EFFECT OF VARIOUS ACID PRETREATMENT ON PHYSICOCHEMICAL PROPERTIES AND SENSORY PROFILE OF DUCK FEET GELATINE AND APPLICATION IN SURIMI

by

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LIST OF ABBREVIATIONS

DFG	Duck feet gelatine
DFCl	Duck feet gelatine treated with hydrochloric acid
DFAa	Duck feet gelatine treated with acetic acid
DFLa	Duck feet gelatine treated with lactic acid
DFCa	Duck feet gelatine treated with citric acid
CBG	Commercial bovine gelatine
CFG	Commercial fish gelatine
SDS-PAGE	Sodium dodecyl polyacrylamide gel electrophoresis
FTIR	Fourier transform infrared spectroscopy
QDA	Quantitative descriptive analysis
EM	Expressible moisture
TPA	Texture profile analysis

LIST OF SYMBOLS

kg	Kilogram
g	Grams
mg	Milligrams
mm	Millimetre
mm/s	Millimetre per second
ml	Millilitre
L	Litre
%	Percentage
nm	Nanometre
L*	Lightness
a*	Redness
b*	yellowness
Hyp	Hydroxyproline
Pro	Proline
°C	Degree Celsius
M	Molarity
w/v	Weight per volume
w/w	Weight per weight
<	Less than
>	More than
≤	Less or equal than
≥	More or equal than

KESAN RAWATAN AWAL ASID YANG BERBEZA TERHADAP SIFAT FIZIKOKIMIA DAN PROFIL SENSORI GELATIN KAKI ITIK DAN APLIKASI DALAM SURIMI

ABSTRAK

Gelatin diperolehi daripada kaki itik telah dinilai sebagai alternatif kepada gelatin mamalia dan marin. Gelatin kaki itik (DFG) telah dikaji melalui dua fasa. Dalam fasa pertama, kaki itik telah dirawat dengan 0.1 M asid hidroklorik (DFCl), asid asetik (DFAa), asid laktik (DFLa), dan asid sitrik (DFCa). Ciri-ciri fizikokimia dan profil sensori semua DFG dibandingkan dengan gelatin komersial daripada lembu (CBG) dan gelatin komersial daripada ikan (CFG). Hasil DFG yang paling tinggi adalah DFCa dengan 3.17%, sementara itu nilai pH dan kelikatan DFG adalah di antara 5.33-5.81 dan 13.07-17.90 mPa.s. Analisis proksimat DFG bagi kandungan air, protein, abu dan lemak adalah dalam lingkungan 6.29-10.24%, 86.40-89.67%, 0.58-1.35% dan 0.57-1.20%. Kekuatan gelatin bagi DFG adalah jauh lebih tinggi contohnya DFCa dengan 322.63 g berbanding CBG (216.63 g) dan CFG (247.97 g). Semua DFG mempunyai suhu lebur yang tinggi di antara 42.79- 48.08 °C. Warna DFG lebih legap dan kurang telus berbanding CBG dan CFG dengan nilai L*, a* dan b* masing-masing di antara 19.03-20.88, -0.36 ke -1.34 dan -1.71 ke -3.61. Sementara itu, berat molekul dengan menggunakan SDS-PAGE menunjukkan corak jalur untuk semua DFG adalah sama dan sempit iaitu sekitar 200 kDa bagi ß, sekitar 130 kDa bagi α1 dan sekitar 115kDa bagi rantaian helix α2. Cap jari FTIR bagi semua DFG adalah serupa dan sama dengan CBG dan CFG. DFCa mempunyai asid imino paling tinggi dengan 23.01% berbanding dengan DFG lain. Profil sensori

dengan menggunakan analisis deskriptif kuantitatif (QDA) juga menunjukkan bahawa penerimaan keseluruhan serbuk gelatin dan gel daripada DFG adalah lebih lebih baik dibandingkan dengan gelatin komersial. Dalam fasa kedua, sebanyak 0.5% hingga 3.0% DFG telah dimasukkan ke dalam sardin surimi untuk menilai ciri-ciri tekstur gel surimi. Hasil memasak gel surimi meningkat dengan ketara dengan peningkatan peratusan DFG dalam sardin surimi. Penambahan sebanyak 0.5% DFG dapat meningkatkan ujian lipatan daripada 1.00 kepada 2.00, dan tambahan sebanyak 3% DFG dapat meningkatkan ujian lipatan dari 1.00 kepada 4.00. Ini jelas menunjukkan bahawa dengan peningkatan tahap DFG dalam surimi gel maka skor ujian lipatan juga bertambah. Nilai tekanan kelembapan bagi surimi gel yang ditambah dengan 0.5% hingga 3.0% DFG menurun dengan ketara sebanyak 62.37%, 76.66%, 59.32% dan 82.35% masing-masing bagi DFCl, DFAa, DFLa dan DFCa. Kekuatan gel juga turut meningkat dengan ketara jika penambahan sebanyak 0.5% hingga 3.0% DFG ditambah ke dalam surimi gel. Penambahan sebanyak 0.5% DFCa dapat meningkatkan kekuatan gel kontrol sebanyak 53.77% dan dengan penambahan sebanyak 3.0%, kekuatan gel meningkat sebanyak 292.48%. Untuk warna gel surimi, DFCa dengan 0.3% mempunyai nilai kecerahan tertinggi dengan 59.71. Secara keseluruhan, sifat-sifat tekstur gel surimi yang ditambah dengan gelatin kaki itik dapat meningkatkan surimi sardin kepada gred yang lebih baik. Oleh itu, keputusan telah membuktikan bahawa kaki itik boleh dijadikan sebagai alternatif untuk penghasilan gelatin.

EFFECT OF VARIOUS ACID PRETREATMENT ON PHYSICOCHEMICAL PROPERTIES AND SENSORY PROFILE OF DUCK FEET GELATINE AND APPLICATION IN SURIMI

ABSTRACT

Gelatine derived from duck feet was evaluated as an alternative to mammalian and marine gelatine. Duck feet gelatine (DFG) was studied through two phases. In the first phase, duck feet was treated with 0.1 M hydrochloric acid (DFCl), acetic acid (DFAa), lactic acid (DFLa), and citric acid (DFCa). The physichochemical properties and sensory profile of all extracted DFG were compared with commercial bovine (CBG) and commercial fish gelatine (CFG). The highest yield of DFG was from DFCa with 3.17%, meanwhile the pH and viscosity of DFG was ranged between 5.33 to 5.81 and 13.07 to 17.90 mPa.s, respectively. The proximate analysis of DFG was in the range of 6.29 to 10.24%, 86.40 to 89.67%, 0.58 to 1.35% and 0.57 to 1.20% for moisture, protein, ash and fat, respectively. The bloom strength showed that DFG had significantly higher bloom strength such as DFCa with 322.63 g compared to CBG (216.63 g) and CFG (247.97 g). All DFG had highest melting temperature around 42.79 to 48.08 °C. Colour of DFG are more opaque and less transparent compare to commercial bovine and fish gelatine with L*, a* and b* value ranged from 19.03 to 20.88, -0.36 to -1.34 and -1.71 to -3.61, respectively. Meanwhile, the molecular weight using SDS-PAGE shows that DFG for all treatments were narrow with similar band pattern of ß-sheet around 200 kDa, α1 around 130 kDa and α2 helix chains around 115kDa. The FTIR fingerprints of all DFG were similar and comparable with commercial bovine and fish gelatine. DFCa

had the highest imino acid with 23.01 compared to other DFG. Sensory analysis using quantitative descriptive analysis (QDA) also showed that the overall acceptability of gelatine powder and gel from DFG were more preferable compare to commercial gelatine. In phase two, 0.5% to 3.0% of DFG was incorporated into sardine surimi to see the texture properties of surimi gel. Cooking yield of surimi gel increased significantly with increasing percentage of DFG in sardine surimi. The addition of 0.5% DFG increased the folding test from 1.00 to 2.00 and the addition at 3% DFG increased the folding test from 1.00 to 4.00. It showed that by increasing the level of DFG in surimi gel significantly increased the folding test score. The expressible moisture for surimi gel added with 0.5% to 3.0% of DFG decreased significantly by 62.37%, 76.66%, 59.32%, and 82.35% for DFCl, DFAa, DFLa, and DFCa, respectively. The gel strength also increased significantly by the addition of 0.5% to 3.0% DFG in surimi gel. The addition of 0.5% DFG increased the gel strength of DFCa by 53.77% and at 3.0%, the gel strength increased by 292.48%. For colour of surimi gel, DFCa with 0.3% had the highest whiteness value with 59.71. Overall, the texture properties of surimi gel incorporated with duck feet gelatine able to improve the sardine surimi to a better grade. Therefore, the results have been proven that duck feet can be alternatives for gelatine production.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Gelatine's parent molecule is collagen, which is obtained from animal tissues, specifically skins and bones. Gelatine extraction requires an alkaline, acid or enzyme treatment to achieve the cleavage of collagen cross-links followed by extraction with warm water. Gelatine has unique properties which are the ability to form thermo-reversible gels and solubility in water. These properties contribute to a wide range of application in the food, pharmaceutical, photographic, cosmetic, and others (medical, matchstick and paintball) (Schrieber and Gareis, 2007; Haug and Draget, 2009). Gelatine acts as an additive to improve elasticity, consistency, and stability of foods (Kaewudom *et al*., 2012). Gelatine can also used in foodstuffs to enhance protein levels and to reduce carbohydrate levels in foods formulated for diabetic patients (Karim and Bhat, 2009).

The global gelatine production was 348.9 kilo tonnes in 2011 (Transparency Market Research, 2013). Major productions of gelatine are mainly produced from porcine and bovine sources. Muslims and Jews are prohibited from consuming porcine and any porcine related products. Even though bovine sources gelatine is permitted by Islam, it is only acceptable if the sources were prepared according to religious requirement. Besides that, the outbreaks of Bovine Spongiform Encephalopathy (BSE) had gained peoples' attention to finding other alternatives of gelatine from other sources. There has been an interest in the market of gelatine derived from fish and poultry. Tan *et al.* (2014) mention that according to the Food and Agriculture Organisation of the United Nations, duck meat is the third most

widely produced poultry in the world after chicken and turkey. Poultry such as chicken, turkey, and duck are alternatives to mammalian and marine sources gelatine. As poultry skin contains lots of fats, duck feet are used as the raw material to extract gelatine (Schrieber and Gareis, 2007).

At the same time, the use of poultry by-products is important for environmental sustainability and for providing additional revenue to the poultry industry (Jayathilakan *et al*., 2012). Value-added products from the processing of poultry by-products have attracted researchers to investigate it as raw materials for gelatine product. Previous studies of the extracted gelatine from poultry showed that the main sources used are chicken skin (Kim *et al*., 2012; Norizah *et al*., 2013) and chicken feet (Almeida and Farias, 2012; Widyasari and Rawdkuen, 2014). Only several researches focus on gelatine from duck feet alone (Park *et al*., 2013; Yeo *et al*., 2013; Kim *et al*., 2014). Previous studies showed that, duck feet gelatine can improve the cooking yield and texture properties of low fat sausages (Yeo *et al.*, 2013) and the sensory properties of frankfurter with duck feet gelatine showed a similar satisfaction score for flavour, tenderness, juiciness and overall acceptance compared to regular frankfurter (Yeo *et al.,* 2014). Therefore, further research about the quality of duck feet gelatine processes are necessary.

Previous study on poultry, especially duck feet gelatine showed that only hydrochloric acid (Yeo *et al.,* 2013; Yeo *et al.,* 2014; Kim H.W. *et al.*, 2014) and combination of hydrochloric acid and sodium hydroxide (Park *et al.,* 2013) were used as a pretreatment. Acid and alkaline are more preferred because the enzyme is expensive (Pitpreecha and Damrongsakkul, 2006) to use in gelatine pretreatment. Meanwhile, preliminary study of physicochemical properties of duck feet collagen by Huda *et al.* (2013) used 5% lactic acid. Hence, the present study uses different

acid to study the quality of duck feet gelatine produce by hydrochloric acid, acetic acid, lactic acid and citric acid as a pretreatment. The differences in acid with the same concentration (0.1 M) used in gelatine pretreatment might produce different yield and physicochemical properties of DFG. Then, all different types of DFG were added into sardine surimi in order to see the improvement of texture properties in surimi gel. The data from this study could be used as a reference for the application of duck feet gelatine in terms of their physicochemical properties, sensory profile, and their potential application in food products. Thus, it can give an opportunity and hopes for a gelatine producer to get other alternative sources of gelatine and to minimise the waste material from poultry industries.

1.2 Objectives

The objectives of this study are;

1. To determine the effect of various acid pretreatment on yield, physicochemical properties (pH, viscosity, proximate composition, bloom strength, melting temperature, colour properties, molecular weight, fourier transform infrared spectrum, and amino acid composition) and sensory profile (appearance, odour, and texture) of duck feet gelatine.
2. To study the potential application of duck feet gelatine from various acid pretreatment as an additive to improve cooking yield, texture properties (expressible moisture, folding test, gel strength and texture profile analysis) and colour of sardine surimi gel.

CHAPTER 2

LITERATURE REVIEW

2.1 Poultry industry

Food and Agriculture Organization of The United Nations showed that the global poultry meat production and consumption are growing with the annual growth of 3.6%, and about 78 million tonnes of poultry meat were produced (Du *et al.,* 2013). According to McKee (2007), poultry is defined as any type of domesticated fowl primarily raised for meat. Chicken and duck based poultry can be considered as one of the best income produced poultry since it can provide eggs and meat. Chicken and turkey dominate the world poultry industry, but in parts of Asia, ducks are commercially more important. While some areas in Europe, there are more geese than turkeys (Maurer, 2003). Most of the ethnic dishes in Asian countries are made of poultry such as China's Peking duck, Kungpao chicken, steamed, and grilled lemon chicken; Thailand's curry chicken, chicken satay and chicken macadami; India's chicken Tikka and chicken Biryani; Africa's roasted and barbecued chicken; Mexico's mole and pibil chicken; Brazil's Xim-xim and Colombia's ajiaco (Guerrero-Legarreta & Hui, 2010). For poultry by-product such as chicken skin, it is used to make animal meal, whereas a smaller proportion is used as a source of fat, especially for preparing soups and to incorporate into meat emulsions (Cliche *et al.,* 2003). According to Guerrero-Legarreta and Hui (2010), the poultry industry in Malaysia is well positioned to provide halal processed poultry to other Islamic countries and Muslim consumers worldwide.

2.1.1 Chicken

Around the world, there are at least 25 billion of chicken, this is the highest population of any bird in the world. The male chicken called as cock, but in some countries such as Australia, it is known as a rooster. For female chicken, it is called as hen and the fluffy yellow babies are called chicks (Anonymous, 2008a). The chicken was breed for their egg and meat. They are belonged to the *Galliformes* order and it is believed to originate from the red jungle fowl (*Gallus gallus*) (Poultryhub, 2015a). The weight of male chicken was about 2.3 kg to 4.8 kg, while for female chicken it was around 1.8 kg to 4.3 kg, depended on their species (Anonymous, 2015a). Chicken is an omnivore animal, it is usually scratching on the ground in search of seeds, berries and insects, but chicken has also been known to eat larger animals such as lizard sand or even small mice (Anonymous, 2008a). Broiler is a chicken that was farmed for their meat. They can live for more than six years, but for broiler chickens, it only takes less than 6 weeks to reach slaughter size. Meanwhile, for organic chicken meat, it will be slaughtered at the age of 14 weeks (Wikipedia, 2015a).

2.1.2 Turkey

Around the world, about 5.6 million tonnes of turkey meat were produced each year and 60% of it is from North America, which is the native home of turkey (Poultryhub, 2015b). According to Maurer (2003) and Remignon (2004), turkey originates from Mexico where it was first domesticated. Turkey is omnivore animal, it primarily eats nuts, seeds, fruits, berries, and insects, but it also eats small amphibians, reptiles and even rodents (Anonymous, 2008b). In developed

countries, turkeys are commonly found as meat producing birds. Turkeys are traditionally bought as whole carcasses at Christmas, Easter or Thanksgiving with a range of 2.5 to 5.0 kg dressed weight (Remignon, 2004). Turkey's meat is very similar to chicken with a very little fat and high protein content. Remignon (2004) mention that according to the Food and Agriculture Organization of the United Nation, main countries that produce turkeys in 2002 are the United States and France with 49% and 14%, respectively.

2.1.3 Duck

Duck (*Anas platyrynchos*) was first domesticated over 4000 years ago. Duck is like chicken and turkey, they are omnivore animals that feed on small fish, aquatic plants, insect and grub. According to Huda *et al.* (2010), although chicken and turkey dominate the world poultry industry, about 700 million ducks are kept around the world. Ducks are one of the most widespread birds in the world as they are found on every continent. It has the ability to eat food both in water and on dry land (Anonymous, 2008c). There are three most commercial types of duck, which is Pekin, Muscovy and Mule duck (a cross between Muscovy and Pekin) (Poultryhub, 2015c). The most famous duck breed is a Pekin duck (Yan, 2004). All Pekin ducks (Plate 2.1) are generally killed between seven to eight weeks of age for whole carcass marketing. Table 2.1 shows the scientific classification of Pekin duck. The proximate analysis of 100 g duck feet had about 48.5% moisture (Anonymous, 2012), 11% fat and 27% of protein content (William, 2008). Adult Pekin ducks were weight between 3.6 and 5.0 kg with average eggs laying of 200 per year. Pekin duck has a yellow bill and creamy white feathers with orange shanks and toes. Sometimes, it has yellowish

tinge feathers, especially for ducks that are not exposed to sunlight and breed indoors. Duck eggs are easy to hatch compared to other birds as it can adapt in a variation of temperature and humidity (Anonymous, 2015b).

Table 2.1 Scientific classification of Pekin duck

Pekin duck (*Anas platyrhynchos domestica)*
Order : Anseriformes
Family : Anatidae
Genus: Anas
Species : *Anas platyrhynchos*

Plate 2.1 Pekin Duck

Malaysia is one of the top main producers of duck meat and the average duck meat production around the world from the year 1992 to 2012 showed that the Asia region contribute to 80.5% of the total productions (FAO, 2014). In Asia, domesticated ducks are preferred as a source of meat and eggs compared to chicken

and turkey. According to DVS (2014), the state of Perak produces about 48,302.93 metric tonnes of duck meat in 2011 and they export around 4,256,748 birds to Singapore. Perak Duck Food Industries is the only company that obtained a logo of "Veterinary Health Mark" (VHM) in their products. The logo is a mark of quality and safety given to plants processing livestock products which was awarded under the Veterinary Inspection and Accreditation Programme from Department of Veterinary Services. Ducks do not require a pre-emptive antibiotic to remain healthy, so they carry the antibiotic-free label (Schneller, 2010). When the demand for duck meat in the market increase, a number of duck by-products also increase (Huda *et al.*, 2013; Park *et al.*, 2013). According to Adeola (2006), ducks are one of the fastest growing and most efficient producers of animal protein. Besides that, only few further processed products are prepared from duck compared to chicken and turkeys because the duck meat has a good natural flavour and does not require further processing to improve its appeal (Remignon, 2004).

2.2 Poultry waste

Poultry offal comprises of heads, feet, and inedible viscera (intestines, lungs, pancreas, spleen, and the reproductive organs) (Maurer, 2003; Yan, 2004; Zhu *et al*., 2010). Worldwide, more than 55.5 billion broilers were slaughtered in 2009 and yielded about 16.5% of the total offal including heads, feet and inedible viscera (Heuze *et al.,* 2013). Du *et al.* (2013) reported that, according to the Food and Agriculture Organisation, it is estimated that around 22 to 30% of the poultry production is considered to be by-products. In recent years, the demand for chicken is increased and lead to an oversupply of by-products (Cliche *et al*., 2003). According to Maurer (2003), there are several ways to handle poultry offal. Usually,

all offal except the blood and sick birds is floating in the water from the processing areas in an accumulation area for removal by trucks. This waste can be minimized by utilizing it as a source for gelatine production. Meanwhile, according to William (2010), the most common method to dispose animal by-products was buried in landfills, but it is costly and risky because of human and environment contamination. Normally, animal byproducts were composted for agriculture purposes or used to make animal feed (Bolan *et al*., 2010).

Poultry by-product contains large amount of gelatine (Park *et al*., 2013; Lee *et al.,* 2012). According to Karim and Bhat (2009), it was expected that poultry skin and bones will be one of the main gelatine sources in the near future. Gelatine from poultry gave significantly higher bloom strength with 355 g as compared with bovine gelatine with 229 g (Norizah *et al*., 2013). As poultry skins contain a lot of fats and the concentration of collagen is low, Schrieber and Gareis (2007) suggested using other material from poultry such as their feet.

2.2.1 Duck Feet

The duck has webbed foot called palmate. There are three toes that webbed, which are the front toes that connected to the small and elevated hind toes. The webbed feet of duck play an important role as a paddle to push against the water when they swim (Anonymous, 2008c). Duck feet are perfect for paddling through water, but less suited to walk on the ground (Anonymous, 2015b). The picture of duck feet is shown in Plate 2.2. According to Sara (2012), dehydrated duck feet (Figure 2.1) are sold as delicious treats for animals such as dogs because duck feet not only provide a long lasting chew satisfaction but also a great source of protein

supplement. Duck feet were also used in preparing some appetizer and delicacies to be served in some restaurants. Huda *et al.* (2013b) reported that duck feet collagen can improve the physicochemical properties of low-grade surimi. According to Park *et al.* (2013), duck feet are a good source of proteins and functional collagen materials, especially in 'mustard duck feet' which was originated in China and Southeast Asia. Limited studies were done on poultry especially their feet. Table 2.2 shows details from the previous study that focuses on gelatine extracted from poultry feet.

Plate 2.2 Raw duck feet

Figure 2.1 Dehydrated duck feet
(Source: Anonymous, 2015c)

Table 2.2 Gelatine extracted from poultry feet

Raw material	Objective	Reference
Chicken feet	To examine the effects of liming conditions of chicken feet on the yield and physicochemical properties of gelatine, and to determine the optimum levels of these variables for the production of chicken feet gelatine. The effects of neutralizing conditions also were examined.	Lim *et al.* (2001)
Chicken feet	To characterise the gelatine from the chicken feet by FTIR (Fourier transform infrared) spectroscopy.	Almeida *et al.* (2012)
Chicken feet	To investigate the effect of chicken feet gelatine and wheat fibre levels on the quality characteristics properties of semi dried chicken jerky.	H.-Y. Kim *et al.* (2012)
Chicken feet	To extract gelatine from different parts of chicken feet, identifying the best characteristic related to ash content and subsequent characterization as to proximate composition, texture profile, colour and gel strength (*Bloom*), and to verify the behaviour of gelatine gels at different concentrations aiming to obtain a product with characteristics adequate to consumption.	Almeida and Lannes (2013)
Chicken feet	To extract gelatine from chicken feet using two different methods (acid extraction and ultrasound assisted extraction).	Widyasari and Rawdkuen (2014)
Duck feet	To investigate the effects of extracting method such as water bath (WB), pressure cooker (PC) and microwave oven (MO) on quality characteristics of the duck feet gelatine and to improve utilization of duck feet as a novel source of gelatine.	Park *et al.* (2013)
Duck feet	To determine the effect of duck feet gelatine concentration on the physicochemical, textural and sensory properties of duck meat jellies	H.-W. Kim *et al.* (2014)
Duck feet	To investigate the effect of duck feet gelatine gel as a fat replacer on the physicochemical, textural, and sensory properties of low-fat frankfurters.	Yeo *et al.* (2014)

2.3 Gelatine

One-third of total proteins in the body is the skin, tendon and connective tissues which are composed of collagen. Gelatine is collagen derivative and it comes from the animal origin, which is obtained from collagen protein through acid or alkaline hydrolysis. Figure 2.2 shows the denaturation of collagen to form gelatine. Basically, it consists of twenty kinds of amino acid that is essential for our body (Lee *et al*., 2012). Gelatine is a digestible protein that contains all essential amino acids except tryptophan and it differs from other hydrocolloid because most of them are polysaccharide (Mariod & Adam, 2013). Depends on the method use to extract gelatine, amino acids compositions are varied from species to species.

Gelatine is widely used in food industry as an additive for stabilizing, gelling and emulsifying. Gelatine is used in pharmaceutical, photographic, cosmetic, and technical products. In addition, it can be used as a dietetic food, salt reducer, a flocculating agent, and protein enrichment and in adhesives. In the pharmaceutical industry, gelatine is generally used in the production of soft and hard capsules, tablets, haemostatic sponges, blood plasma substitutes, suppositories, and vitamin encapsulation.

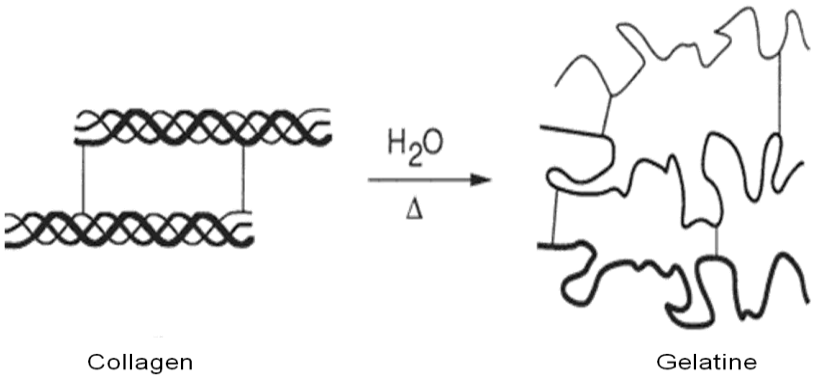

Figure 2.2 Denaturation of collagen to gelatine
(Source: Anonymous, 2015d)

Generally, the sources of gelatine are mammalian from porcine and bovine (Kittiphattanabawon *et al.,* 2010; Lee *et al*., 2012). According to Gómez-Guillén *et al.* (2011), porcine skin (46%) is the most abundant source of gelatine, followed by bovine hide (29.4%) and porcine and cattle bones (23.1%). For these reasons, few alternatives of mammalian gelatine have gained attention (Badii & Howell, 2006; Karim & Bhat, 2009) because the main gelatine sources from porcine and bovine create a controversy due to religious factors and diseases. Alternative for porcine and bovine gelatine include the marine sources (fish and jellyfish) and poultry (chicken and duck). Aguirre-A´lvareza *et al.* (2012) suggest that fish and poultry can replace the mammalian gelatine (bovine and porcine) but it depends on the expected characteristics of the final product such as stability, viscosity, colour, and consistency.

2.3.1 Alternative sources of gelatine

Fish gelatine has one major advantage because fish gelatine is not associated with the risk of Bovine Spongiform Encephalopathy (BSE) and food and mouth diseases. Fish gelatine is acceptable in Islam, and can be used with minimal restrictions for Jew and Hindu. Furthermore, fish skin, which is a major by-product of the fish-processing industry, had caused waste and pollution. This could provide a valuable source of gelatine (Badii & Howell, 2006). Nagai and Suzuki (2000) showed that collagen contents in the fish skin waste of Japanese sea bass, chub mackerel, and bullhead shark were 51.4%, 49.8%, and 50.1% (dry basis), respectively. Gelatine has been extracted from the skins and bones of various warm-water (e.g., tuna, catfish, and tilapia and cold-water (e.g., cod, Alaska Pollock, and

salmon) fish. Jamilah and Harvinder (2002) found that, the extraction yield of fish gelatine is lower than mammalian gelatine, giving approximately between 6% and 19% (grams of dry gelatine per 100 g of clean skin). The lower extraction yield of fish gelatine could be due to the loss of extracting collagen through leaching during the series of washing steps or due to incomplete hydrolysis of the collagen. In addition, Intarasirisawat *et al.* (2007) reported that some heat-stable proteases endogenous to the skin are involved in the degradation of gelatine molecules (specifically β and α chain) during the extraction process at elevated temperatures, which contribute to lower bloom strength.

Imino acids (proline and hydroxyproline) content in fish gelatine is low compared to mammalian gelatines, but warm-water fish gelatines have a higher imino acid content than cold-water fish gelatine (Eastoe & Leach, 1977 Muyonga *et al.* (2004a) reported that proline and hydroxyproline contents contribute about 30% of amino acids in mammalian gelatine, 22–25% in warm-water fish gelatines (Tilapia and Nile perch) and 17% in cold-water fish gelatine (cod). As shown in Table 2.3, imino acid content of fish skin is the lowest compared to mammalian and poultry. It was reported that aquatic animal collagen such as fish collagen contains lower imino acids compared to mammal's collagen (Montero & Gomez-Guillen 2000; Lin & Liu, 2006). Fish gelatines have lower gelling and melting temperatures, but relatively higher viscosities compared to mammalian (Leuenberger, 1991). Typical gelling and melting points for fish gelatines range from 8 to 25 ºC and 11 to 28 ºC, but typical gelling and melting points for porcine and bovine gelatines range are from 20 to 25 ºC and 28 to 31 ºC. Aguirre-A´lvareza *et al.* (2012) stated that the intrinsic properties of gelatine from different origins were affected by parameters inherent to the

manufacture of gelatine such as bloom strength, specific charge, isoelectric point and viscosity. Also, the origin of the raw material used in the gelatine extraction process account for the wide range of gelling temperatures. In addition, the raw material from marine sources failed to pull people's attention due to the allergic factor. The study showed that fish and shellfish are included in the eight types of food that contribute for more than 90% of allergy reactions (Nieuwenhuizen *et al.,* 2006).

Table 2.3 Amino acids composition of gelatine produce from different raw material

Amino acids (%)	Bovine skin[a]	Porcine skin[b]	Fish Skin[c]	Chicken skin[d]
Alanine	8.41±0.10	8.60	9.28±0.60	10.08±0.02
Arginine	5.09±0.04	8.30	8.00±0.78	5.57±0.00
Aspartic acid	3.29±0.01	6.20	5.69±0.34	2.11±0.02
Cysteine	0.47±0.00	0.10	0.06±0.00	0.16±0.00
Glutamic acid	5.43±0.03	11.30	10.65±0.14	5.84±0.01
Glycine	37.05±0.11	26.40	24.36±0.06	33.70±0.02
Histidine	ND	0.90	0.81±0.01	0.30±0.01
Hydroxyproline	10.67±0.11	13.50	6.10±0.13	12.13±0.02
Isoleucine	1.01±0.01	1.40	1.49±0.01	1.15±0.00
Leucine	1.89±0.01	3.10	2.22±0.01	2.63±0.00
Lysine	4.86±0.05	4.10	3.55±0.05	4.66±0.00
Methionine	0.22±0.13	0.80	1.65±0.00	0.07±0.00
Phenyalanine	1.60±0.02	2.10	2.13±0.00	1.77±0.00
Proline	12.66±0.14	16.20	12.8±2.21	13.42±0.01
Serine	2.93±0.08	2.90	5.62±1.39	2.20±0.00
Threonine	0.82±0.03	2.20	2.31±0.40	1.01±0.00
Tyrosine	1.16±0.01	0.40	0.60±0.00	1.22±0.01
Valine	2.07±0.02	2.50	1.94±0.00	1.94±0.02
Imino acid (Hyp + Pro)	23.33	29.70	18.90	25.55

ND: not detected
Source: [a, d](Norizah *et al.,* 2013); [b] GMIA, (2012); [c](Nikoo *et al.*, 2014)

2.3.2 Poultry gelatine

Chicken and duck based poultry can be considered as one of the best income produced poultry since it can provide eggs and meat. New gelatine source such as poultry skin, feet, and bone have increased to replace mammalian resources

(Gudmundsson, 2002; Karim & Bhat., 2009; Schrieber & Garies, 2007). The chicken skin contains approximately 75% type I and 15% type III collagens (Abedin & Riemschneider, 1984). Lin and Liu (2006) reported that collagen extracted from chicken broiler feet had higher hyproxyproline (Hyp) and proline (Pro) content and exhibited higher thermal stability. Table 2.3 showed the amino acid content of poultry which is chicken compared with porcine, bovine and fish. The present of hydroxyproline of amino acid which is found only in a few connective tissue protein collagen or gelatine, makes it different (Campbell & Farrell, 2006). Chicken gelatine showed similar properties with mammalian gelatine and it contains a high value of certain amino acid. In Table 2.3, it showed that chicken skin gelatine has high imino acid with 25.55% compared with bovine (23.33%) and fish (18.90%), this composition is important for gelling effect (Gómez-Guillén *et al.,* 2011) and it plays an important role in gel strength (Wangtueai & Noomhorm, 2009).

Cheng *et al.* (2009) noted that silky fowl feet are a useful industrial by-product, as the collagen extraction yield is 7.3% and collagen content is 516.6 mg/g. Cheng *et al.* (2009) also noted that collagen extracted using acetic acid-pepsin solubilisation method showed the highest yield and collagen content. Cliché *et al.* (2003) showed that yields obtained in 100 kg of chicken skin could generate between 860 g of collagen with telopeptides and 950 g without telopeptides. Norizah *et al.* (2013) noted that the yield of chicken skin gelatine is 16% based on dry weight basis. Meanwhile, Park *et al.* (2013) found that gelatine extracted from duck feet gave a yield of 3.02%, 3.31% and 0.75% for extraction using water bath, pressure cooker and microwave oven, respectively. In addition, duck feet gelatine can be used as a fat replacer in frankfurters (Yeo *et al.*, 2014) and as a useful ingredient for

manufacturing cold-cut meat product (Kim e*t al.,* 2014). Thus, poultry can be a potential alternative gelatine source.

2.3.2 Functional and characteristic of gelatine

Gelatine is one of the water soluble polymers that can be used as an ingredient to improve the elasticity, consistency and stability. It is unique because of it special properties that are thermo-reversible and melt in the mouth (Morimura *et al.,* 2002). Production of gelatine can be divided into three main steps; pre-treatment of the raw material, extraction of gelatine from collagen and drying or purification of the extracted gelatine (Benjakul *et al*., 2012; Park *et al*., 2013). Pre-treatment is important to derive gelatine from collagen by breaking down the non-covalent bonds which disrupt the protein structure. There are two types of gelatine which is type A and type B. The type A is derived using acid pretreatment and it has isoinic point of seven to nine, while type B is the results of an alkaline pretreatment with isoionic point of four to five (Gómez-Guillén *et al*., 2011).

The quality of gelatine depends to a large extent on its rheological properties mainly the gel strength and viscosity, but other characteristics, particularly transparency, the absence of colour and flavour and easy dissolution are also important. The physicochemical properties of gelatine are principally determined by their amino acid sequence. The imino acid which is proline and hydroxyproline are important in gelatine renaturation during gelling (Mariod & Adam, 2013). Meanwhile, the molecular weight of gelatine may be affected by the hydrolysis process that contributes to the splitting of the peptide chains (Norizah *et al.,* 2013).

The most important properties of gelatine are bloom value or bloom strength. It is measured in grams and it must be tested under standard conditions by depressing the surface of gelatine gel (concentration of 6.67% that was matured at 10 °C for 16-18 h) using a cylindrical plunger (Nikoo *et al.,* 2014; Norizah *et al.,* 2013). Commercial gelatine usually has a bloom strength, range from 50-300 g which can determine the grade of gelatine. According to Schrieber and Gareis (2007), high bloom strength means that it had stronger gelling power, so smaller amount of gelatine are needed to get the desired firmness for finished product. The properties of bloom strength are related to α and β chain components in gelatine. Also, bloom strength is related to the viscosity which play an important role in the food industry as it is a guide to gel behaviour (Mariod & Adam, 2013).

2.3.4 Gelatine extraction

All gelatine process consist of three main stages which is pretreatment of the raw material, extraction of the gelatine and purification and drying (Karim & Bhat, 2009). Prior to extraction, gentle chemical treatment is necessary to break down the cross-linked nature of collagen into gelatine. Other than acids and alkaline for pretreatment, enzyme or combination of enzymes and chemicals are used for cleaving the cross-links (Scherieber and Gareis, 2007).

2.3.4.1 Acid pretreatment

Gelatine that was produced from acid pretreatment known as a type A gelatine. Normally, the raw material is soaked in cold dilute mineral acid with pH 1.5 to 3.0 for 18 to 24 h depending on the raw material used (Haug and Draget, 2009).

Then, after treatment, the raw material is washed with running tap water to neutralise. The gelatine will be extracted from pre-treated raw material by heated in water between 50 °C and about 100 °C (Scherieber and Gareis, 2007). A previous study by Tavakolipour (2011) stated that organic acids like lactic and acetic acids are better than sulphuric and hydrochloric because both of it are stronger acid which can cause denatured of collagen proteins. This might resulted in low gelatine quality but possibly gave higher yield. Moreover, the yield of acidic gelatine (7.5%) is higher than alkaline gelatine (6.5%). According to Scherieber and Gareis (2007), phosphoric and organic acids are suitable for gelatine pretreatment, but they are more expensive and tend to influence the odour and taste of the final product negatively. Previous study of poultry gelatines is using an acid treatment such as hydrochloric acid (Kim H.Y. *et al.,* 2012; Park *et al.*, 2013; Yeo *et al.,* 2014) and acetic acid (Almeida *et al*., 2012; Almeida & Lannes, 2013). There is also treatment using both acid and alkaline (Du *et al.,* 2013; Norizah *et al.*, 2013). Several studies had discussed on using a different acids solution in gelatine and collagen extraction (Khiari *et al.,* 2011; Liu *et al.,* 2001; Cheng *et al.*, 2009). Khiari *et al.,* (2011) stated that there is no difference in molecular weight distribution of gelatine extracted using organic acids (acetic acid, lactic, malic, and tartaric acid) and suggested to use citric and malic acids due to better physicochemical properties. Meanwhile, high yield of collagen resulted from using an acetic acid treatment compared to citric, hydrochloric and lactic acid (Cheng *et al.,* 2009). But according to Liu *et al.* (2001), the best condition to extract collagen are using lactic acid rather than hydrochloric, acetic and citric acids.

Acids are grouped into inorganic acid (hydrochloric acid and sulphuric acid) and organic acid (acetic, lactic and citric acid). The substance is classified as an acid if it forms hydrogen (+) ions in aqueous solution when it was ionised (Anonymous,

2015e). The acidity of a solution is measured in terms of the pH of the solution. The lower the pH means higher acidity due to a high concentration of positive hydrogen ions in solution. Hydrochloric acid is a clear and colourless strong mineral acid with a chemical formula of HCl. It was produced by dissolving hydrogen chloride in water (Wikipedia, 2015b). Lots of products use hydrochloric acid in their processing. High fructose corn syrup (HFCS) is the major product of a food industry that uses hydrochloric acid. Hydrochloric acid consumed in the HFCS industry is used to regenerate the ion exchange resins that are employed to remove impurities. Hydrochloric acid is also used to acid-modify cornstarch and to adjust the pH of intermediates, final product and wastewater. Hydrochloric acid is used in acidulating crushed bones for the manufacture of gelatine and as an acidifier for products such as sauces, vegetable juices, and canned goods (Anonymous, 2012). Besides that, hydrochloric acid is also used as chemical reagent especially for the chemical industry and for household cleaning (Wikipedia, 2015b).

Acetic acid is a weak acid that included in an organic compound. Acetic acid, also known as ethanoic acid that has a sour taste, pungent smell and colourless. It is the main component for vinegar (3- 9% of acetic acid) with a chemical formula of CH_3COOH. Although it is a weak acid, but the concentrated acetic acid is corrosive. (Wikipedia, 2015c). Generally recognised as safe for use in foods. A research study in Japan suggests that acetic acid could help to control high blood pressure and fat accumulation (Hirshson, 2011). Acetic acid also use as a condiment food flavouring enhancer. It is widely to produce pickles and sushi rice. In addition, acetic acid can be found in many different types of vinegar such as malt vinegar, white vinegar, wine vinegar, cane, date and palm vinegar (Hudson, 2012). Meanwhile, lactic acid is a carboxylic acid with pKa less than 1 which make it easy to deprotonates compared

with acetic acid (Wikipedia, 2015d). The chemical formula is $C_3H_6O_3$. Lactic acid is naturally present in many foodstuffs. Lactic acid is found primarily in sour milk, such as yoghurt, kefir or cheese (Das and Goyal, 2012). Lactic acid is used in food industry such as in meat, poultry and fish to extend the shelf life; for beverage as acidity regulator in soft drinks and fruit juices; pickled vegetables to prevent spoilage and also in dairy product as acidification agent (Anonymous, 2015f). Citric acid also known as the 2-hydroxy-1,2,3-propanetricarboxylic acid is a weak organic acid with a chemical formula of $C_6H_8O_7$. Citric acid occurs naturally in citrus fruits It is used as preservative to extend the shelf life and prevent spoilage as well as to enhance the flavour with the slightly sour taste to the food (Dykes, 2014). Citric acid can also used as cleaning and chelating agent (Wikipedia, 2015e).

2.3.4.2 Alkaline pretreatment

Gelatine that was produced from alkaline pretreatment known as a type B gelatine. The conditioning process takes from a few days and up to 6 months (Scherieber and Gareis, 2007; Haug and Draget, 2009) but by agitation, the conditioning process can be speeded up. During the alkaline treatment process, the solution is constantly enriched with dissolved non-collagenous protein and other substances, therefore repeated changes of solution over the months, at first daily and later on a weekly basis should be done. Subsequently, the treated material is washed free of alkali by neutralising it with the addition of acid. Then, serial of washing was done to remove the neutral salts produced during this process. The main alkaline use for gelatine pretreatment was sodium hydroxide. Previous study by Du *et al.* (2013) use a combination of sodium hydroxide and sodium bicarbonate to produce gelatine from turkey and chicken head. The other studies use a combination of alkaline and

acid treatment, for example, sodium hydroxide and hydrochloric acid (Kim H.W. *et al.,* 2012), sodium hydroxide and sulfuric acid (Norizah *et al.*, 2013) and sodium hydroxide and acetic acid (Widyasari & Rawdkuen, 2014).

2.3.4.3 Enzyme pretreatment

The biological preparation of gelatine containing material is a fairly new process. The enzymatic hydrolysis for gelatine production is also of interest since the time of the treatment was shorter than in the chemical process, but the enzyme was expensive (Pitpreecha & Damrongsakkul, 2006). Apart from other methods use for gelatine production, enzymatic extraction is used to produce high purity of gelatines (Rowlands & Burrows, 2000). Previous study to produce gelatine used enzyme protease (Hosseini-Parvar *et al.,* 2009; Zhang *et al.,* 2011) and enzyme papain (Pitpreecha & Damrongsakkul, 2006). The grass carp fish scales were pretreated by 0.22% protease A 2G at 30.73 for 5.52 hours to produce gelatine with 276 g bloom strength (Zhang *et al.,* 2011). Result shown that, the grass carp fish scale gelatine had lower imino acids, but a higher α-chain and β-component contents, which corresponded well to the low melting point (26.9 ◦C). Meanwhile, study by Pitpreecha and Damrongsakkul (2006) use crude extracted enzyme from papaya latex to produce gelatine from raw hide. The bloom strength of gelatine obtain was relatively low, especially at 75 °C, which corresponded to the condition for the highest enzyme activity. According to Pitpreecha and Damrongsakkul (2006), at the condition for the highest enzyme activity, short chain gelatine or low molecular weight gelatine was obtained from the hydrolysis. Alternatively, high molecular weight gelatine could be obtained from the hydrolysis of which the condition corresponded to low enzyme activity. This was because peptide bonds of collagen

were extremely cleaved by enzymatic hydrolysis reaction much more than by acid and alkaline. Another study by Hosseini-Parvar *et al*. (2009) extract gelatine from cattle bone by using 2, 6 and 10 ppm Neutrase solution which is an alkaline protease obtained from Novo Nordisk Co., (Bagsvaerd, Denmark) for 8, 12 and 16 hours at 50 °C. According to Hosseini-Parvar *et al*. (2009), due to different bond breakage during enzymatic gelatine extraction that is a function of pH, enzyme concentration, time of enzymatic treatment and extraction temperature, the extracted gelatine is composed of a distribution of proteins with varying lengths.

2.3.5 Application of gelatine in food products

Gelatine is used primarily in food and according to Benjakul *et al*. (2012), gelatine is widely used in the food industry as ingredients to improve the elasticity, consistency and stability of foods. Usage of gelatine depends on processing, bloom strength, and the desired viscosity. In the market, gelatine is available in a wide range of bloom strength. Too much gelatine in products results in a brittle gel and the gel is not smooth (Rakes & Laaman, 2011). According to Mariod and Adam (2013), gelatine was among the first commercial raw materials that are suitable as a contact preservative for meat and meat products. Gelatine as a hydrocolloid added in food products can be divided into four main groups which is confectionery, dairy and dessert, meat and fish products and refining. Table 2.4 shows several applications of gelatine in a food product based on their function, bloom strength, and viscosity with how much dosage to add on product. Other than that, gelatine is still the best medium used for making photographic emulsions (GMIA, 2012).

Table 2.4 Application of gelatine in food products

	Function	Bloom strength	Viscosity	Dosage on product (%)
Confectionery				
Gelatine gums	-Gelling agent -Texture -Elasticity	180-260	Low-high	6.0-10.0
Chewable sweets	-Aeration -Chewability	100-150	Medium-high	0.5-3.0
Marshmallows	-Aeration -Stabilisation -Gelling agent	200-260	Medium-high	2.0-5.0
Coating	-Film forming -Binding	120-150	Medium-high	0.2-1.0
Dairy and desserts				
Gelatine dessert	-Gelling agent -Texture	180-260	Medium-high	1.5-3.0
Yoghurt	-Prevents syneresis -Texture -Gelling agent	200-250	Medium-high	0.2-1.0
Puddings and creams	-Texture -Thickening /gelling agent	200-240	Medium-high	0.2-2.0
Ice creams	-Texture -Stabilisation	100-200	Low-medium	0.2-1.0
Icing	-Thickening/ gelling agent	220-280	Medium-high	0.5-1.0
Meat and fish				
Canned meat	-Texture	250-280	Medium-high	1.5-3.0
Frozen cooked meat	-Meat binding	200-240	Medium-high	0.5-3.0
Low fat spreads	-Stabilisation of emulsion -Texture feeling in the mouth	130-180	High	0.3-2.0
Wine and juice fining				
	-Clarification	80-120	Low-medium	5-15g/L

(Source: PBGelatins, 2009)