	112	117	112	123
Valine	22	26	22	18	15
Leucine	24	24	24	21	23
Isoleucine	11	10	11	11	8
Methionine	6	4	4	3	9
Phenylalanine	13	14	14	13	13
Proline	115	132	124	96	119
Polar					
Hydroxyproline	104	91	93	60	79
Serine	35	35	33	63	35
Threonine	17	18	18	24	24
Glutamine	48	48	72	72	69
Tyrosine	4	3	1	9	2
Asparagine	16	16	46	48	48
Electrically charged					
Aspartic acid	29	29	-	-	-
Glutamic acid	25	25	-	-	-
Lysine	28	27	28	28	25
Hydroxylysine	5	6	4	5	8
Arginine	51	49	48	49	47
Histidine	4	4	4	11	6

[a]Babel, 1996.
[b]Product Information from Norland Products Inc., 1999.
[c]Sarabia, Gómez-Guillén, & Montero, 2000.

2.1.4 Physicochemical Properties

Gelatin exhibits a wide range of physicochemical properties that confer great versatility and larger number of functional properties to be applied in various products. Over the years, scientists have shown that these physicochemical properties are greatly related to the chemical structure of the gelatin (Piez & Gross, 1960; Harrington & Rao, 1967; Leuenberger, 1991; Sarabia, Gómez-Guillén, & Montero, 2000; Haug, Draget, & Smidsrød, 2004). This section listed several important physical and chemical properties of gelatin.

2.1.4 (a) Physical Properties

2.1.4 (a)(i) Solubility and Viscosity

Gelatin has good solubility and exhibits high water-binding capacity, i.e., gelatin is readily swelled up to 10 times its own weight upon contact with cold water forming large swollen particles and rupture into solution upon heated above the melting point (Harris, Normand, & Norton, 2003). This behavior is owing to the pyrrolidine content of gelatin and the presence of ionic charges along the chain (Haug & Draget, Gelatin, 2011). Gelatin solutions are generally having low viscosity compared to other hydrocolloids and therefore easy to handle, even at low temperatures of 50–70 °C (Stevens, 2010). At temperature above 40 °C, gelatin solutions exhibit the properties of a Newtonian fluid behavior. However, the viscosity of gelatin solutions depends greatly on temperature, concentration, ionic content, pH and molecular weight (Stevens, 2010).

2.1.4 (a)(ii) Gel Strength/Bloom

A gelatin solution sets below the gelling temperature, which is dependent on the gelatin type, concentration, ionic content, gel strength and viscosity (Stevens, 2010). This gelation process is due to the rigidity factor that is attributed to the formation of junction zones, where the individual molecular chains rearrange into an ordered network of helical arrangements (Haug & Draget, 2011). The gel strength of gelatin is measured with a standard method using specific concentration and procedures (GMIA, 2013). The gel strength is often expressed as Bloom, which ranges from 50 to 300 g. Gelatin is commonly divided in three categories for commercial products, i.e., low Bloom (less than 125 g), medium Bloom (150–200 g), and high Bloom (above 220 g) (Stevens, 2010).

2.1.4 (a)(iii) Gelling and Melting Point

Scientists have proven that the thermal stability of a gelatin is related with its pyrrolidine content, and there is evidence suggesting that hydroxyproline located in the third position of the triplet is the major stabilizer due to its hydrogen bonding ability (Haug & Draget, 2011). Above a minimum concentration of 0.5%, gelatin solutions gel when cooled (Ziegler & Foegeding, 1990). However, when gelatin is heated to temperatures over 25–35 °C, gelatin gels will melt. This is the unique melt-in-the-mouth property illustrated by gelatin (Mariod & Adam, 2013). Moreover, the gelatin gels exhibit 'gel-to-sol' and 'sol-to-gel' changes repeatedly several times without loss of gel characteristics, which indicate the unique thermo-reversibility characteristic (Stevens, 2010).

2.1.4 (b) Chemical Properties

Gelatin exhibits an amphoteric behavior owing to the functional groups on the amino acids, and the terminal amino and carboxyl groups (Stevens, 2010). Gelatin carries a positive net charge in the presence of hydrogen ions (acid medium), whereas gelatin shows a negative charge in the presence of hydroxyl ions (basic medium) (Harris, Normand, & Norton, 2003).

2.1.4 (b)(i) Isoelectric Point (*p*I)

The *p*I corresponds to the pH at which gelatin has a neutral charge with no net migration takes place in an electric field (Harris, Normand, & Norton, 2003). At *p*I, the positive charges from amine (NH_3^+) groups equal the negative charges on the carboxyl (COO^-) groups (Stevens, 2010). The *p*I of gelatin is dependent on the raw materials (skin, bones, pork, beef, etc.), raw material pre-treatment during

manufacturing by the acid (Type A gelatin) or alkaline (Type B gelatin) condition (Harris, Normand, & Norton, 2003). Type A gelatin normally exhibits a *p*I in the range of 6–9.5, whereas Type B gelatin has a *p*I ranging from 4.5–5.6 (Stevens, 2010; Harris, Normand, & Norton, 2003). Many functional properties of gelatin are affected near the *p*I because of electrostatic attraction of oppositely charged groups, i.e., either maximum or minimum. Therefore, the physical properties such as swelling, viscosity and gelation show minimum values at the *p*I, whereas turbidity, gel strength, foaming ability and syneresis exhibit a maximum valueat the *p*I (Stevens, 2010).

2.1.5 Functional Properties and Food Applications

Food manufacturers often use gelatin as an ingredient in their formulation because this ingredient provides several technological benefits not only for single use but also could be the combination at the same time (Stevens, 2010). Table 2.3 listed several functional properties of gelatin for food applications.

2.1.5 (a) Gel Formation

Gelatin is often used in confectionery for its gelling and stabilizing properties, such as regulation of sugar crystal growth (Harris, Normand, & Norton, 2003; Stevens, 2010). In dairy industry, gelatin is applied to prevent syneresis and adsorb the water released by other hydrocolloids, owing to its superior water-binding ability (Jones, 1977; Haug & Draget, 2011). In yoghurts and mousses, the addition of gelatin helps control the texture by reinforcing the milk protein network (Jones, 1977; Haug & Draget, 2011). Another outstanding feature of gelatin that needs to be emphasized is the sensory characteristics, i.e., its thermo-reversible characteristic