PROPERTIES OF EDIBLE FILMS PREPARED FROM FISH SKIN GELATIN ADDED WITH DIFFERENT FATTY ACIDS AND THEIR SUCROSE ESTERS

by

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Alhamdulillah. Thank God for all the blessings.

This thesis is specially dedicated to my late father, Ahmad Busran. I hope I make him proud.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
ASTM	American Society for Testing and Materials
ATR-FTIR	Attenuated total reflectance-Fourier transform infrared
BSE	Bovine Spongiform Encephalopathy
C	Carbon
CBG	Commercial beef skin gelatin
CHBG	Commercial halal bovine gelatin
CTRL	Control
E	Young's modulus
EAB	Elongation at break
EFG	Extracted fish gelatin
FA	Fatty acid
FASE	Fatty acid sucrose ester
FTIR	Fourier transform infrared
G	Glycerol
GMIA	Gelatin Manufacturers Institute of America
GRAS	Generally Recognized as Safe
HLB	Hydrophilic-lipophilic balance
P	Plasticizer (Percent)
PA	Palmitic acid
PASE	Palmitic acid sucrose ester
SA	Stearic acid

SASE	Stearic acid sucrose ester
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	Scanning Electron Microscopy
SPSS	Statistical Package for the Social Sciences
TS	Tensile strength
WVP	Water vapour permeability
WVTR	Water vapour transmission rate

LIST OF SYMBOLS

%	Percent
^oC	Degree celcius
mg	Milligram
g	Gram
kg	Kilogram
mm	Milimetre
cm	Centimetre
m	Metre
s	Second
min	Minute
h	Hour
µl	Microlitre
ml	Millilitre
V	Volt
kV	Kilovolt
mol	Mole
M	Molar
N	Newton
Pa	Pascals
MPa	Megapascals
mPa.s	Milipascals seconds
kDa	Kilodalton
rpm	Revolutions per minute

w/w	Weight/ weight
w/v	Weight/ volume
v/v	Volume/ volume
O/ W	Oil in water
vs.	Versus
α	Alpha
β	Beta
γ	Gamma
a_w	Water activity
pI	Isoelectric point
T_g	Glass transition temperature

SIFAT-SIFAT FILEM BOLEH MAKAN YANG DIHASILKAN DARIPADA GELATIN KULIT IKAN DITAMBAH DENGAN PELBAGAI ASID LEMAK DAN DERIVATIF ESTER SUKROSANYA

ABSTRAK

Di antara biopolimer-biopolimer yang digunakan untuk menghasilkan filem-filem boleh makan, gelatin telah mendapat banyak perhatian kerana kebolehannya yang baik dalam membentuk filem. Walau bagaimanapun, filem-filem gelatin sangat hidrofilik justeru modifikasi adalah diperlukan untuk mengurangkan kelemahan ini. Dalam kajian ini, gelatin ikan telah diekstrak daripada kulit-kulit ikan Tilapia dan sifat-sifat fiziko-kimianya ditentukan. Hasil gelatin ikan yang diekstrak (EFG) ialah sebanyak 20.2 % dengan kandungan protein iaitu 91.5 %, kelembapan 7.0 %, dan abu 1.3 %. Kekuatan bloom EFG ialah 241.1 g, dianggap tinggi dan juga menunjukkan kehadiran protein yang memiliki berat molekul yang tinggi seperti rantaian-rantaian α dan β dalam corak protein elektroforesis. Spektrum FTIR bagi EFG menunjukkan kumpulan-kumpulan amida (A, B, I, II dan III) dalam lingkungan nombor-nombor gelombang yang sama seperti ditunjukkan oleh gelatin-gelatin komersial daripada sumber lembu. Gelatin ikan yang diekstrak (EFG) kemudian digunakan untuk menghasilkan filem-filem boleh makan berasaskan protein diplastikkan dengan gliserol. Gliserol telah ditambah pada kepekatan, 5, 10 dan 15 % berdasarkan berat gelatin. Penggunaan EFG sebagai matrik biopolimer berjaya menghasilkan filem-filem gelatin yang bebas terbentuk dan stabil. Penambahan gliserol sebagai bahan pemplastik telah mengurangkan kekuatan tensil (TS) dan modulus Young (E) tetapi meningkatkan pemanjangan pada titik putus (EAB) filem-

filem gelatin berbanding filem yang tidak diplastikkan. Walau bagaimanapun, dengan penambahan kandungan gliserol dari 5 ke 15 %, filem-filem yang diplastikkan juga menunjukkan penambahan kebolehtelapan air (WVP) dan kelegapan tetapi pengurangan kelarutan. Justeru bagi menambah baik sifat-sifat filem terutamanya sifat-sifat penyekat air, modifikasi ke atas filem-filem gelatin yang diplastikkan telah dilakukan dengan penambahan asid palmitik (PA) atau asid stearik (SA), atau ester-ester sukrosanya; ester sukrosa asid palmitik (PASE) atau ester sukrosa asid stearik (SASE) sebagai pengganti bagi gliserol pada pelbagai nisbah. Kesan-kesan penambahan asid lemak (FAs) atau ester sukrosa asid lemak (FASEs) tersebut ke atas sifat-sifat fizikal, mekanikal dan morfologikal filem-filem berasaskan gelatin telah dikaji. Penambahan semua FAs atau FASEs tersebut telah berjaya menambah baik sifat-sifat penyekat air dan cahaya filem-filem gelatin dengan cara menambahkan kelegapan dan mengurangkan WVP. Pengurangan WVP terbesar telah dilihat pada tahap 15 % bahan pemplastik, dengan peratusan pengurangan sebanyak 62.5, 60.1, 62.6 dan 68.7 % masing-masing untuk filem-filem yang ditambah dengan PA, SA, PASE dan SASE. Secara keseluruhannya, nilai TS tertinggi telah terlihat pada filem-filem; PA (62.3 MPa), SA (74.8 MPa), PASE (72.6 MPa) dan SASE (68.7 MPa). Nilai E filem-filem yang ditambah dengan FAs atau FASEs juga adalah lebih tinggi berbanding filem-filem yang diplastikkan dengan gliserol sahaja (pada semua kepekatan gliserol). Keputusan tersebut menggambarkan bahawa filem-filem yang lebih kuat/keras telah dihasilkan dengan penambahan FAs atau FASEs. Nilai EAB telah berkurang dengan kenaikan jumlah bahan-bahan hidrofobik dalam filem-filem gelatin, kecuali filem yang ditambah dengan PA pada kepekatan 15 % bahan pemplastik, di mana filem 15P-PA75 telah menunjukkan nilai EAB tertinggi secara signifikan. Kajian-kajian isoterma serapan menunjukkan bahawa filem-filem gelatin menampilkan lengkung-lengkung berbentuk J dengan penambahan penyerapan kelembapan mengikut turutan filem-filem yang ditambah dengan SA < PA < SASE < PASE. Keseluruhannya, filem-filem yang ditambah dengan FAs atau FASEs telah menunjukkan peningkatan sifat-sifat penyekat air dan cahaya dan juga kekuatan filem yang tinggi. Tambahan itu juga, filem-filem yang ditambah dengan FASE menunjukkan WVP yang lebih rendah tetapi kurang fleksibel berbanding filem-filem yang ditambah dengan FA. Kesimpulannya, filem-filem tersebut boleh digunakan sebagai bahan-bahan pembungkus sekunder bersama-sama dengan penggunaan bahan-bahan pembungkus sintetik, dengan potensi-potensi pengaplikasian sebagai bahan-bahan pembungkus berpenyekat air dan cahaya untuk makanan-makanan berkelembapan rendah seperti kekacang, bijirin dan rempah-ratus.

PROPERTIES OF EDIBLE FILMS PREPARED FROM FISH SKIN GELATIN ADDED WITH DIFFERENT FATTY ACIDS AND THEIR SUCROSE ESTERS

ABSTRACT

Among biopolymers used to prepare edible films, gelatin has received much attention for its good film forming ability. However, gelatin films are very hydrophilic thus modification is needed to reduce this drawback. In this study, the fish gelatin was extracted from Tilapia fish skins and its physico-chemical properties determined. The yield of extracted fish gelatin (EFG) was 20.2 % with 91.5 % protein, 7.0 % moisture, and 1.3 % ash contents. The bloom strength of EFG was 241.1 g, considered relatively high and also showed the presence of high molecular weight proteins such as α and β chains in the electrophoresis protein pattern. FTIR spectra of EFG showed amide groups (A, B, I, II and III) in regions of wavenumbers similar to those of commercial gelatins from bovine sources. Extracted fish gelatin (EFG) was then further utilized to produce glycerol plasticized protein based edible films. Glycerol was added at concentrations, 5, 10 and 15 % based on weight of gelatin. The use of EFG as biopolymer matrix successfully formed free-stand and stable gelatin films. Addition of glycerol as plasticizer reduced tensile strength (TS) and Young's modulus (E) but increased elongation at break (EAB) of gelatin films compared to unplasticized gelatin film. However, with increasing amount of glycerol from 5 to 15 %, the plasticized films also exhibited increasing water vapour permeability (WVP) and opacity but decreasing solubility. Thus to improve the film properties especially the water barrier properties, modification on the plasticized gelatin films was carried out by addition of palmitic acid (PA) or stearic acid (SA), or their sucrose esters; palmitic acid sucrose ester (PASE) or stearic acid sucrose ester (SASE) as substitutes for glycerol at various ratios. The effects of adding these fatty acids (FAs) or fatty acid sucrose esters (FASEs) on the physical, mechanical and morphological properties of gelatin based films were investigated. Addition of all these FAs or FASEs successfully improved water and light barrier properties of gelatin films by increasing opacity while decreasing WVP. Greatest WVP reduction was observed at 15 % plasticizer level, with percentage reduction of 62.5, 60.1, 62.6 and 68.7 % for PA, SA, PASE and SASE added films, respectively. In general, highest TS values were observed in films: PA (62.3 MPa), SA (74.8 MPa), PASE (72.6 MPa) and SASE (68.7 MPa). The E values of all FAs and FASEs added films were higher than those of films plasticized with glycerol only (at all glycerol concentrations). These results indicated stronger/stiffer films were formed with addition of the FAs or FASEs. The EAB decreased with increasing amounts of these hydrophobic materials in gelatin films, except for PA added film at 15 % plasticizer concentration, in which 15P-PA75 film showed significantly highest EAB. Sorption isotherm studies showed that gelatin films exhibited J-shape curves with increasing moisture uptake in the order of SA < PA < SASE < PASE added films. Overall, films added with the FAs or FASEs showed improved water and light barrier properties as well as high film strength. In addition, FASE added films exhibited lower WVP but less flexible than FA added films. In conclusion, these films can be used as secondary packagings to complement the use of synthetic packagings, with potential applications as water and light barrier packaging materials for low moisture foods such as nuts, cereals and spices.

CHAPTER 1

INTRODUCTION

1.1 Background

For many years, petroleum based synthetic packagings have been widely used for many purposes, either for industrial purposes or household essentials. These commercially available plastics materials are considered practical for their light weight, strong, cheap and durable as well as easily kept and transported compared to traditional metals, woods, leathers or glasses. Currently, there are at least twenty types of plastics with different grades and varieties (Thompson *et al.*, 2009). Among the most commonly found plastics are high density polyethylene, low density polyethylene, polypropylene, polystyrene, polyvinyl chloride and polyethylene terephthalate (Encinar & González, 2008). Plastics are often added with additives such as fillers and stabilizers to improve their performance, including thermal resistance and material pliability. Plastics are known to have good mechanical handling for the users. However, they have few but very unfortunate disadvantages mainly for their non-biodegradability and non-renewability.

For these reasons, plastics are among the major contributors to municipal solid wastes amounting to about 10 % (Acomb *et al.*, 2014) resulting in serious environmental problems to both water and land area in the world. Among the major problems include huge landfills required, higher sources of litters especially in urbanized area, greater risk of drainage failure, and increase in marine debris (Sharma *et al.*, 2014). These problems are not just affecting technical and economic aspects, but also social being and stability of the ecosystem. Recently, one of the issues that become a public interest is the physical hazard caused by ingestion of plastic fragments by marine animals and wildlife such as turtles, fishes and birds. According to Thompson *et al.* (2009), the amount of plastics used globally is more than 260 million tonnes per year, representing around 8 % of global oil production. Sivan (2011) reported that usage of plastics derived from polyethylene alone has reached about 140 million tonnes per year. In recent years, there is a rapid growing interest in the development and use of biobased packaging materials to prolong the shelf life and improve the food quality besides addressing the environmental issues related to disposal problems of conventional synthetic food packaging materials and the need to develop more ‘ecofriendly’ and biodegradable materials. Thus, biodegradable materials such as biopolymers mixed with plasticizers have received much attention in the development of edible films and coatings which could offer competitive mechanical properties for potential replacement of petroleum based commodity plastics.

Edible films and coatings have long been used in few types of products such as fruits and sausages to protect them from deterioration and quality loss (Debeaufort *et al.*, 2000). According to Fakhouri *et al.* (2009), edible films can be made into three major types which are simple films, multilayer films and composite films. These films can be prepared using various types of biopolymers i.e. proteins, lipids and polysaccharides. Lipids such as carnauba wax, cocoa butter substitutes, shellacs, terpenes, lecithin and fatty acids were reported as suitable biopolymer matrixes (Morillon *et al.*, 2002). Polysaccharides such as sage seed gum (Razavi *et al.*, 2015), sodium alginate, pectin (Galus & Lenart, 2013), locust bean gum, kappa-carrageenan (Martins *et al.*, 2012), methyl cellulose (Jiménez *et al.*, 2010; Pérez-Gago *et al.*, 2005), agar, arabinoxylan, cassava starch (Gutiérrez *et al.*, 2015; Phan The *et al.*, 2009) and tapioca starch (Chillo *et al.*, 2008) were also studied for their potentials to form edible films. Proteins make excellent oxygen, aroma and oil barrier films and coatings at low to intermediate relative humidity. Among proteins that have been studied as biopolymer film formers were isolated hazelnut meal protein (Aydemir *et al*., 2014), shrimp muscle protein (Gómez-Estaca *et al*., 2014), whey protein (Ramos *et al*., 2013; Pérez *et al*., 2011; Ozdemir & Floros, 2008), soy protein isolate (Cho *et al*., 2010; Kokoszka *et al*., 2010; Ou *et al*., 2005), β lactoglobulin (Sothornvit & Krochta, 2001), gelatin (Cao *et al*., 2009; Vanin *et al*., 2005; Sobral *et al*., 2001) and zein (Santosa & Padua, 1999).

Recently, production of edible films made from biopolymers e.g. gelatin derived from low market by-products of food processing industry has received much attention. In fish processing industry, skins, bones, fins, scales and swimbladders are among the major by-products (Shakila *et al*., 2012a). They are considered as wastes and being discarded which may then contribute to environmental pollutions (Hosseini *et al*., 2013). Tilapia fishes alone have been reported as among the most widely cultured fish species with overall production of this species had reach more than 4 million tonnes in 2010, and expected to increase up to 7.3 million tonnes by 2030, globally (Anon, 2014) thus contribute to major waste of fish resources (Niu *et al*., 2013). Since fish skins, the by-products of food processing can be used to produce gelatin, this allow greater utilization of these wastes while at the same time avoiding the ‘grow to throw’ culture of bioresources and promote better product management. Gelatin produced from these sources later could be used as biopolymers to produce edible films which not only provide alternative to synthetic packagings and reduce the reliability on petroleum based products, but also enhance sustainability of the food industry.

Many studies have been done on protein based films including those made of gelatin (Gómez-Guillén *et al.*, 2007; Chambi & Grosso, 2006; Paschoalick *et al.*, 2003; Fairley *et al.*, 1996). Though these films exhibit good mechanical properties, they are found to be hydrophilic and lack of water barrier properties. On the other hand, lipid based films have poor mechanical properties but excellent water barrier properties. On this basis, several attempts to incorporate lipids into protein based films to minimize or overcome these drawbacks have been widely reported. Shellhammer and Krochta (1997) and Gontard *et al.* (1994) used waxes to improve wheat gluten and whey protein based films, respectively. Kim and Ustunol (2001) incorporated butterfat into whey protein isolate based films. Commonly known unsaturated fatty acids such as oleic acid were also studied in zein based films (Lai & Padua, 1998). Essential oils derived from cinnamon, ginger (Atarés *et al.*, 2010) and citrus plants such as lime, lemon, bergamot, and kaffir lime were also studied for their effects on protein based films (Tongnuanchan *et al.*, 2012). Composite films combining fish protein isolate and gelatin as the main biopolymers were also added with essential oils from basil leaf (Arfat *et al.*, 2014). Saturated fatty acids (e.g. stearic acid and palmitic acid) were also studied by few other authors (de la Caba *et al.*, 2011; Taqi *et al.*, 2011; Zahedi *et al.*, 2010; Yoshida & Antunes, 2004; Lai & Padua, 1998) in various types of protein based films. Thus, additional research on protein-lipid composite film formation and properties is needed to improve the performance and reduce the cost of protein based films and coatings as moisture barriers.

Nevertheless, to the best of our knowledge, very little information regarding the use of fatty acid sucrose esters (FASEs) and their effects on the properties of gelatin based films has been reported. Previous studies done on the use of fatty acid sucrose esters in edible films are majority focus on polysaccharide films (Chen *et al*., 2009; Tzoumaki *et al*., 2009; Villalobos *et al*., 2009; Villalobos *et al*., 2006; Phan The *et al*., 2002, Diab *et al*., 2001) and protein films of plant based (Tzoumaki *et al*., 2009; Xu *et al*., 2001; Gontard *et al*., 1994). However, very limited studies on animal protein based films added with FASEs with the exception of the study reported by Jongjareonrak *et al.* (2006). Jongjareonrak *et al.* (2006) reported the use of these hydrophobic materials in gelatin films using high plasticizer concentration (50 % of glycerol) and protein content of 3 % of the film forming solutions. Research on the effects of plasticizer types and amounts may provide opportunities for achieving desired mechanical properties while further improving the barrier properties. Increasing protein interactions (e.g. chain cross-linking) can reduce the water vapour permeability (WVP) by several factors. Thus, in this study, we aimed to investigate the use of lower plasticizer, glycerol concentrations (5, 10 and 15 %), in the development of edible films using gelatin extracted from fish skins.

Gelatin based films were produced by casting method and characterized for their mechanical, water vapour permeability (WVP), solubility, morphological and optical properties. Lipids and glycerol were added as the plasticizers. Two types of fatty acids, palmitic acid and stearic acid (described in Chapter 4) and their sucrose esters, palmitic acid sucrose ester and stearic acid sucrose ester (described in Chapter 5) were added as glycerol substitutes at different levels into the gelatin based films. It was hypothesized that the films added with these fatty acids and their sucrose esters could exhibit good film forming ability (e.g. no cracks, strong but not brittle, and easily handled) and improve the films' water barrier properties. The overall research methodology is given in Figure 1.1. In addition, the influences of carbon lengths, structures and compositions of fatty acids and their sucrose esters on the films' properties were elucidated in this study.

1.2 Research objectives

The general objective of this study is to prepare edible films based on extracted Tilapia fish skin gelatin with good physical and mechanical properties by conducting modification of the films using fatty acids and their sucrose esters. The specific objectives of this study are;

- To extract and characterize gelatin from Tilapia fish (*Oreochromis niloticus*) skins.
- To prepare gelatin based films added with stearic and palmitic fatty acids and their fatty acid sucrose esters as glycerol substitutes at different levels.
- To investigate the physical, mechanical and morphological properties of the modified gelatin based films.

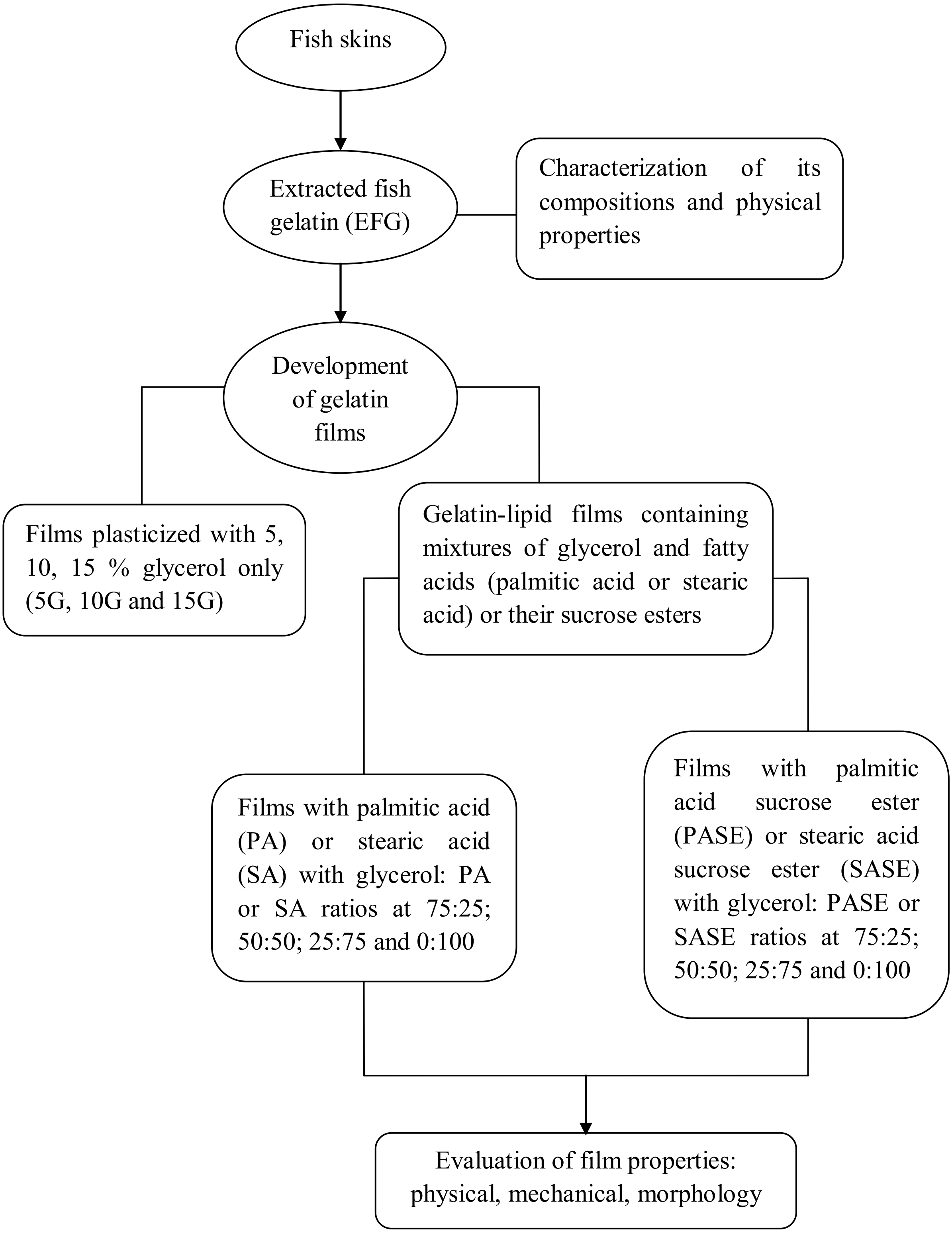

Figure 1.1 Flow chart of overall research methodology.

CHAPTER 2

LITERATURE REVIEW

2.1 Gelatin

Gelatin is not a naturally occurring product. It is very well known as a product of partial hydrolysis of fibrous water insoluble protein, collagen, existed in many connective tissues such as tendons, ligaments, and cartilages (Bourtoom, 2008). Hydrolysis of collagen to produce gelatin can occur under thermal, physical or chemical processes. Production of gelatins usually involves the use of acidic and alkaline solvents during pre-treatment processes, in order to remove the non-collagenous materials and promote swelling of the tissues. Gelatin obtained from the acid treated precursor is known as Type A while gelatin from the alkaline treated precursor is known as Type B. Gelatin is built up of twenty types of amino acids as its natural monomers. Gelatin consists of a repeated sequence of Glycine-X-Y, resembling its parent collagen. Two well known imino acids, proline (Pro) and hydroxyproline (Hyp) are responsible for gelatin's secondary structures. Usually, Pro favors the X position while Hyp favors the Y position (Díaz-Calderón *et al.*, 2014).

During gelatin extraction process, the triple helix structure of its parent collagen will be denatured. However, upon cooling process the structure can be recovered either in full or partial triple helix structure (Bigi *et al.*, 2004; Ross-Murphy, 1997) or in the form of random coils, turns etc. The presence of gelatin can be identified by determining its specific functional groups mainly amides, at different absorbances and wavenumbers, using Fourier transform infrared (FTIR) spectroscopic analysis. Generally, gelatin would exhibit several amide groups including Amide A (around 3300 cm^{-1}), Amide B (around 3100 cm^{-1}), Amide I

(1600-1690 cm^{-1}), Amide II (1480-1575 cm^{-1}), and Amide III (1229-1301 cm^{-1}) (Sjahfirdi *et al.*, 2012). The conversion of collagen to gelatin may result in a mixture of protein chains with varying masses. Among the protein chains involve in the formation of gelatin are α chains, β (dimers of α chain) and γ (trimers of α chain) components, weighing about 90, 180 and 300 kDa, respectively (Rbii *et al.*, 2011). Due to these unique structures, gelatin offers wider functional properties and potential applications amongst all polymers and hydrocolloids. Since many years ago, gelatin has been extensively used as gelling, emulsifying, stabilizing and foaming agent in many food products such as confectionaries, jellies, soups, juices etc. Gelatin has also been incorporated as one of the materials used in cosmetics, drugs and pharmaceutical products (Rivero *et al.*, 2010).

2.1.1 Properties of gelatin

The most incredible property of gelatin is its ability to form thermo reversible gel with a melting point close to human body temperature (Limpisophon *et al.*, 2010; Cao *et al.*, 2009). Gelatins exhibit good film forming properties and good barrier against oxygen and aromas especially at low and intermediate relative humidity (Hosseini *et al.*, 2013). Gelatins are also reported to be effective carriers of bioactive components (Gómez-Guillén *et al.*, 2007). In addition, gelatins also have polyampholytic properties, which act as a base under acidic condition and as an acid under basic condition. Depending on the presence or absence of charged or polar groups, amino acids of gelatins can be hydrophiles or hydrophobes (Zandi *et al.*, 2007).

Gelatin properties are influenced by several factors including the origin of raw materials (Kołodziejska & Piotrowska, 2007), extraction conditions (e.g. pH, temperature and types of acids used) (Bigi *et al.*, 2004), presence of other components, gel maturation time and temperature (Kołodziejska *et al.*, 2004), amino acid compositions, molecular weights and molecular weight distribution (Bougatef *et al.*, 2012). Fish gelatins contain lower amounts of imino acids (proline and hydroxyproline) but higher amounts of serine and threonine (Karim & Bhat, 2009). The imino acid contents of fish gelatins are about 22-25 % for warm water fish and 17 % for cold water fish, whereas the mammalian gelatins contain approximately 30 % of the imino acids (Muyonga *et al.*, 2004a). Due to lower amounts of imino acids, fish gelatins have lower denaturation temperatures than the mammalian gelatins (Grossman & Bergman, 1992). However, cross-linking is higher in animal gelatins than fish gelatins, and also higher in warm water fish gelatins compared to cold water fish gelatins (Pérez-Mateos *et al.*, 2009), responsible by their imino acid contents. In addition, amino acids like proline and hydroxyproline also responsible to gelatins' renaturation levels, which vary with different sources and species (Haug *et al.*, 2004).

A comparative study between cold water fish gelatins and mammalian gelatins by Avena-Bustillos *et al.* (2006) showed that the cold water fish gelatins have lower hydroxyproline, proline, valine and leucine contents, but higher glycine, serine, threonine, aspartic acid, methionine and histidine contents than the mammalian gelatins. However, both cold water fish and mammalian gelatins have comparable amounts of alanine, glutamic acid, cysteine, isoleucine, tyrosine, phenylalanine, homocysteine, hydroxylysine, lysine, and arginine contents (Avena-Bustillos *et al.*, 2006). Another study conducted by Haug *et al.* (2004) also concluded that the main difference between fish gelatins and mammalian gelatins is their imino acid contents, in which lower imino acid contents of fish gelatins are responsible for lower gel modulus, and lower gelling and melting temperatures of fish gelatins. Typically, the gel strengths (indicated by the Bloom values) of fish gelatins range from 0 to 270 g, porcine and bovine gelatins are 200 to 240 g, with some warm water fish gelatins show the values so close to that of mammalian gelatins (Karim & Bhat, 2009). However, the cold water fish gelatins derived from cod, salmon (Arnesen & Gildberg, 2007), Alaska pollock (Zhou *et al*., 2006) and hake (Montero & Gómez-Guillén, 2000) have been reported to show much lower Bloom values, ranging from 70 to 110 g. In respect to the gelling and melting temperatures, fish gelatins exhibit lower values than the mammalian gelatins. Fish gelatins show the gelling temperatures of approximately 8 to 25 ^{o}C, compared to 20 to 25 ^{o}C for porcine and bovine gelatins. The melting temperatures are ranging from 11 to 28 ^{o}C for fish gelatins, whereas the values are from 28 to 31 ^{o}C for both porcine and bovine gelatins (Karim & Bhat, 2009).

When used as biopolymer to produce edible films, the properties of gelatin films especially their mechanical behaviours are closely related to the gelatin sources (Tongnuanchan *et al*., 2012). Films made from warm water fish gelatins are usually exhibit greater mechanical strength than those made from cold water fish gelatins (Weng *et al*., 2014). Other factors such as renaturation levels or triple helix contents of the gelatins may also affect the film properties (Yakimets *et al*., 2005). Gelatin itself has strong cohesiveness between its polymer molecules (Arvanitoyannis *et al*., 1998). Through drying process, three dimensional networks between intermolecular microcrystalline junctions will be formed and may produce brittle films. Thus, plasticizers are commonly added to reduce the brittleness.

2.1.2 Prospect of gelatin production

Gelatin exists abundantly and considered as low cost biopolymer (Acosta *et al.*, 2013; Vanin *et al.*, 2005). Global market of gelatins had worth USD 1.77 billion in 2011 and expected to grow at a Compound Annual Growth Rate (CAGR) of 6.75 % from 2012 to 2018. In terms of production, gelatins were produced around 348.9 kilo tonnes in 2011 globally and expected to reach 450.7 kilo tonnes in 2018 (Anon, 2013). The major sources of gelatins in commercial market are mammalian products which are pig skins (42.12 %), bovine hides (28.67 %) and bovine bones (27.43 %). Only small amount (1.78 %) of gelatins is derived from non-mammalian sources (Anon, 2013). Since the burst of Bovine Spongiform Encephalopathy (BSE) disease, foot-and-mouth disease and some religious restriction mainly among Muslims and Jews, utilization of gelatins based on other sources has gain a lot of attention.

With abundant existence of fish processing by-products such as skins, bones, fins, scales and swimbladders, known to possess considerable amount of connective tissues and good sources of protein, exploration on these materials to produce valuable food components i.e. gelatin has been widen. Among these by-products, fish skins have been found to be the most suitable candidates to produce gelatin due to their structural properties which contain dermis and epidermis layer, with dermis possesses a lot of collagenous components (Yata *et al.*, 2001). Skins from tropical fish species i.e. Tilapia have been reported to produce excellent quality gelatins with less fishy odour and acceptable appearance (Grossman & Bergman, 1992).

2.2 Introduction to edible films

Edible film is defined as a thin layer of material which is safe for consumption and provides a barrier to moisture, oxygen and solute movement for the foods in order to preserve the products' qualities (Nemet *et al.*, 2010; Bourtoom, 2008). The material can be a complete food coating or can be disposed as continuous layer between the food components (Kowalczyk *et al.*, 2014). Commonly, coatings are applied to the foods in solution form while films are first formed into sheets before applied to the foods as wrappers (Falguera *et al.*, 2011).

Just like synthetic packagings, edible films can also improve product's shelf life and quality. These films have offer great potential to partially or totally replace the use of synthetic packagings (Fernández *et al.*, 2007). Edible films can be prepared from polysaccharides, lipid, protein or combination of them (Bruno *et al.*, 2008; Di Pierro *et al.*, 2006; Quezada-Gallo *et al.*, 2000; Pérez-Gago *et al.*, 1999). For these biopolymers, edible films are not just biodegradable and renewable but also consumable and considered non-toxic (Debeaufort *et al.*, 1998). Among the most significant advantage of edible films over synthetic packagings is the films can be consumed together with the packaged foods (Wittaya, 2013) thus help to reduce the disposal problems and recycling costs of the used materials (Navarro-Tarazaga *et al.*, 2008; Viña *et al.*, 2007). In addition, edible films offer greater compatibility with functional additives purposely added into the films to improve their properties (Wittaya, 2013). Edible films have also been reported to exhibit comparable protective effects to the synthetic packagings in evaluation of storage under controlled atmospheres (Park, 1999).

2.3 Preparation methods of edible films

Edible films can be prepared in few ways, depending on properties of the film forming materials, costs, technologies and equipments needed, target applications etc.

2.3.1 Dry process

The dry process is based on thermoplastic properties of the biopolymers at low amount of water. This process includes compression, injection, extrusion, blown extrusion and injection moulding (Andreuccetti *et al.*, 2012). Usually, plasticizers are added into the films before heating above the glass transition temperature (T_g) of the polymers (Dhanapal *et al.*, 2012). Using the dry process, edible films can be formed as separated layers (in bilayer or multilayer films) which able to overlay each other (Quezada-Gallo *et al.*, 2000).

2.3.2 Wet process

The wet process or commonly known as casting involves hydration and dissolution of the biopolymers in solvent (Nur Hanani *et al.*, 2012; Pommet *et al.*, 2005). Casting is preferable since it requires only one drying step thus less cost and less time needed. Casting also allows the use of only a small amount of raw materials with no requirement of special equipments during manufacturing process (Nur Hanani *et al.*, 2012). Moreover, emulsion films prepared using this method can be applied on foods at room temperature and not selective to any surfaces due to combination of both hydrophilic and hydrophobic parts of the films (Quezada-Gallo *et al.*, 2000). Other methods such as dipping, spraying and brushing are also widely reported. However, these methods are commonly applied for forming coating materials for foods such as fruits, vegetables, meat cuts, and poultry products.

2.4 Types of edible films

Edible films are prepared using biomaterials such as hydrocolloids (polysaccharides and proteins) and lipids, either used alone or combined together to form films. These films can be classified as active films, when added with various types of active ingredients to enhance their functional properties.

2.4.1 Polysaccharide based films

Polysaccharide films have excellent barrier against rancidity and surface browning. However, these films have been reported to have poor water barrier, but can be used as sacrifying agents to retard moisture loss (Dhanapal *et al.*, 2012). The use of polysaccharides such as cellulose and its derivatives, alginate, pectin, starches, carrageenan, chitin and chitosan in film making is very well known. Celluloses with thermal gelation properties form gel upon heating, but liquefy upon cooling (Murray, 2000). Agars also exhibit reversible gel which reset upon cooling but melt upon heating (Dhanapal *et al.*, 2012). Film forming ability of starches is due to their amylose contents (Claudia *et al.*, 2005), with higher amylose contents would result in more flexible, heat-sealable, and water soluble as well as greater barrier to gases and oils (Dhanapal *et al.*, 2012). Chitosan is considered as excellent gas barrier. It forms films with good mechanical and antimicrobial properties (Bourtoom, 2008). Alginates derived from seaweeds also form good films, with addition of cationic minerals such as calcium and magnesium as gelling agents (Dhanapal *et al.*, 2012). Other seaweed derived products, carrageenans, also exhibit gelling ability by forming polysaccharide-double helices structure throughout the drying process of the films (Karbowiak *et al.*, 2006).

2.4.2 Protein based films

Protein films have good mechanical strength but tend to be very hydrophilic especially at high relative humidity (Bourtoom, 2008). Proteins are preferable amongst other film formers since they may act as supplement to the foods (Bourtoom, 2009), have pleasant organoleptic properties and provide good mechanical handling (Ma *et al.*, 2012). Many studies have been done on protein films using proteins based on animal or plant sources (Artharn *et al.*, 2009). Protein from rapeseed has been utilized to produce edible films (Jang *et al.*, 2011). Film forming materials using soy based products such as soy protein isolate (Zhang & Mittal, 2010), soy milk, soy flour and fractionated soy protein (Cho & Rhee, 2004) have been studied. The use of canola protein isolate to produce protein films is also reported by Manamperi *et al.* (2011). Films based on wheat gluten can be prepared either using casting technique (Zhang & Mittal, 2010) or thermo pressing technique (Mangavel *et al.*, 2004). Corn zein based films have been studied, but found to be brittle (Kim *et al.*, 2004; Wang *et al.*, 2004). Pea protein is also described as potential film former with its properties are much affected on the processing temperature (Choi & Han, 2002). Gelatins derived from animal tissues are also reported as good film formers but exhibit hydrophilic properties especially at high relative humidity (Limpisophon *et al.*, 2010; Jongjareonrak *et al.*, 2006).

Proteins have been known for their unique structural characteristics contributed by their monomers. Proteins able to form bonds at different positions and thus have many potential functional properties (Wittaya, 2013; Nur Hanani *et al.*,

2012) and film forming ability, depending on various extrinsic and intrinsic conditions e.g. co-polymers, plasticizers, additives and process involved (Vanin *et al.*, 2005). In film making, under heat, alkaline or acidic treatment, proteins undergo partial denaturation of their original structures. Consequently, few possible types of bonds including hydrogen, ionic, hydrophobic and covalent bonds could be formed (Pol *et al.*, 2002) thus affecting the film properties. However, in terms of heat treatment, the effects of heating on film properties i.e. water barrier are seemed to be more complex. For example, based on the fact that denatured proteins will unfold and expose more sulfhydryl and hydrophobic groups, Kowalczyk *et al.* (2014) hypothesized that heating the film forming solutions prior to casting would result in less permeable film. However, they found no effects of heating on water vapour permeability of pea protein films. Meanwhile, a study by Hoque *et al.* (2010) showed that heating the gelatin solutions (40-70 ^{o}C for 30 min) resulted in higher water vapour permeability of the films compared to unheated solutions. Thus, it is believed that other factors such as amino acid compositions, molecular structures and organization as well as polymer interactions are also responsible for film properties.

2.4.3 Lipid based films

Lipids have been widely used to coat fresh fruits, sweets and meat cuts (Bourtoom, 2008). Lipid films have excellent water barrier properties and commonly used to retard moisture migration through foods. However, films based on lipids alone are brittle, thus lipids are combined with other polymers such as polysaccharides and proteins as mechanical supports to reduce the drawback. Lipid based films can be categorized into monolayer, bilayer, multilayer, or emulsion films. Monolayer lipid based films are usually prepared by immersing the support materials into molten lipid to obtain homogeneous lipid layer (Martin-Polo & Voilley, 1990). Bilayer or multilayer lipid based films are prepared by depositing lipid layer onto the pre-formed support films (Debeaufort *et al.*, 2000). Meanwhile, emulsion films require dispersion of the lipid molecules in the continuous support matrix using casting technique (Avena-Bustillos *et al.*, 1997). Among the most commonly used lipids to prepare these lipid based films are waxes, shellac, glycerol monostearate, fatty acids, oils, and cocoa butter (Morillon *et al.*, 2002). Previous studies by other authors have reported waxes as the most resistant to water vapour while lipids with higher unsaturation degree exhibit the least (Fabra *et al.*, 2008; Morillon *et al.*, 2002). However, the use of waxes in food application has been questioned mainly for their effects on human health. In view of that issue, other types of lipids especially fatty acids have been utilized for edible film making (Jiménez *et al.*, 2010).

2.4.4 Composite films

Composite films are formed by combining two or more materials to produce films with superior qualities. Various types of polysaccharides have been combined with lipids in order to reduce the hydrophilic behaviour of polysaccharide films. Methylcellulose-oleic acid emulsion films showed lower water vapour transmission with increasing amounts of oleic acid (Ghanbarzadeh & Almasi, 2011). Rice starch-chitosan films also showed better water barrier when added with oleic acid, palm oil or margarine (Bourtoom & Chinnan, 2009). Unlikely, oleic acid did not significantly affect water vapour permeability of corn starch films, compared to saturated palmitic acid and stearic acid (Jiménez *et al.*, 2012). Wu *et al.* (2003) reported that increasing amounts and carbon lengths of saturated fatty acids resulted in better water barrier of starch-alginate films. Methylcellulose films added with alkanes or triglycerides also showed better water barrier, with properties were affected by crystallinity and hydrophobicity of the lipids (Quezada-Gallo *et al.*, 2000). Galus and Lenart (2013) observed that composite films based on sodium alginate and pectin showed lower water vapour sorption compared to alginate films alone. Rubilar *et al.* (2015) prepared films based on hydroxypropyl methylcellulose and whey protein isolate added with oil. The films obtained showed slightly lower water vapour transmission rate in the presence of oil. However, a study by Hosseini *et al.* (2015) revealed that fish gelatin-chitosan based films added with essential oil showed higher water vapour permeability. Generally, most of these authors concluded that film properties were affected by compatibility and internal arrangement of the blended components.

2.4.5 Active films

Antioxidants may enhance the functional properties of edible films by binding with free radicals to prevent oxidation process (Pokorny, 2007). Antioxidants can be classified as primary or secondary agents due to their different mechanism of actions in the films (Reische *et al.*, 2002). The use of synthetic antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) is very common. However, due to health and safety issues, the use of natural sources has gained much attention. Various types of antioxidants such as plant extracts (Li *et al.*, 2014; Zeng *et al.*, 2013), essential oils (Perdones *et al.*, 2014; Bonilla *et al.*, 2013), α-tocopherol (Jiménez *et al.*, 2013), ascorbic acid (Pérez *et al.*, 2012; Bastos *et al.*, 2009) and citric acid (Robles-Sánchez *et al.*, 2013; Atarés *et al.*, 2011) have been incorporated into edible films and shown to minimize oxidation process. Edible films have also been added with antimicrobial agents to retard food spoilage (Sorrentino *et al.*, 2007). Acetic acid and propionic acid were added into chitosan films to inhibit growth of *Enterobacteriaceae* and *Serratia liquefaciens* (Ouattara *et al.*, 2000). Cagri *et al.* (2002) incorporated *p*-aminobenzoic and sorbic acid into whey protein isolate films to inhibit *Listeria monocytogenes*, *Escherichia coli O157:H7* and *Salmonella enteric*. Essential oils derived from oregano or pimiento were also added into milk protein films and studied for their antimicrobial effects against *E. coli* and *Pseudomonas spp.* in beef muscle slices (Oussalah *et al.*, 2004).

2.5 Properties of edible films

Edible films are designed and applied to provide protection to the food products. Having good mechanical properties and ability to promote easy handling are among the most important characteristics to justify suitable applications of edible films. In addition, compatibility of the edible films with additives purposely added into the films may also enhance their functional properties.

2.5.1 Protective barriers

As a part of food packaging materials, edible films should act as protective barriers against small molecules like water vapour, oxygen and aromas that potentially alter the food components and create undesirable effects (Eça *et al.*, 2014). Among the most challenging requirement for their effectiveness is to minimize water vapour exchange between internal and external environment of the food systems while at the same time maintaining the desired moisture contents of the foods (Coupland *et al.*, 2000). These characteristics are very important in order to prevent loss of texture as well as chemical and enzymatic deterioration of foods (Bourtoom & Chinnan, 2009). Film permeability has always been related to small molecules especially gases and water vapour. High permeability indicates poor barrier properties and vice versa. Permeation occurs through activated diffusion where molecules are transferred through concentration gradients in random molecular motions (Siracusa, 2012). According to Lai and Padua (1998), permeation of films involves three steps mechanisms; (1) absorption of the small molecules into the polymers (2) penetration of the molecules through the polymers to new distinct regions (3) desorption of the molecules from the polymer surfaces, and evaporation or relocation throughout other processes, as illustrated in Figure 2.1.

Penetration or diffusion of the molecules through films is affected by polarity, sizes and shapes of the molecules (Siracusa, 2012) as well as chemical compositions, structures, tortuous path, compatibility (Lai & Padua, 1998), amount of free volume, glass transition temperature (T_g), degree of unsaturation, cross-linking and crystallinity of the film components (George & Thomas, 2001). Other factors such as temperature, humidity, steric effects, plasticizers, fillers, preparation methods and thickness also affect the transport of molecules through films (George & Thomas, 2001). In films involving polymer blends, formation of homogeneous film matrix cannot simply restrict transport of molecules. Penetration of molecules through homogeneous films is more affected by molecular interaction between the film components, compared to other factors such as interfacial behaviour and physical states of the polymers (i.e. glassy or rubbery) as observed in heterogeneous films. To explain film permeability, two types of models; microvoid model and micropathway model have been introduced by Ukai *et al.* (1976) and Krochta (1990), respectively. Microvoid model proposes that mass transfer occurs through microvoids which are developed during drying process and exist between hydrophobic and hydrocolloid matrix. However, micropathway model suggests that mass transfer occurs through hydrocolloid matrix itself, due to its high susceptibility to vapours.

Apart from minimizing mass transfer across the food systems, edible films may also enhance the appearance and glossiness of the food surfaces. Usually, films with high transparency are desirable for application as superficial food coatings (Gontard *et al*., 1994). However, low transparency or opaque films are somehow important in relation to maintaining the qualities of the food components such as preventing the light oxidation. Generally, light barrier properties of edible films are influenced by rearrangement of the film components during drying process (Villalobos *et al*., 2005), interaction between the film components (Valenzuela *et al*., 2013; Gontard *et al*., 1994), nature of crystallization (Quezada-Gallo *et al*., 2000) and alteration of the refractive index across the films (Valenzuela *et al*., 2013; Jiménez *et al*., 2012).

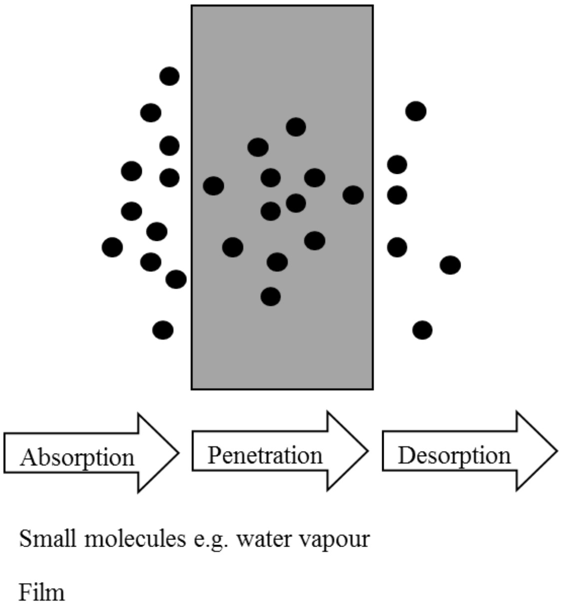

Figure 2.1 Mechanism of transport of molecules through films.

2.5.2 Mechanical supports

Edible films have been reported to promote better workability and easy handling of foods. Thus, mechanical properties of edible films themselves are very important to estimate their potential protective layers towards physical damage during transportation and storage of foods. Since edible films are prepared from biopolymers, their mechanical properties actually resemble the polymers' behaviours. Mechanical properties of a polymer are correlated with its five basic molecular properties which are molecular weight, van der Waals volume, glass transition temperature (T_g), cohesiveness, angle and distance of the individual units (Seitz, 1993). When polymers are used to form edible films, these molecular properties also impart the properties of the films. Among the most common parameters used to evaluate the mechanical properties of films are tensile strength, Young's modulus and elongation at break which can be obtained from a graph of stress vs. strain of the samples. Tensile strength measures the amount of stress to break the samples, Young's modulus corresponds to the slope of stress vs. strain curve, and elongation at break measures the amount of strain on the samples when they break (Carraher, 1996).

2.5.3 Carriers of functional ingredients

Edible films can be used as carriers for functional food ingredients such as flavouring agents, colorants, spices, probiotics and nutraceuticals (Martín-Belloso *et al.*, 2009). Active compounds such as antioxidants or antimicrobial agents are also purposely added into edible films to extend the shelf life and preserve the qualities of fresh or processed foods. In order to secure their functionality, edible films must have considerable release mechanisms for these ingredients. Thus, film solubility can be an important indicator for functionality of edible films. Good solubility reflects high edibility of biopolymer films. However, too high solubility could contribute to harder handling of the films especially when in direct contact with foods. Films could easily lose their structures and thus their protection ability. The mechanisms of films' dissolution made from biopolymers are affected by the polymers, involving two main processes which are solvent diffusion and chain disentanglement (Miller-Chou & Koenig, 2003). It is believed that when the polymers are compatible with the solvents, the solvents will diffuse into the polymer and cause swollen layers or cracks that promote polymer solubility in the solvents. As for the emulsion films, the structure, homogeneity, and hydrophobicity of the films also impart solubility of the films (Wu *et al.*, 2003).

2.6 Modifications of edible films

Properties of edible films are closely related to their film components (Bourtoom & Chinnan, 2009). Among the most important characteristic of edible films for feasible applications is to provide excellent water barrier, which is the attribute of lipid films. However, application of lipid films is restricted by their poor mechanical properties (brittle and easily form cracks). In contrast, protein and polysaccharide based films have poor water barrier but better mechanical properties than lipid films. Thus, modifications of these films are needed to improve their properties for wider applications. During film preparation, plasticizers are always added into the film forming solution to improve mechanical properties of the final products. In addition, modifications of edible films using chemical methods, enzymatic methods, radiation, or combination with more hydrophobic materials are also practiced, in order to improve their workability, handling, and barrier properties.