Extraction and characterization of gelatine from chicken skin

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Extraction and characterization of gelatine from chicken skin

ROBIATUN AIN BT MAIDIN

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

DECEMBER 2013

SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature :
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To my parents and siblings

ACKNOWLEDGEMENT

I am grateful and would like to express my sincere gratitude to my supervisor Dr. Syed Mohd Saufi bin Tuan Chik for his germinal ideas, invaluable guidance, continuous encouragement and constant support in making this research possible. He has always impressed me with his outstanding professional conduct, his strong conviction for science, and his belief that a degree program is only a start of a life-long learning experience. I appreciate his consistent support from the first day I applied to be under him. I am truly grateful for his progressive vision about my training in science, his tolerance of my naive mistakes, and his commitment to my future career. I also sincerely thanks for the time spent proofreading and correcting my many mistakes.

I acknowledge my sincere indebtedness and gratitude to my parents for their love, dream and sacrifice throughout my life. I cannot find the appropriate words that could properly describe my appreciation for their devotion, support and faith in my ability to attain my goals. Special thanks should be given to my classmate friends. I would like to acknowledge their comments and suggestions, which was crucial for the successful completion of this study.

ABSTRACT

Gelatine is mixture of peptides and protein produce by partial hydrolysis of collagen from the animal skin, connective tissue and bones. Gelatine has gelling, foaming and emulsifying properties that contribute to a wide range of applications in the food, pharmaceutical, photographic and cosmetic industries. In the current study, gelatine was extracted from the chicken skin and was characterized in term of yield, molecular weight, melting point and viscosity. Two different pre-treatment methods using acetic acid and nitric acid were used during preparation of gelatine. The yield of gelatine using acetic acid and nitric acid pre-treatment are 11.19 %(w/w) and 9.18%(w/w) respectively based on dry weight basis. Both gelatines showed the same molecular weight pattern range from 53 to 250 kDa. The viscosity of gelatine using acetic acid and nitric acid pre-treatment are 3.3 mPa.s and 2.8 mPa.s respectively.

ABSTRAK

Gelatin adalah campuran peptida dan protein hasil daripada hidrolisis separa kolagen berasal dari kulit haiwan, tisu perantara dan tulang. Gelatin mempunyai sifat elastik, berbuih dan pengemulsi yang menyumbang kepada pelbagai aplikasi dalam makanan, farmaseutikal, industri fotografi dan kosmetik. Di dalam kajian ini, gelatin telah diekstrak daripada kulit ayam dan dicirikan dari segi hasil, berat molekul, takat lebur dan kelikatan. Dua kaedah yang berbeza dengan menggunakan asid asetik dan asid nitrik dikaji semasa penyediaan gelatin. Hasil gelatin menggunakan asid asetik dan asid nitrik sebagai rawatan memberi hasil 11.19% (w / w) dan 9.18% (w / w), masing-masing berasaskan berat kering. Manakala bagi ujian berat molekul gelatin, kedua-dua molekul gelatin menunjukkan corak berat molekul yang sama 53-250 kDa iaitu berada di dalam lingkungan berat molekul gelatin seperti di pasaran. Nilai kelikatan gelatin menggunakan asid asetik dan asid nitrik pra-rawatan adalah 3.3mPa.s dan 2.8 mPa.s.

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LIST OF SYMBOLS

^0C	: Degree celcius
%	: Percentage
BSE	: Bovine Spongiform Encephalopathy
BSA	: Bovine serum albumen
FMD	: Foot-and-Mouth Disease
g	: gram
GME	: gelatine manufactures of Europe
H_2SO_4	: sulphuric acid
hr	: hour
HCl	: hydrochloric acid
kDa	: kilodalton
kN	: Kilonewton
kg	: kilogram
l	: liter
lb	: pound
M	: molarity
mg	: milligram
ml	: milliliter
mm	: millimeter
NaCl	: sodium chloride
NaOH	: sodium hydroxide
Nm	: nanometer
Pa.s	: Pascal second
α	: Alpha
β	: Beta
γ	: Gamma
μ	: Micro
Tm	: melting temperature
UV	: ultraviolet
vs	: Versus
w/v	: Weight / volume
v/v	: Volume / volume

CHAPTER I

INTRODUCTION

1.1 Research background

Gelatine is mixture of peptides and protein produce by partial hydrolysis of collagen from the animal skin, connective tissue and bones. It is a translucent, colourless, brittle when dry, flavourless solid substance. Gelatine has unique properties as a gelling agent because it can form liquid and gel based on the temperature change. Gelatine will softens and form liquid when being heating and turn back into gel during cooling. This property was known as thermo reversible gel. The melting temperature for gelatine is below 35°C which is below human body temperature. This property make it unique in terms of its fit sensory aspects, especially flavour release that are need for some food industry (Baziwane and He, 2003; Boran and Regenstein, 2009; Choi and Regenstein, 2000). Other gelling agents such as starch, alginate, pectin, and agar are carbohydrates and their gels cannot melt below body temperature because have high melting temperatures (Williams, 2007).

Gelatine has been widely applied in food, pharmaceutical, photographic, and cosmetic industries (Karim and Bahat, 2009; Yang et al., 2007; Zhou and Regenstein, 2004). In food industry gelatine is used as ingredients to improve elasticity, consistency and stability of food like deserts, candies, bakery product, jellied meats, ice cream and dairy products. Gelatine also used as stabilizer to modify the taste of the food product. Gelatine is added to yogurt to reduce and increase firmness. Gelatine also recommended enhancing protein level in food stuffs and suitable in body-building foods. Different concentrations of gelatine would give a wide range of textures in food products. Gelatine is compatible with milk proteins and can improve the taste of cakes and marshmallow.

In pharmaceutical industry, it can be used for encapsulation, production of hard and soft capsules, wound dressing and emulsions (Djagny et al., 2001). In photographic application, gelatine is use for lighting equipment which is the colour gel use to change the beam colour. For cosmetic usage, gelatine can make as styling gel usually use by swimmer to hold their hair in place because gelatine not dissolve in cool water or pool. It also can be used in nail polish remover and make up application. Other than that, a lot of beauty product nowadays uses collagen in their product for whitening, repair skin damage and some good for repairing our tissue in body.

Alkali and acid treatment is required before the hydrolysis of collagen into gelatine. The function of alkali treatment is to remove non-collagenous proteins and pigment. Another function is to weakening the collagen structure leading to higher quality of gelatine. In most of the acid extraction process, citric acid is used because it does not change the texture of gelatine in term of colour or odour. Acid treatment will effectively removes odours and colour from the raw material (Boran and Regenstein, 2009; Zhang et al., 2007). There are two types of gelatine which is type-A and type-B gelatine. Type-A gelatine is produced from acid-treated collagen and type-B is produced from alkali-treated. Acidic treatment very suitable for less cross-linked collagens that usually use for pig skins whereas alkali treatment is used for more complex cross-linked sources such as bovine hides. Whether organic acid or inorganic acid can be used for extract collagen directly from animal tissue but the different is amount of collagen that can be extract and the quality of collagen produce. Examples of organic acid are acetic, citric and lactic acid (Sadowska et al., 2003). Hydrochloric is the example of inorganic acid that employed for extraction of collagen. However inorganic acid give worse performance compare to organic acid (Skierka and Sadowska, 2007).

1.2 Problem statement and motivation

Most of the available gelatines have been produced from mammalian resources, either pig skins or cowhides (Simon et al., 2002). Gelatine from mammalian sources such as from bovine and pig skins account for 46% of the world gelatine output, followed by bones and hooves, representing 23% and 29% of the total gelatine

production, respectively and only the remaining percentage, i.e. 1% comes from marine sources (Gómez et al., 2002). Mammalian gelatine has been use because it have high melting, gelling point and it is thermo reversible (Gudmundsson, 2002). The cow bone is most preferred collagen source for producing high-quality gelatine (Rowlands and Burrows, 2000). Gelatine extraction from fish by-products are seldom used because they are mainly used for animal feed supplements due to their small size (Gildberg, 2002).

Traditional gelatine productions are manufactured from mammalian resources such as pork skin, cattle hides and cattle bones (Cho et al., 2005). Based to the report Gelatine Manufacture of Europe, 95% gelatine is made up from hide porcine and bovine and the rest from bones of porcine and bovine. Gelatine produce from pig skin cannot be used for some food due to aesthetic and religion objections (Judaism, Islam and Jews) for example. Muslims are prohibited to consume animals that are not properly slaughtered according to Syariah law. Therefore, gelatines that are produced from bovine source cannot be consumed by Muslims if the animals that are used to make those gelatines are not properly slaughtered according to Syariah law. But if beef gelatines are prepared based on religious requirement it accepted as a food additive (Badii and Howell, 2006). From that, the increases market for halal food has gained attention from both researchers and industry (Karim and Bhat, 2009).

However due to the outbreak disease BSE (bovine spongiform encephalopathy) known as mad cow disease and foot-and-mouth disease (FMD) commonly derived from mammalian part, the search from other sources of gelatine has been continuously investigate. Researchers are not only continually searching for an alternative to gelatine, and also to find new sources of gelatine. Within the past few years, there has been increased interest in the market in gelatine derived from fish and poultry. Poultry skin and bones are expected to yield gelatine in the near future, but commercial production is currently limited by low yields (Schrieber and Gareis, 2007).

Nowadays a lot of research has been conducted to make a gelatine from fish, however it has been limited application due to the gel formed is less stable and had a weak rheological properties compare to gelatine extracted from land mammals (Shahiri

et al., 2010). However, there is too little research concerning production of gelatine from chicken skin. At present, the fish gelatine production is very low, yielding about 1% of the annual world gelatine production of 270,000 metric tonnes (Jamilah and Harvinder, 2002). Nowadays, there is great request for Halal products so chicken is the best selected sources for halal product. Furthermore, the production of gelatine from chicken skin can be beneficial to the food industries since most of the chicken in Malaysia is Halal certified. Therefore, the study of gelatine from chicken, such as skin is interest as sources of collagen to extract gelatine. Instead of being waste that can cause pollution to environment, chicken skin can be used for production of gelatine. The waste not only causes pollution but also it emit defensive odour (Takeshi and Nobutaka, 2000). Those gelatines can be used to replace gelatines that are produced from bovine and porcine sources.

1.3 Objective of the research

The main objective of this research is to extract and characterize the gelatine produced from chicken skin

1.4 Scopes of the research

In order to fulfil the research objective, the following scopes has been outlined.

i. To produce gelatine from chicken skin.
ii. To study the effect of, two different solutions which are acetic acid and nitric acid during the preparation step on the gelatine properties.
iii. To characterize the chicken derived gelatine in terms of molecular weight, melting point, yield and viscosity.

CHAPTER II

LITERATURE REVIEW

2.1 Collagen

Collagen is one of the most abundance proteins present in the bodies of mammals. Collagen is major dominant structure in the living body. It is tasteless and colourless solid substance derived from the fibrous protein collagen. About one half of total body made up of collagen. Collagen is mostly found in fibrous tissues such as tendons, ligaments and skin (collagen), and is also abundant in corneas, cartilages, bones, blood vessels, the gut, and intervertebral discs (Brinckmann et al., 2005). Collagen is one of the key structural proteins found in the extracellular matrices of many connective tissues in mammals; the whole-body protein making up about 25% to 35% of content (Muyonga et al., 2004).

Collagen played an important role to support the body structure of animal. It connects and supports other body tissues such as skin, bone, tendons, muscles, connective tissue and cartilage. It also supports the internal organs and is even present in teeth. Collagen works strongly elastin in supporting the body's tissues (Madison, 2011). Even the blood vessels depend on both collagen and elastin. It works hand-in-hand with elastin in supporting the body's tissues. This combination of collagen and elastin is very important in many parts of the body including lungs, bones, and tendons. It also supports the internal organs and is even present in teeth. Basically, it gives body tissues form and provides firmness and strength.

Collagen molecules are arranged with a 35-nm gap between molecules head-to-tail and are found in larger structures having staggered bundles, that is, adjacent collagen molecules are not aligned with each other (Gutsmann et al., 2003). Charged and uncharged residues are found to be periodically clustered along the sequence of collagen at about every 230 residues, which is around 67 nm, although this distance

may vary somewhat among different tissue sources of collagen (Holmes et al., 2001). The ending fibril can be from 20 to 400 nm in diameter and is stabilized by four covalent cross-links per collagen molecule, two at either end of the molecule. This suggests that the collagen molecules are aligned such that the maximum electrostatic and hydrophobic interactions occur between different molecules as shown in Figure 2.1.

Collagen is generally considered as incomplete protein since the concentration of some essential amino acids is low in collagen and consequently, in gelatine (Belitz et al., 2004; Nelson and Cox, 2005). Therefore, gelatine is mixture of fractions composed entirely of amino acids joined by peptide linkages to form polymers that have molecular mass from 15,000 to 400,000 and not a single chemical entity.

Collagen can be extracted from pig, bovine, fish and chicken. Different sources of collagen will result in different physical properties. However, in the industry, the main sources of the collagen are become limited to those that obtained from pigs and bovine skin and bones (Takeshi et al., 2002). Collagen from mammals for example bovine and pig is different than collagen extracted from fish. The properties of collagen markedly vary with the habitat, species, and part of fish being isolated (Falguni et al., 2010).

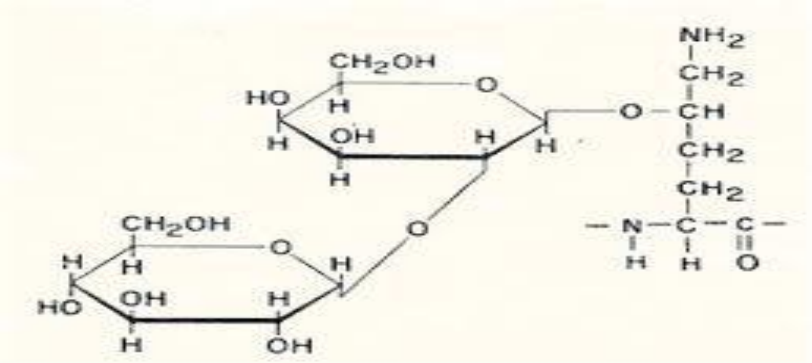


Figure 2-1: Structure of collagen

Treatment process such as alkali and acid treatment will be subjected for the collagen from by-product of land animal. After all the process, the structure of collagen will break down and the product produce is known as gelatine. The processes involve in the production of gelatine for commercial manufacturing of gelatine are extraction, filtration and clarification, evaporation, sterilization, drying, grinding and sifting, and storage (Gomez-Gullien et al., 2009).

There exist at least fourteen general types of collagen. The most familiar type I, the predominant genetic type that consist of three polypeptides chain. Two chains identical are call α1: the third beings call α2. Type I collagen is the type occurs widely, primarily in connective tissue such as skin, bone, and tendons. Usually collagen type I is widely used in food industries, cosmetic, pharmaceutical, biomedical, and tissue engineering due to its excellent biocompatibility and biodegradability (Liu et al., 2010). Whereas collagen types I, II, III, and V are called fibril- forming collagens and have large sections of homologous sequences independent of species, among which first three types are known to be chemotactic (Chevallay and Herbage, 2000). Type II is the type of collagen occurs practically exclusively in cartilage tissue. Then type III are strongly dependent on age: very young skin can contain up to 50%, but in the course of time this is reduced to 5–10% (Gelse et al., 2003). For type IV collagen, being present in basement membrane, the regions with the triple-helical conformation are interrupted with large non-helical domains, as well as with the short non-helical peptide disturbance. Other types of collagen are present in very low amounts only and mostly organ-specific (Schrieber and Gareis, 2007).

Collagens molecules from which gelatine are derived are composed of three α-chains intertwined in the so-called collagen triple helix. This particular structure is due to the almost continuous repeat of the (Gly-X-Y) sequence and each chain is generally more than 1000 residue long. Glycine is the most abundant acid in gelatine which is X and Y mostly proline and hydroxyproline. Usually every molecules contain two or even three different α chains, described as α 1, α 2 and α 3, with the difference lying in the amino acids present in X and Y positions of the triplets. About 25% of dry gelatine contains proline and hydoxyproline that stabilize its structure (Russell et al., 2007). This triplet of amino acids allows collagen chains to twist into a helical structure. Each

collagen molecule contains 3 chains twisted around each other to form a triple helix as shown in Figure 2.2.

Collagen comprises a triple helix structure issue which forms fibres, arranged in bundle, which make up of connective matrix. The triple helix structure is stabilized by intra-chain hydrogen bonds and all the main chain N-H and C = O groups are involved in these types of interactions. The triple helix gives collagen a rigid structure. It maintains the mechanical integrity of tissues. Less amino acid content should result in a less statically hindered helix and may affect the dynamic properties of gelatine.

The size of triple-helix is about 300 nm in length, and the chain has a molecular weight of approximately 105 kDa (Papon et al., 2007). When process of acid or alkaline hydrolysis, a mild derivative process occurs and the fibrous structure of collagen is broken down irreversibly due to the rupture of covalent bonds. Denaturation of soluble collagen due to the breakdown of hydrogen and probably electrostatic bonds in hot water (40 °C) takes place by destroying the triple helical structure of collagen to produce one, two or three random chain gelatine molecules that give a solution in water of high viscosity. It will destabilize the triple helix by means of a helix to coil transition and leading to conversion into soluble gelatine (Gomez-Guillen et al., 2005).

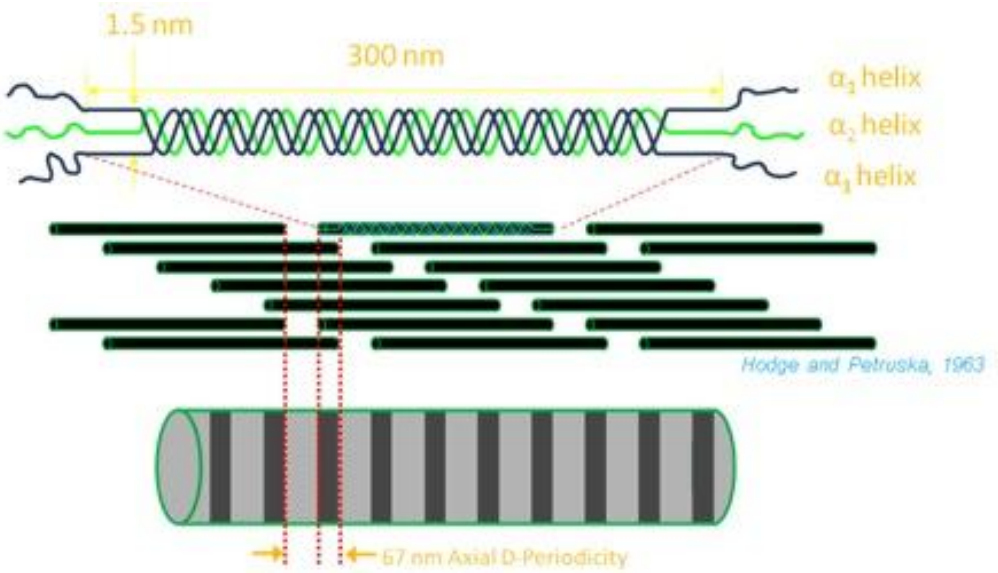


Figure 2-2: Schematic diagram for collagen molecule

2.2 Gelatine

Gelatine is the product of thermal denaturation of insoluble collagen by partial hydrolysis process with various molecular weights (MWs) and isoionic points (IEPs) (Gomez-Gullien et al., 2009). Collagen denaturation causes separation of rods and total or partial separation of the chain (Papon et al. 2007). This is because of destruction of hydrogen bonds, causing loss of the triple helix conformation, and following denaturation, the polymers exist in a coiled form. During the process of gelatine, raw animal material is treated with dilute acid or alkali, resulting in partial cleavage of the crosslinks: the structure is broken down to such an extent that ‘‘warm-water-soluble collagen’’, then gelatine is formed (Schrieber and Gareis, 2007).

The degree of crosslinking in gelatine is highly variable. It depends on collagen type, tissue, animal species and also age. The properties and gelling abilities of gelatine, involving a partial denaturation of denatured collagen molecules depend on all these parameters since gelatine is derived from denatured collagen (Gomez-Gullien et al., 2009). The properties of the resulting gelatines are greatly influenced by the two main factors that are the initial collagen characteristics and the precise treatment process. The properties of the resulting gelatine are influenced by the source and type of collagen (Binsi et al., 2009).

In many aspects the chemical composition of gelatine are similar, to its parent molecule. However gelatine is not composed of one size of collagen fraction or peptide chain but is a combination of many fractions varying in size, including the whole α -chain of the tropocollagen molecule (a trimmer of around 330 kDa that aggregates to form the larger collagen structures) and hydrolytic fragments of parts of the α -chains of different lengths. Gelatine is a mixture of different polypeptide chains including α-chains, β (dimers of α-chain) and γ (trimmers of α-chain) components with a molar mass of around 90, 180 and 300 $\times 10^3$ g/mol, in aqueous solutions (Rbii et al., 2011). Higher gel strength is showed by gelatine which contains more α-chains. Therefore, all the processing steps of this gelatine should avoid extensive degradation of peptide structure in order to obtain high gelling strength (Liu et al., 2008). The properties of the resulting gelatine are depending on the sources and type of collagen (Binsi et al., 2009).

2.3 Application of gelatine

Gelatine, one of the most popular biopolymers, is widely used in food, pharmaceutical, cosmetic, and photographic applications because of its unique functional and technological properties. The most common application of gelatine is used as a jellying agent. In sugar jellies industries, gelatine will give the gel and delay crystallisation of the sugar in the jellies. Recent years, the gelatine is added with Arabic gum in the production of tougher jellies. (Boran et al., 2010; Gómez-Guillén et al., 2011; Kittiphattanabawon et al., 2010). Another function of gelatine is to reduce the moisture content in sugar jellies.

Gelatine is a water-loving material which act as hydrophilic properties and can absorb up to ten times its weight in water (GMAP, 2011). Thermally reversible gels with water are formed from an aqueous solution of a few per cent gelatines and the gel-melting temperature (<35°C) which is below body temperature, which gives gelatine products unique organoleptic properties and flavour release. Due to the thermo reversibility properties, this process gives the gelatine gel its unique “melt-in-mouth” quality (Boran et al., 2010). Gelling agents other than gelatine sources such as starch, alginate, pectin, agar and carrageenan are all polysaccharides from plant sources, but their gels are lack of the melt-in-mouth and elastic properties of gelatine gels.

Gelatine is one of component the most accepted biopolymers and is extensively utilized in food because of its unique functional and technologies properties (Karim and Bhat (2009). Gelatine has been used as a beverage clarifier a fining agent for white wine, as a beer clarifier and to clarify fruit and vegetable juice especially to clarify apple juice and pear juice. Moreover, gelatine also utilized in confections mainly for providing chewiness, texture, and foam stabilization. It is low-fat spreads to provide creaminess, fat reduction, and mouth feel. Gelatine is a dairy product to provide stabilization. In ice cream, stabilizer is used to prevent the formation of coarse ice crystals and gelatine was the easiest stabilizer used. It also decreases the rate of melting, give body and a firm smooth texture and baked goods to provide emulsification, gelling, and stabilization.

Gelatine, being low in calories, is normally recommended for use in foodstuffs to enhance protein levels, and is especially useful in body-building foods. In addition, gelatine is also used to reduce carbohydrate levels in foods formulated for diabetic patients (Gilsenan and Ross-Murphy, 2000). Nutritionally, both collagen and gelatine are low quality of protein that can improve quality of skin and finger nails (Meler, 2006). Skin is made up of collagen and as our age increase, production of collagen drops off and skin sags because it get thinner, weaker and less resilient. This is automatic related to amino acid content. There are specific amino acids content in skin's structure such as glycine, proline, hydroproline and alanine decrease with age and bad diet (King 2011).

In the pharmaceutical industry, gelatine is widely used for the manufacture of hard and soft capsules, plasma expanders, and in wound care. Karim and Bhat (2009) also suggested that gelatine with low melting point could be used in dry products for microencapsulation. Gelatine also been used as a matrix for implants, in inject table drug delivery microspheres, and in intravenous infusions. In fact collagen has already found significant usage in clinical medicine over the past few years, such as injectable collagen for repair tissue defects, haemostasis, burn and wound dressings, hernia repair, bioprostetic heart valves, vascular grafts, a drug –delivery system, ocular surfaces, and nerve regeneration (Lee et al., 2001). There are also reports in which live attenuated viral vaccines used for immunization against measles, mumps, rubella, Japanese encephalitis; rabies, diphtheria, and tetanus toxin contain gelatine as a stabilizer (Gimenez et al., 2005). Gelatine can form fibres with extra strength and stability by self-aggregation and cross-linking, which makes it useful in drug delivery systems (Lee et al., 2001).

In cosmetic and health care products, gelatine is used as a gelling ingredient in face creams, body lotions, shampoos, hair sprays, sun screens and bath salts and bubbles. The types of fish are influence the pharmaceutical application for example Codfish gelatine are used for evaluation of allergen city of commercial and food-grade fish gelatine (Hansen et al., 2004). For Pacific codfish skins the application in pharmaceutical is to investigation of changes of antioxidant activity in skin tissue and

the arrangement of collagen fibres using ultraviolet radiation induced skin photo aging (Hou et al., 2009).

Collagen and health benefits related with it have led to establishment of collagen-supplement industry. Nowadays collagen supplement are meant to mainly improve skin appearance and being image-obsessed society and got high demand (Jamie 2009). Field of sport nutrition is another area that increasing worldwide demand for hydrolysed collagen. Collagen can automatically boost lean muscle gain, decrease recovery time, rebuild damage joint structure without surgery and improve cardiovascular performance on athletes. Therefore, a lot of athletes and body builders use hydrolysed collagen as clean sources for muscle gain, tendon and ligament repair, fast recovery time and maximum performance (King 2011).

In photographic industry, gelatines are needs for film coating, colour paper, graphical and X films, and printer ink. The unique chemical and physical properties of gelatine make it an important component in the photographic industry. Gelatine serves many useful purposes in the preparation of silver halide emulsions in the production of photographic film. Such gelatines have been reported to have a good film formation and emulsifying properties (Schrieber and Gareis, 2007).

2.4 Properties of gelatine

There are a lot of properties effects the quality of gelatine for example physical attributes and chemical characteristic. Physical attributes include gel strength, viscosity, melting and gelling temperature. The quality of gelatine is measured by the gel strength or Bloom value, including low (<150), medium (150–220) to high Bloom (220–300); commercially, high viscosity gelatine is preferred and fetches a higher price. The chemical properties of gelatine are affected by amino acid composition, molecular weight distribution and triple helix formation (Gomez-Guillen et al., 2002). Amino acid composition is similar to that of the parent collagen, thus influence by animal's species, breed, age, manner of feeding the animal, storage conditions of raw materials and type of tissues. The differences in molecular weight distribution were also affected its

chemical properties which result from the variation in the nature or extraction conditions (Zhou and Regenstein, 2006).

The source and type of collagen also influence the properties of the gelatine (Binsi et al., 2009). The principal raw materials used in gelatine production are cattle bones, cattle hides, and pork skins but mostly from pig. Other than this source, there are alternative raw material that can be used in gelatine production, including by-product from chicken and fish processing industries. For production of large amount high-quality of gelatine, fish skins have received lot of attention from researcher as alternative raw material. Therefore, studies on various species of fish skin gelatine have been a famous research for the production of high quality gelatine.

One of the ways to improve gelatine is by manipulating the characteristics of gelatine by addition of salts. Fish gelatine properties can be modified through addition of enhancer like salts, glycerol, variation of pH and in combination of other ingredient such as sucrose (Koli et al., 2011; Sarebia et al., 2002). Saline ion will cause the collagen to interact with water molecules and folding indirectly. In addition, when the fish skins have been washing using NaCl and KCI at 0.8 M, it will result in a higher gelling ability and stability on fish gelatine (Gimenez et al., 2005). Choi and Regenstein (2000) also stated that melting point of gelatine decreased directly as the concentration of NaCl went up to 14%. NaCl is very sensitive to fish gelatine because the concentration NaCl is able to break both of hydrophobic and hydrogen bonds. Thus preventing the stabilization of the gel junction sites, either by prevent hydrogen bond formation or by modify the structure of liquid water.

Other than amino acids, properties of gelatine also contain moisture, ash, calcium, copper and iron. The moisture content of gelatine is different at different pH. The moisture content is increasing if the pH is increasing (Chen et al., 2007; Fishman et al., 2000). However the composition such as ash, calcium, copper, and iron must be in lower amount in gelatine. This is because the composition will give low quality of gelatine. For example, if more than 2 ppm of iron content in gelatine, it will show grey strain on food product. In addition, colour of gelatine also depends on the raw material

extracted (Pan et al., 2003). The official standard of good quality of gelatine is to be free of objectionable taste or offensive odour and colour.

Except from amino acid composition, other factors such as functional properties of are gelatine also influenced by the distribution of the molecular weights and compositions of its subunits. An important factor affecting the quality of fish gelatine is the environmental condition of the fish species. Generally, collagen and gelatine, prepared from low temperature fish species contain lower amounts of proline and hydroxyproline, lower number of hydrogen bonds and have a lower melting point than species from a higher temperature environment. During gelatine process, the conversion of collagen to gelatine yields molecules of varying mass, due to the cleavage of inter-chain covalent crosslinks and the unfavourable breakage of some intra-chain peptide linkages (Zhou et al., 2006).

The properties of gelatine are strongly depend strongly to pH in the reaction mixture and on the charge balance (determined by the gelatine pectin ratio), which will influence the degree of electrostatic associations and ionic interactions in the gelling system (Farris et al., 2009). Not only species or tissue from which it extracted are influenced the physicochemical properties, but also by the severity of the manufacturing method (Gilsenan and Ross-Murphy, 2000).

An optimization of the tissue extraction procedures and a better knowledge of the properties of fish-skin gelatine could be helpful in extraction of gelatine from fish (Gomez-Guillen et al., 2002). Based on research, fish gelatine has known limited application because the gels formed tend to be less stable and to have worse rheological properties compare to gelatines from land mammals (Shahiri et al., 2010). These limitations because of gelatine in cold water fish contain less proline than in warm blooded animals.

2.5 Amino acid

Gelatine usually contains 90% protein, 18 types of amino acids and 7 essential for people to consume (Ali, 2010). The high quality of gelatine are contains high protein, low ash and heavy metal, small molecular weight, easy absorption and utilization, high biological value, promoting absorption of vitamin and mineral. Table 2-1 show the comparisons of amino acid content in gelatine derive from several type's fish such as Cod skin, Alaska Pollock skin, Megrim and Tilapia skin compare to pork skin.

Table 2-1: Composition of amino acid in fish skin

Amino acid	**Cod skin[a]**	**Pollock skin[b]**	**Megrim[a]**	**Tilapia skin[c]**	**Pork skin**
Alanine	96	108	123	123	112
Arginine	56	51	54	47	49
Aspartic acid	52	51	48	48	46
Cysteine	0	0	-	0	0
Glutamic acid	78	74	72	69	72
Glycine	344	358	350	347	330
Histidine	8	8	8	6	4
Hydroxylysine	6	6	5	8	6
Hydroxiproline	50	55	60	79	91
Isoleucine	11	11	8	8	10
Leusicine	22	20	21	23	24
Lysine	29	26	27	25	27
Methionine	17	16	13	9	4
Phenylalanine	16	12	14	13	14
Proline	106	95	115	119	132
Serine	64	63	41	35	35
Theorinine	25	25	20	24	18
Tryptopthan	0	0	-	0	0
Tryrosine	3	3	3	2	3
Valine	18	18	18	15	26

Reference: [a] Gomez et al., (2000), [b] Zhou et al., (2006),[c] Sarabia et al,.(2000)

Amino acid composition will affect chemical properties of gelatine which is similar to that of the parent collagen, thus influence by animal's species and type of tissues (Zhou and Regenstein, 2006). In gelatine, all the amino acids are present except tryptophan and have low in methionine, cystine and tyrosine due to the degradation during hydrolysis (Jamilah and Harvinder, 2002). Although some differences in amino acid composition are apparent across collagens derived from different sources, there are certain features that are common to and uniquely characteristic of all collagens.

There are only mammalian gelatine contain large amounts of hydroxyproline and hydroxyl sine, and the total amino acid (proline and hydroxyproline) content is high (Gilsenangher and Ross-Murphy, 2000). The high amino acid content in gelatines from mammalian and warm water fish is considered to be related to a lower critical concentration and higher melting point. Secondly a higher molecular weight MW (300 kDa) gelatine is known to have a higher Bloom value than low MW gelatine.

Thermal stability of amino acid content was reported to have a major influence in the collagen (Prabjeet et al., 2011 and Falgani et al., 2010). There is a well-known almost linear relationship between the hydroxyproline content and the denaturation temperature of the collagen. When hydroxyproline content is lower the denaturation temperature also lowers (Hickman et al., 2000). As the amount of hydroxyproline contain increases, rheological properties and gel strength of gelatine also increase (Prabjeet et al., 2011).

The composition of amino acids is of particular importance regarding both gelatine gel strength and melting point (Badii and Howell, 2005). Proline and hydoxyproline will be influenced by the raw material of gelatine used. Gelatine from warm-blooded and from warm water fish give have higher collagen compare to cold-water fish because contains of higher amino acid and increase proline and hydroxyproline. Although less imino acid contain in cold-water fish compare to warm water fish and mammalians, the contents of amino acid, molecular weight and gelatine viscosity maybe will be higher contain for cold-water fish (Gómez-Guillén et al., 2002; Gudmundsson, 2002). High content of hydrophobic amino acid have similar effect to gelatine even though it is less prominent (Badii and Howell, 2005).

In general, imino acid content in fish collagens is lower compare than mammalian collagens, and this may be the reason for the denaturation at low. Overall, fish gelatines have lower concentrations of amino acids (proline and hydroxyproline) compared to mammalian gelatines, and warm-water fish gelatines (such as bigeye-tuna and tilapia) have a higher amino acid content than cold-water fish (such as cod, whiting and halibut) gelatines (Choi Regenstein, 2000; Gómez-Guillén and Montero, 2001; Jamilah and Harvinder, 2002; Muyonga et al., 2004). The proline and hydroxyproline contents are around 30% for mammalian gelatines, 22–25% for warm-water fish gelatines (tilapia and Nile perch), and 17% for cold-water fish gelatine (cod) (Muyonga et al., 2004).

2.6 Gel strength

Another important property of gelatine is gel strength. Mammalian gelatine has higher gel strength compare to fish gelatine (Choi and Regenstein, 2000). Meanwhile, cold-water fish have the lowest physical properties compare to mammalians and hot water fish. According to Normand et al. (2000), the gel strength will decrease with increasing extraction temperature. Higher temperature will caused protein degradation and lowering the gelatine production.

Some species from warm-water fish show higher gel strength such as grass carp and tilapia. Gelatine from salmon showed the gel strength about 108 g (Arnesen and Gildberg 2007) compared to gelatine from cod (70–90 g) (Gómez-Guillén et al., 2002) and Alaskan pollock (98 g) (Zhou, Mulvaney, Regenstein, 2006). Based on bloom strength, yellow fin tuna show slightly same content of gelatine with mammalian gelatine. Yellow fin tuna have higher melting and boiling point compare to others fish gelatine. Due to the high gelatine content and gel strength, yellow fin tuna could be a good source of fish gelatine (Gilsenan and Ross-Murphy, 2000, Choi and Regenstein, 2000 and Gudmundsson, 2002). Lower content of imino acid, proline and hydroxyproline will influence the gel strength.

2.7 Viscosity

Compared to gel strength, viscosity is not as well correlated with textural properties. The viscosity is usually affected by molecular weight distribution. Gelatine that contains high molecular weight fractions give high viscosity but that does not exactly mean that their gel strengths also be high. For example gelatine samples from fish skin, show high viscosity but giving low gel strength compared to that of pork skin gelatine due to the carefully controlled extraction conditions and consequently, the presence of higher molecular weight protein fractions (Boran and Regenstein, 2009). Atlantic salmon and Atlantic cod skin gelatine had higher viscosities than pork skin gelatine while giving lower gel strengths than pork skin gelatine (Arnesen and Gildberg, 2007). Normally, fish skin gelatines are expected to have a lower viscosity compared to that of gelatines obtained from porcine and bovine sources with similar molecular weight distributions.

2.8 Preparation of gelatine

The world usage of gelatine are estimated about 200,000,000 kg per year and it is mainly obtained from pork skin accounting for the highest (46%) of output and followed by bovine hides (29.4%), bones (23.1%) and other sources (1.5%) (Badii and Howell, 2006; Choi and Regenstein, 2000; Larraza´bal and Camacho, 2008). Gelatine extraction from fish by-products from freshwater are seldom used as a source because they are mainly used for animal feed supplements due to their small size (Gildberg, 2002). At present, there are two processes to extract gelatine from animal raw material (e.g. skins and bones) an alkaline process, giving high quality products for photographic applications, and an acid process, which is faster but leads to a lower quality product for food use. This lower quality is related to a lower mean molecular weight, caused by chain degradation reactions interfering with the gelatine extraction. The collagen from by-product of land animal will be subjected to treatment process such as alkali and acid treatment.

All processes of manufacturing gelatine consist of three main stages: pre-treatment of the raw material, extraction of the gelatine, and purification and drying.

Furthermore, manufactured gelatine is often blended to produce trade-quality gelatine, with specific properties for specific applications (de Wolf, 2003). Extraction of gelatine from raw material such as from bovine and porcine can be done through acid, basic or enzymatic treatments, all of which are assisted by heat and water. For fish and pig skin, the treatment with acid is preferentially used. Type of acid that usually used are hydrochloric acid, sulphuric acid, phosphoric acid and acetic acid. This is because fish and pig skin have less fully cross-linked collagen. Whereas, in bovine hides alkaline treatment is suitable for extraction process because of more complex collagens found (Gómez-Guillén et al., 2002). The use of high pressure is needed for example pressure above 200 MPa can facilitate swelling by destabilizing acid-soluble cross links and also accelerating hydrolysis of collagen (Gómez-Guillén et al., 2005).

The commonly Type A gelatine is generally adjusted to an acidic pH which is below the isoelectric point at pH 6 – 7 is produced from acid treatment, while a Type B gelatine is also adjusted to a pH that is acidic, but in this case the pH is above the isoelectric point pH 5 is produced from alkali treatment (Karim and Bhat, 2009). Usually type B gelatines produce from bovine skin by alkaline treatment, while type A gelatine produced from porcine skin by acidic treatment. Conversely, the treatment with alkaline yield type B gelatine which is harder and more viscous than type A gelatin and has a pH between 5 and 7; its main application is in stabilizing other food hydrocolloids (Wasswa et al., 2007). During the preparation process, collagen is thermo sensitive and is often extracted under room or lower temperature.

It is necessary to understand the effects of extraction conditions for obtaining higher recovery of collagen. After all the process, the structure of collagen will break down and the product produce is known as gelatine. However, the gelatine produce has different behaviour due to acid and alkali process. It showed that the process or treatment is playing an important role in choice of gelatine. In addition, by manipulating pre-treatment and process condition, the quality of gelatine can be controlled to the desired standard (Gudmunsson, 2002). Table 2.2 shows some extraction procedure to produce gelatine from various sources.

Table 2-2: Extraction procedure to produce gelatine from various sources.

Sources	**Pre-treatments**	**Extraction procedure**	**Reference**
Channel catfish (Ictalurus punctatus)	Alkaline solution NaOH followed by an acid solution acetic acid	Cleaned skins was treated with NaOH (1:6 w/v) for variable time. Then the, samples were drained using cheesecloth and rinsed with tap water (related two times). Afterwards the samples were treated with acetic acid (1:6 w/v) for variable times, followed by draining using cheesecloth and rinsed with tap water (1:6 w/v) (three times) (samples maintained at 4 °C). After the above pre-treatments, ion-free water was added to the flasks and samples were extracted in a water bath for variable times.	Yang et al. (2007)
Horse mackerel	Frozen fish skins were thawed and washed with warm water and dried between two layers of filter paper at hen cut into 2–3 cm pieces.	Fish skin pieces (250 g) were extract with (0.125w/v) sodium hydrogen carbonate ($NaHCO_3$) and 200 g ice to remove fat. Then the sample were extract with (0.02w/v) NaOH. The resultant wash skin were extract with (0.02w/v) sulphuric acid. Then the skin pieces were mix with (0.07 w/v) citric acid. Finally gelatine were extracted from the fish skin pieces with distilled water in a 45°C water bath.	Choi and Regenstein (2000).
Cleaned dark megrim (Lepidorhombus boscii) skins	The skin is store at temperature -20°C	Skins was stir with 0.2 M sodium hydroxide (1: 6 w/v) and (repeated three times). The samples were rinsed with tap water after each step. Then the skin are swollen with 0.05 M acetic acid (1:10 w/v) and extracted with distilled water overnight at 45C.	Sarabia et al., (2000)
marine snail (Hexaplex trunculus)	meat of snails is blend and store at −20 ◦C.	Snail meat were soak in 0.02 M NaOH ratio of 1:6 (w/v). The solution was change every 30 min. The gelatine was extract from the washed pelleted tissue with acetic acid at pH 4.0 with a solution ratio of 1:6 (w/v) 9 hour at 60°C.	Zied Zarai et al,. (2012)

Table 2.2: Continued

Sources	Pre-treatments	Extraction procedure	Reference
Animal bones	Hard bones were chosen because the particles obtained after crushing and sieving	600 g of bone powder were mixed with hydrochloric acid. The ossein is wash three times with tap water. Wet ossein was mixed with demineralized water for 1 h at controlled temperature (75 8C) and pH (2.25).The pH was controlled by addition of phosphoric acid. The samples (5 ml) were taken during extraction and centrifuged: the solid fraction was injected into the reactor and analysed by refractometry to determine the gelatine content.	Nicolas-Simonnot et al., (1997)
Unicorn leatherjacket (Aluterus monoceros)	skin is cut into small pieces then placed in polyethylene bags and stored at 20 °C until use.	Skin was soaked in 0.1 M NaOH with a skin/solution ratio of 1:10 (w/v). Then the skin was soaked in 0.2 M acetic acid or 0.2 M phosphoric acid with a skin/solution ratio of 1:10 (w/v). The swollen skin was soaked in distilled water (45 C) with a skin/water ratio of 1:10 (w/v) in a temperature-controlled water bath.	Mehraj Ahmad and Soottawat Benjakul (2011)
Nile tilapia (Oreochromis niloticus)		Skin was soak in 0.4 (w/v) NaOH at the skin/solution ratio of 1:7 (w/v). Later the skin was soaking in 0.4 (v/v) HCl aqueous solutions. Finally, the skin was extrac with distilled water for 1.5 h at 70°C at the Skin /water ratio of 1:2 (w/v).	Songchotikunpan et al (2008)
Brownbanded bamboo shark (Chiloscyllium punctatum) and blacktip shark (Carcharhinus limbatus)	The clean sharkskin is cut into small pieces (1.0 x1.0 cm2) for gelatin extraction and kept at °20 C until use.	Skin was mix with 0.1 M NaOH at a solid/alkali solution ratio of 1:10 (w/v). Then the sample was mix with 1 M HCl with a solid/solution ratio of 1:10 (w/v). The demineralized skin was swollen by mixing the skins with 0.2 M acetic acid at a ratio of 1:10 (w/v). The swollen skin was mix with distilled water at different temperatures (45, 60 and 75 °C) for various times (6 and 12 h) with sample to water ratio of 1:2 (w/v). Then, the sample was freeze-dried using a freeze-dryer.	Kittiphattanabawon (2004)

Table 2.2: Continued

Sources	Pre-treatments	Extraction procedure	Reference
Yellowfin tuna (Thunnus albacares)	Skin is store at 20 °C until used for the experiments.	Skin was wash with running tap water and dip in 0.5 M sodium chloride. Then skin was wash with tap water three times before mix with sodium hydroxide. The sample was mix in 0.1 N acetic acid solution. The solution was dry in an oven initially at 80 °C and then 100°C until reached to a solid state.	Gomez-Guillen and Montero (2001).
Grass carp fish scales	The thawed fish scales is wash with NaCl solutions. Demineralization is achieved with 0.4mol/l HCl solution. After that, the fish scales were dried in a vacuum desiccator.	The fish scales were mix with distilled water, in a ratio of dried material to water of 1:10 (w/v). The pH value of the solution was adjust about 7.0 before putting in enzyme protease. When hydrolysis finish, the fish scales are wash by distilled water for four times. Gelatine was subsequently extract from fish scales in distilled water at temperature 60◦C.	Fengxiang Zhang et al., (2011)
Chicken feet	Chicken feet (broiler) are store in -20°C	Chicken feet were thawed and store at 4°C for 24h, and ground utilizing a 10mm and 0.4mm plate in sequence. The ground chicken feet were mix with 5% different acid solution and with different times. Suspended solution are neutralized to pH 7 using NaoH. The supernatant was discarded and the precipitate is freeze dry.	Lui et al., (2001)
Chicken skin	Frozen chicken are cold in(4-5°C) overnight. After rinsing in excessive water the skins are cut into 2-3 cm pieces and freeze-dried.	14 g defatted dried chicken skin was mix with sodium hydroxide (0.15% w/v). This step was repeat three times. Then the pellets were rinsed with distilled water and mix with ml 0.15% (v/v) sulphuric acid. Again, the resulting pellets were mixed with 0.7% (w/v) citric acid solution. Each treatment was repeated three times. The final extraction was carried out in distilled water at a controlled temperature (45°C).	Badii and Howell (2006)

Table 2.2: Continued

Sources	**Pre-treatments**	**Extraction procedure**	**Reference**
Hoki (Macruronus novaezelandiae) skins	The frozen skins are thawed at 4 °C for 24 h before removing any remaining flesh and scales. Then Hoki skins are then cut into 4-4 cm pieces and then washed with tap water. The skins stored at -20 °C.	Skins was pre-treated in NaCl solutions. These steps were repeat twice. The minced skins were gently stirred with Milli-Q water in a ratio of 1:6 w/v for 10–60 min at controlled temperature of 30, 45 and 60°C in a shaking water bath.	Kołodziejska et al. (2008)

CHAPTER III

METHODOLOGY

3.1 Materials and chemical

Fresh chicken skins were bought from a local market. The chemicals involve during pre-treatment are sodium hydroxide, sulphuric acid citric acid and acetic acid which were purchased from Sigma-Aldrich, Malaysia.

3.2 Chicken skin preparation

Figure 3-1 show the steps involve in preparing gelatine from chicken skin based on the method developed by Badii and Howel (2006) with some modification. The fat in chicken skin was removed using butanol and then thoroughly washed with water to remove any impurities. Cleaned skin was cut into 2-3 cm rectangular and dried using freeze-dryer for about 4-5 days. Dried skin was grinded in blender and sieved to collect a ground particle less than 3um.

3.2.3 Gelatine extraction

The amount of ground skin, approximately 5 g was mixed with 50 ml sodium hydroxide (0.15% w/v) for 40 min at room temperature. After mixing, the solution was centrifuged at 3500 g for 10 minutes and the supernatant was discarded. This alkali pre-treatment step was repeated for three times with freshly prepared NaOH solution. Alkaline treated pellets were rinse a series of solutions of distilled water, 50 ml 0.15% (v/v) sulphuric acid and 0.7% (w/v) nitric acid or acetic acid solution. In each step, the mixing was done for 40 minutes and the previous solution was removed using centrifugation for 10 minutes before adding a new solution. The nitric or acetic acid treatment was repeated three times with freshly prepared acid solution. The extraction was carried out in distilled water at a control

temperature of 45°C overnight without stirring. The resultant mixture was filtered with Whatman filter paper No. 4. The solution ionic strength was checked with a conductivity meter to obtain a value of 50 mS/cm. The pH was adjusted to pH 6.0 with 0.1 M sulphuric acid. The volume was reduced to 1/10 using rotary evaporator at 45°C and then kept in the freezer overnight before being freeze-drying to obtain gelatine powder. Figure 3.2 represent the graphical step involve in preparing the gelatine from chicken skin.

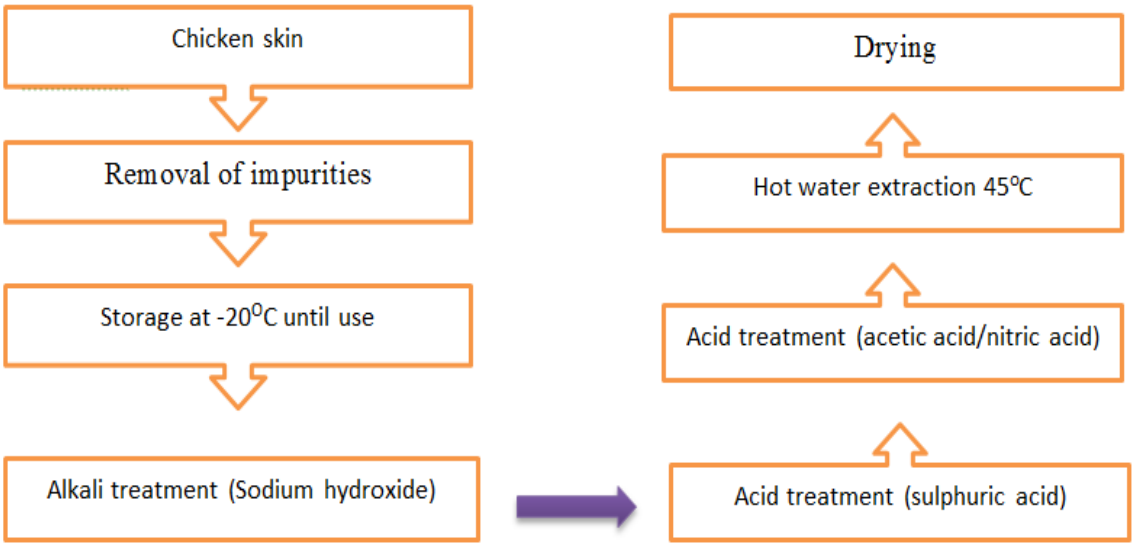
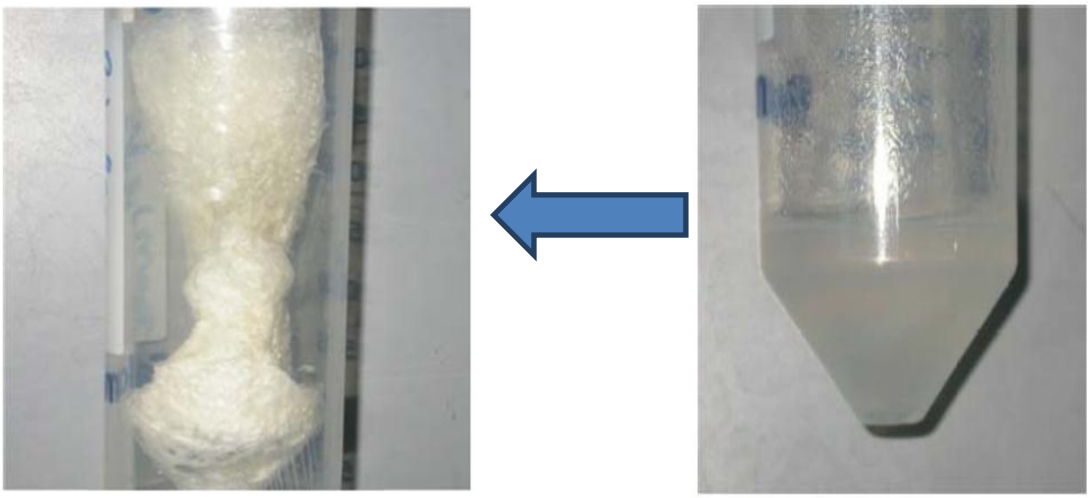


Figure 3-1: Process flow for extraction of gelatine from chicken skin

Chicken Chicken skin



Dry gelatine Gelatine solution

Figure 3-2: Graphical step involve in preparing the gelatine from chicken skin.

3.3 Yield

The percentage of gelatine yield was calculated using Equation 1 (Binsi et al., 2009).

$$\text{Yield (\%)} = \frac{\text{Weight of powdered gelatine}}{\text{Wet weight of fresh skin}} \times 100\%$$

Equation 1

3.4 Melting point of gelatine

Gelatine sample was crushed into fine powder using mortar and passel. It was heated from 200°C to 300°C at the rate of 5 °C /min in the Buchi M565 melting point analyser.

3.5 Viscosity of gelatine

The gelatine viscosity was determined according to the method of Cho et al. (2005). Gelatine solutions (6.67 g/100 ml) was prepared by dissolving the dry powder in distilled water and heating at 60 °C in a shaking water bath for about 20 min. Viscosity was determined using a Brookfield digital viscometer (Model LV-DV-II, Brookfield Engineering; MA, USA) at 60 rpm at 40 ± 1 °C.

3.6 Molecular weight

The molecular weight of gelatine samples was analysed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Readymade NuPAGE® Novex® 4-12% Bis-Tris was used. 13µL gelatines sample were mixed with 5µL NuPAGE LDS 4X sample buffer and 2µL NuPAGE Reducing Agents 10X, and heated at 60°C for about 10 minutes. The gel was mounted into a XCell4 SureLock™ Mini-Cell, filled with NuPAGE MES SDS running buffer and connected to PowerPAC™ HC power supply. 13µL of sample protein ladder was used as a protein marker and 20µL of sample was loaded in each well. The gel was run for 35 minutes at constant voltage of 200V. Proteins were stained with Coomassie Brilliant Blue R 250 (Sigma), 0.125% (w/v) in 10% acetic acid and 40% methanol. Destining was carried out in a solution of 10% acetic acid and 20% methanol in water.

CHAPTER IV

RESULT AND DISCUSSIONS

4.1 Yield of gelatine

Gelatine for chicken skin was carried out with different type of acid uses which is acetic acid and nitric acid for extraction process. The yield of the extracted gelatines was shown in Figure 4-1. The yield obtained, expressed as grams of dry gelatine per 100 g of clean skin, and was different depending on the treatment followed to preserve the skins before gelatine extraction. Loss of water in drying of the skins may promote protein–protein interactions and induce protein aggregation, which may impair collagen swelling and gelatine extraction. The yield of chicken skin gelatine use of acetic acid was higher which is (11.19%)(w/w) compare to chicken skin gelatine that use nitric acid which is only (9.18%)(w/w). The lower yield could be due to the loss of extracted collagen due to incomplete hydrolysis of the collagen (Jamilah and Harvinder, 2002).

Some journal reported that different yield values for the gelatines extracted from fish skins: for black tilapia -5.4% , red tilapia -7.8% (Jamilah and Harvinder, 2002), megrim -7.4%, dover sole -8.3%, cod -7.2%, hake -6.5% (Gómez-Guillén *et al.,* 2002), short fin scads -7.3% (Cheow *et al.,* 2007), big eye snapper -6.5% and brown stripe red (Jongjareonrak *et al.,* 2006). It was found that the yield values of gelatines extracted from chicken skin showed greater yield compare to those of gelatine extracted from fish skin. The different values of yield depend on type of gelatine which is the collagen content and amount of soluble components in the skins, as these properties vary with the species and the age of raw material use (Songchotikunpan *et al.,* 2008).

In this study, a combination of the two pre-treatments was used. Use alkaline and acidic pre-treatments showed effects on removing non collagenous proteins with minimum collagen loss, excluding the effect of endogenous proteases on collagen, causing a significant amount of swelling of chicken skin and securing a high gelatine yield and gel strength by

destroying certain chemical cross linkages that present in the collagen with less breakage of peptide bonds (Zhou and Regenstein, 2005).

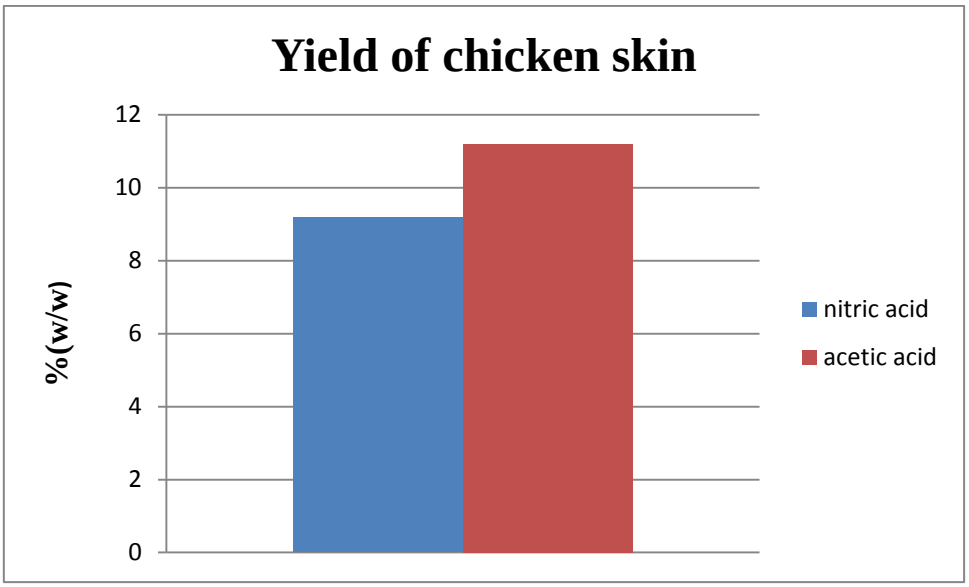


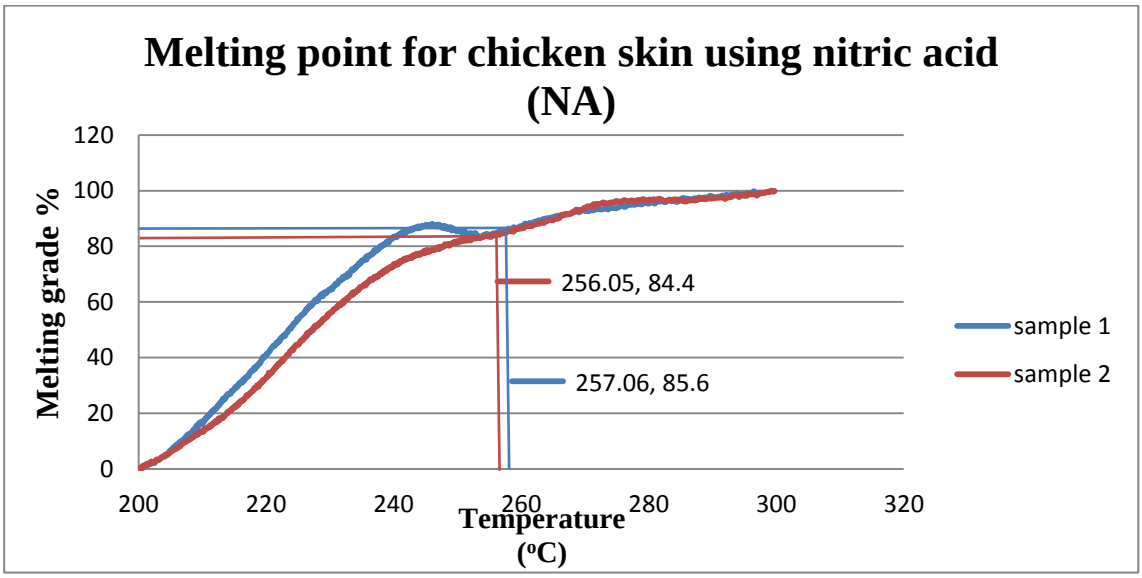
Figure 4-1: The yield of gelatine from chicken skin produced using different acid treatment

4.2 Melting point

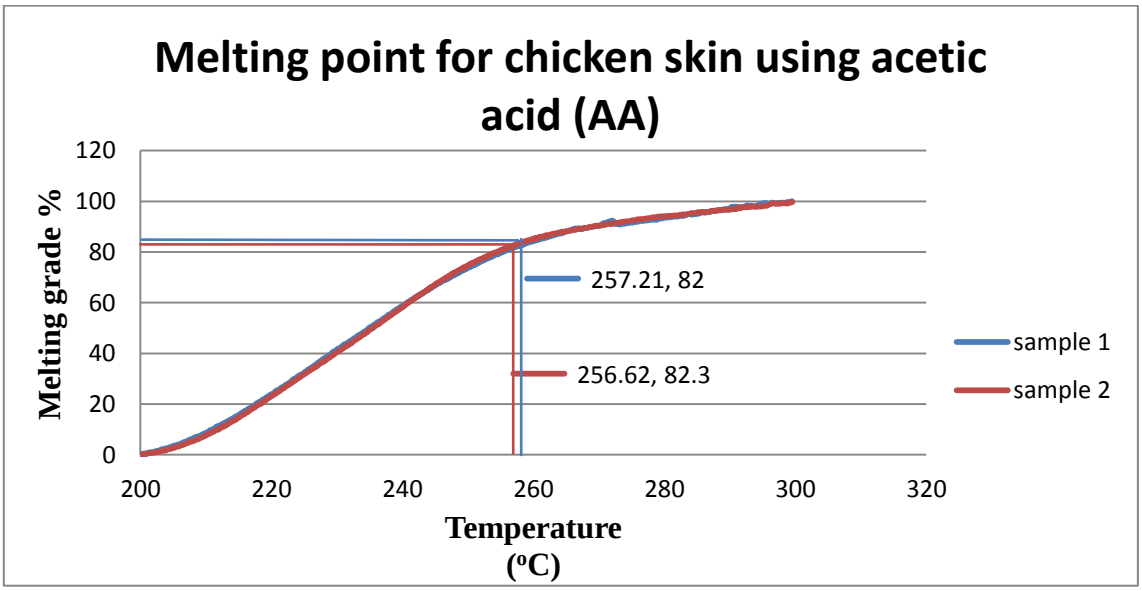
Melting point is one of important physico-chemical properties of gelatine. The melting points were performed with heating temperature from 200 to 300 °C at the rate of 5 °C /min. The melting point was reported based on the average of two samples from the same batch of experiment. Melting point of the gelatine obtained from chicken skin treatment with nitric acid is 256.555°C, whereas melting point for chicken skin treatment with acetic acid is 256.915°C. The commercial bovine and fish gelatine were tested for comparison. Fish gelatine had a melting point of 252.18°C and bovine gelatine had a melting point of 254.555°C. The graph for melting point are shown in Figure 4-2 shows the profile of melting grade % for different gelatine samples.

It is also known that fish gelatine has a lower melting point than mammalian gelatine. Fish gelatines that extracted from cold-water species especially have lower melting points

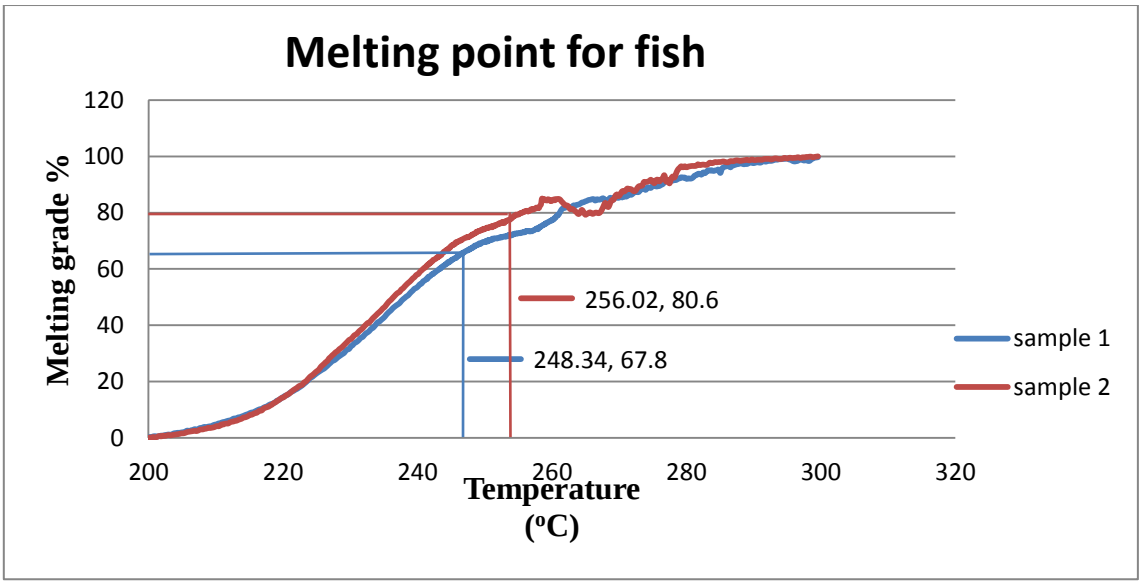
compared to mammalian gelatines. The amino acid composition can also contribute to the melting point characteristics. The lower melting point of fish gelatine is due to the lower amino acid content of fish gelatine, which in turn reduces the propensity for intermolecular helix formation (Choi and Regenstein, 2000). The functional properties of gelatine depend on many factors including the method of preparation and the intrinsic characteristics of collagen (Badii and Howell, 2006).



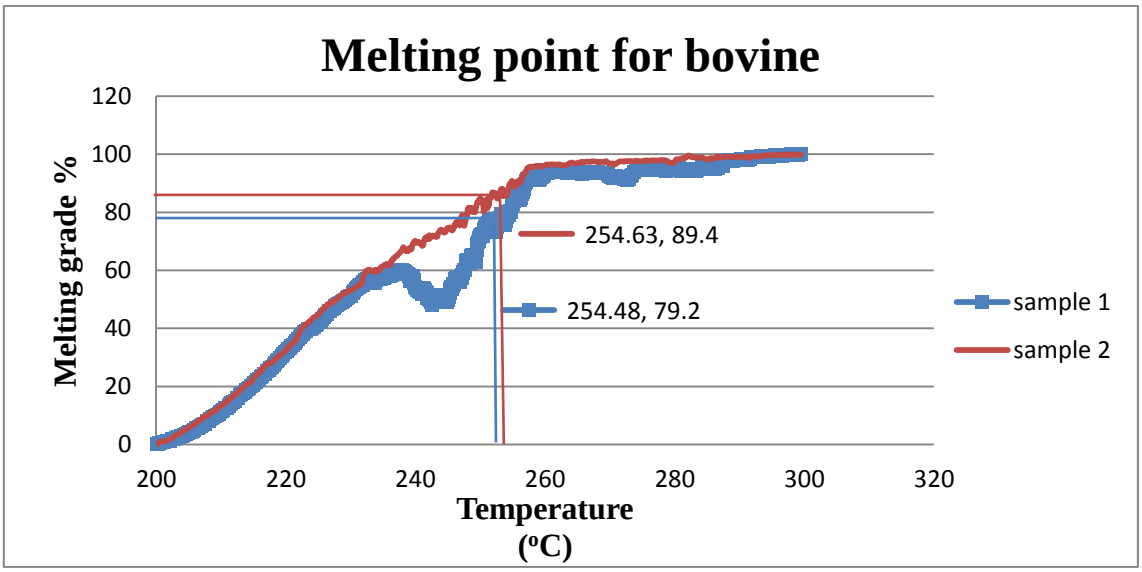
(a)



(b)



(c)



(d)

Figure 4-2: Profile of melting grade % for different gelatine sample prepare (a) chicken skin using acetic acid; (b) chicken skin using nitric acid; (c) commercial fish gelatine; (d) commercial bovine gelatine

The melting point value for different gelatine samples was showed in Table 4-1. The high melting point for chicken skin attributed to the high content of amino acids (Pro and Hyp) (Gomez-Guillen et al. 2002). The proline plays a major role in promoting the formation

of polyroline II helix .The melting point of a gelatine increases with increase in its molecular weight and the maturation temperature (Choi and Regenstein, 2000).

Table 4-1: Melting point for gelatine samples

Gelatin Sample	**Melting Point**
Skin chicken (nitric acid treatment)	256.555^{o}C
Skin chicken (acetic acid treatment)	256.915^{o}C
Fish	252.18^{o}C
Bovine	254.555^{o}C

4.3 Viscosity

Viscosity is the second most important physical property of commercial gelatin .The standard temperature to measure the viscosity of gelatin is 60ºC. The shear viscosity value obtained for different gelatin samples was showed in Table 4-2. The shear viscosity values obtained for chicken skin gelatine with treatment of acetic acid samples (6.67% w/v) was 3.3 mPa s and chicken skin with treatment of nitric acid recorded 2.8 mPa s. Chicken skin based gelatin showed almost similar with commercial fish gelatin. The viscosity of gelatin derived chicken skin is in mid-range of the viscosity of commercial gelatins which in the range from 2 to 7 mPa s (Jamilah and Harvinder, 2001).

Table 4-2: Viscosity of gelatine samples

Gelatin Sample	**Viscosity, mPa.s**
Skin chicken (nitric acid treatment)	2.8
Skin chicken (acetic acid treatment)	3.3
Fish	3.1
Bovine	4.45

Alkaline concentration has had synergistic effect on viscosity with acetic acid concentration and nitric acid. This increase possibly is due to complete opening up of polypeptide chain to random chain and intermolecular hydrodynamic interaction leading to increase viscosity. Minimum viscosity of gelatine that been noted to be in the range of pH 6–8 for many gelatines . The pH that effect on viscosity is minimum at the isoionic point and maximum at pH 3 and 10.5 (Jamilah and Harvinder, 2002). Gudmundsson and Hafsteinsson (2002) reported that the viscosity of the gelatine solutions is mainly controlled by molecular weight and polydispersity.

The viscosity decreased by reducing of gelatine concentration and increasing of temperature. However, NaOH concentration was more effective on viscosity than acid concentration. The most importance in viscosity changes is hydrogen and hydrophilic bond. These will cause a viscosity increase in gelatine solutions. Acid will increasing viscosity by having a main role in demineralizing collagen and degradation peptides chains and establish protein fractions. Hydrolysis peptide bands and facilitate transforming collagen structure to gelatine can be improved with low concentration of Acid. This decreasing of viscosity may be due to presence of low molecular weight peptide component. By increasing Acid concentration, the collagen bands will breakage and changing collagen conformation structure accelerates. Also micro component serve hydrolysis make interaction together and thus viscosity increases. NaOH will decreases viscosity by reducing molecular weight collagen extracted establish intrusive structure and avoid formation crosslink inter chain. Hence increasing NaOH concentration can decrease viscosity.

4.4 SDS-PAGE analysis

The molecular weight distribution of gelatin derived from chicken skin using nitric acid and acetic acid treatment was compared in Figure 4-3. The samples were prepared at different concentration 0.75 – 6 mg/ml. Gelatin derived from chicken skin gelatin using pre-treatment of nitric acid and acetic acid show the same molecular weight pattern. It had three intense bands at molecular weights of approximately 140, 150 and 250 kDa, and faint bands corresponding approximately 53 kDa. Molecular weight for bovine gelatin approximately around 150 kDa. For comparison, the molecular weight pattern of gelatin derived from Nile

tilapia showed 5 bands at approximately 203, 116, 80, 60 and 29 kDa (Songchotikunpan et al., 2008) and gelatin derived from Alaskan Pollock showed 3 bands at 240, 116 and 80 kDa (Chiou et al., 2006). Despite being extracted at a relatively high temperature 70 ◦C, the extracted gelatin contained a relatively greater proportion of high-molecular-weight peptide fractions than those of the commercial gelatin. During freeze-drying of the extracted gelatin, the low molecular weight gelatin fractions might undergo the renaturation gradually, forming a network with more protein– protein linkages than the high molecular weight fractions (Muyonga et al., 2004).

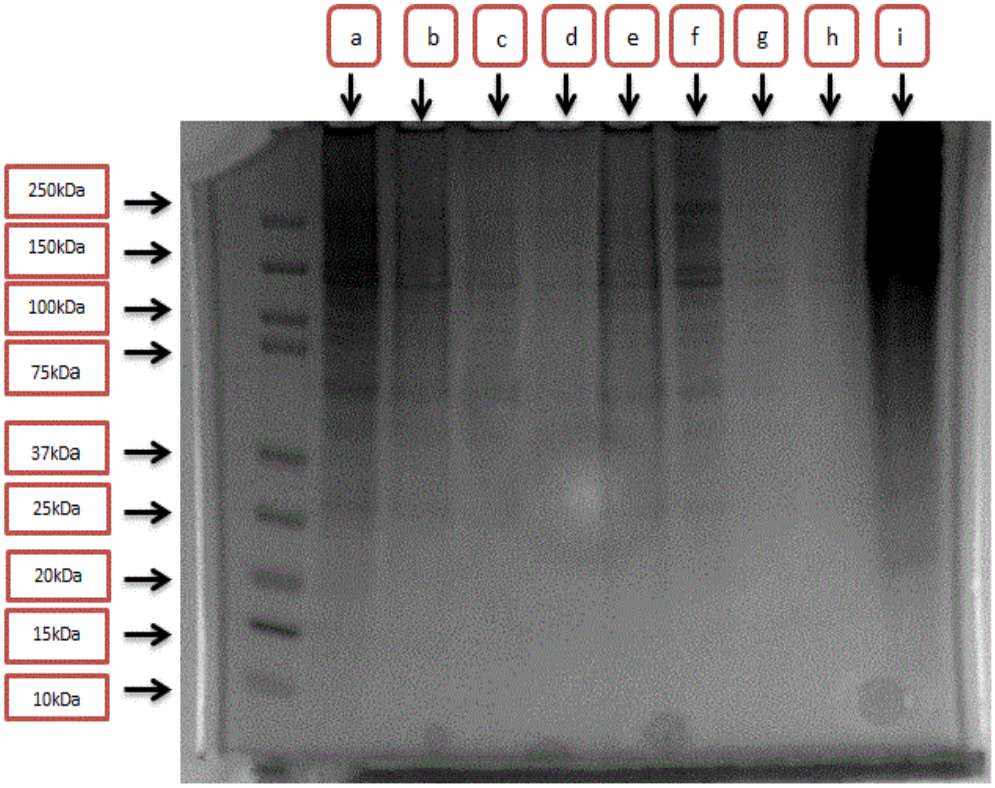


Figure 4-3: SDS PAGE gel chicken derived gelatin at different sample concentration. Chicken skin treated with acetic acid: a – 6 mg/ml, b -3 mg/ml, c -1.5 mg/ml, d - 0.75 mg/ml). Chicken skin treated with nitric acid: e – 6 mg/ml, f - 3 mg/ml, g - 1.5 mg/ml, h - 0.75 mg/ml) and commercial bovine gelatin: i- 6 mg/ml

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The gelatine derived from chicken skin show higher yield and molecular weight compared to the commercial gelatine. For the melting point, chicken gelatine showed higher value than the fish gelatine. The viscosity of chicken gelatine is in the midrange of the commercial gelatine. Gelatine derived from chicken skin can be used in food applications to replace mammalian bovine and porcine gelatine which is not only at risk of contamination with bovine spongiform encephalopathy (BSE), but also got some resistance from koshers and halal consumer.

5.2 Recommendation

There are some recommendations and further study can be done on the extraction of gelatine form chicken skin as below:

i. Gel strength analysis can be test for future study using texture analyzer. Gel strength is important criteria for grading of high quality gelatin.
ii. Determine the chicken skin gelatin amino acid composition. The stability of triple helical structure is proportional to the total content of amino acid proline and hydroxiproline.
iii. Use temperature during pre-treatment and various times for extraction process also can be done to improve the quality of gelatin.

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APPENDICES

Table A-1: Melting point of chicken skin using acetic acid

Temperature ^{o}C	Melting grade %		
	Left	Centre	Right
200.16	0.0	0.2	0.0
200.25	0.4	0.0	0.4
200.74	0.5	1.3	0.5.0
210.07	17.2	13.5	15.0
210.23	17.4	13.5	15.0
210.56	18.0	14.3	16.1
210.98	19.3	15.2	17.1
220.24	41.4	33.2	38.7
220.67	42.9	34.7	39.1
220.92	43.6	34.6	39.7
230.02	64.3	56.0	52.9
230.09	64.7	56.1	53.6
230.18	64.7	56.4	53.5
230.26	65.0	56.4	53.7
230.35	64.5	56.5	53.9
230.43	64.8	56.5	53.8
230.51	65.4	57.1	54.4
240.34	83.3	73.4	65.6
240.41	83.5	73.8	65.6
245.08	87.8	78.1	70.2
247.08	87.7	79.1	71.4
264.09	90.1	89.1	78.9
264.36	90.1	89.5	79.1
264.62	90.3	89.5	79.3
270.6	92.6	94.1	80.8
270.85	93.3	94.5	80.8
272.14	93.3	95.0	81.3
272.38	93.3	95.3	81.4
298.96	99.7	99.5	98.5
299.23	99.9	100	98.7
299.49	99.6	99.7	99.0
299.72	99.9	99.8	99.9

Table A-2: Melting point for fish

Temperature ^{0}C	Melting grade %		
	Left	Centre	Right
200.08	0.3	0.1	0
221.31	16.4	16.5	17.1
222.6	18.2	18.6	19.7
224.08	21.4	21.8	22.7
224.32	21.6	22.2	23.1
227.58	27.5	29.8	30.1
227.83	27.9	30.4	31.3
228.1	28.5	30.8	31.7
243.83	61.0	65.8	68.9
249.31	68.9	73.6	78.0
249.39	68.9	73.7	78.1
250.52	70.3	74.6	79.5
250.6	70.1	74.8	79.5
250.68	70.3	74.7	80.0
250.76	70.1	74.8	79.8
250.84	70.4	75.0	79.9
250.93	70.3	75.0	80.1
251.92	70.9	75.9	80.9
252.01	71.0	75.9	81.2
252.09	71.0	75.9	81.4
252.19	71.1	76.3	81.4
252.28	71.3	76.2	81.5
252.37	71.2	76.2	81.7
252.46	71.3	76.3	81.8
256.02	73.3	80.6	86.3
256.11	73.4	80.5	86.8
256.19	73.3	80.6	87.2
259.75	77.0	84.5	88.1
259.83	77.2	84.2	88-0
259.91	77.2	84.1	88.2
259.99	77.2	84.2	88.0
273.21	88.3	89.5	93.7
273.46	88.3	89.7	93.8
280.3	92.2	96.4	96.8
280.57	92.1	96.5	96.7
287.27	97.0	98.5	98.5
287.52	97.2	98.6	98.4
291.5	98.4	99.0	99.0
299.33	99.6	99.9	99.6

Table A-3: Melting point for bovine

Temperature ^{0}C	Melting grade %		
	Left	Centre	Right
200.11	0	0	0
203.57	2.6	3.7	1.6
206.89	6.3	8.5	7.4
229.14	49.7	52.2	59.8
229.39	50.3	52.5	60.2
233.64	57.5	59.9	64.6
233.72	55.4	59.8	64.4
233.81	55.7	60	64.8
235.24	57.4	61.8	64.1
236.64	58.8	64.1	66.4
238.39	59.2	67.2	67.2
238.48	59.1	66.9	66.9
239.75	56.8	69.4	64.4
241.06	51.9	68.4	64.9
243.3	50.1	71.7	71.2
243.39	50.6	71.6	71.8
245.14	50.7	74.4	74.3
257.48	91.5	95.5	92.9
257.55	91.6	95.6	93.5
257.64	91.9	95.6	93.9
257.73	91.7	95.7	93.5
257.82	91.8	95.7	93.3
257.9	91.9	95.7	93.1
257.99	91.7	95.5	93.4
259.42	92.2	95.7	93.8
259.51	92.2	95.8	93.9
276.7	95	97.9	98
276.97	94.8	97.8	97.8
282.24	94.8	99.5	97.9
282.5	94.8	99.1	97.9
282.77	94.5	98.9	98.4
283.01	94.3	98.9	98.2
283.26	94.2	98.8	98.2
283.5	94.3	98.9	97.9
299.06	99.6	99.8	99.5
299.29	100	100	99.9
299.53	99.8	99.8	99.8

Table A-4: Melting point of chicken skin using nitric acid

Temperature ^{o}C	Melting grade %		
	Left	Centre	Right
200.16	0	0.2	0
200.16	0.2	0.4	0.2
200.25	0.4	0	0.4
207.16	10.9	9.5	9.6
207.42	11.6	9.7	9.7
209.59	16.4	13	14
209.66	16.2	13.1	14.5
209.74	16.9	13.4	15
211.41	20.1	15.5	18.1
211.5	20.8	15.8	17.9
215.67	30.1	23.1	27
215.74	30.5	23.3	27.4
215.82	30.8	24	27.3
220.09	41.4	33.2	37.8
220.16	41.3	33.3	38
220.24	41.4	33.2	38.7
230.67	65.8	57.5	54.8
230.76	65.7	57.4	55.2
230.85	66.3	57.6	54.4
249.97	85.7	81.4	72.9
250.06	85.8	81.9	72.6
277.16	94.9	96.5	82.9
277.4	95.4	96.4	83
277.65	94.9	96.4	83.4
293.47	98.4	98.3	92.5
293.7	98.6	98.2	92.3
293.95	99	98.1	92.6
299.49	99.6	99.7	99
299.72	99.9	99.8	99.9