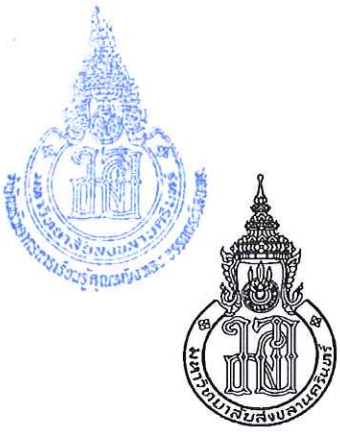

Development of Microfluidized Emulsion Film Based on Gelatin and Palm Oil

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Technology

Prince of Songkla University

2016

Thesis Title	Development of Microfluidized Emulsion Film Based on Gelatin and Palm Oil
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ชื่อวิทยานิพนธ์ การพัฒนาฟิล์มอิมัลชันจากเจลาตินผสมน้ำมันปาล์มโดยกระบวนการไมโครฟลูอิดไดเซชัน

ผู้เขียน นายกฤษณะ นิลสุวรรณ

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บทคัดย่อ

โดยทั่วไปฟิล์มเจลาตินมีสมบัติความต้านทานไอน้ำต่ำ การเติมสารที่มีสมบัติไม่ชอบน้ำ เช่น น้ำมันปาล์มที่พบมากและหาง่ายในประเทศไทย เป็นวิธีหนึ่งที่สามารถลดการซึมผ่านไอน้ำ เพื่อให้เกิดการกระจายหยดน้ำมันในอิมัลชันที่ใช้เตรียมฟิล์ม (FFD) จึงมีความจำเป็นในการใช้สารลดแรงตึงผิวร่วมกับการโฮโมจิไนซ์ที่เหมาะสม จากการศึกษาผลของปริมาณน้ำมันปาล์ม (ร้อยละ 25, 50, 75 และ 100 ต่อปริมาณโปรตีน) และชนิดของสารลดแรงตึงผิว (Tween-20 และเลซิตินจากถั่วเหลือง) ในสภาวะที่เติมหรือไม่เติมกลีเซอรอล (ร้อยละ 30 ต่อปริมาณโปรตีน) ต่อสมบัติของ FFD และฟิล์มเจลาติน พบว่าขนาดหยดน้ำมันมวลรวม (d_{32} และ d_{43}) ของ FFD ที่ประกอบด้วยเลซิตินจากถั่วเหลือง มีค่าใกล้เคียงกัน โดยไม่ขึ้นกับปริมาณน้ำมันปาล์มที่ใช้ ($p > 0.05$) ขนาดหยดน้ำมันมวลรวม (d_{32} และ d_{43}) ของ FFD มีค่าเพิ่มขึ้นเล็กน้อยภายหลัง 12 ชั่วโมง ของการเก็บรักษา การเติมน้ำมันปาล์มในปริมาณเพิ่มขึ้นส่งผลให้ฟิล์มมีค่าการซึมผ่านไอน้ำ (WVP) และค่าความต้านทานแรงดึง (TS) มีค่าต่ำลง แต่มีค่าระยะยืดเมื่อขาด (EAB) เพิ่มขึ้น โดยไม่ขึ้นกับกลีเซอรอลและสารลดแรงตึงผิว ($p < 0.05$) ฟิล์มที่ไม่เติมกลีเซอรอลมีค่า WVP และค่า EAB ต่ำกว่า แต่มีค่า TS สูงกว่าฟิล์มที่เติมกลีเซอรอล ($p < 0.05$) เมื่อเปรียบเทียบค่า WVP และสมบัติเชิงกลของฟิล์มที่ประกอบด้วยสารลดแรงตึงผิวทั้งสองชนิดพบว่าไม่มีความแตกต่างกัน ($p > 0.05$) การเติมน้ำมันปาล์มร้อยละ 50 และ 75 โดยใช้เลซิตินจากถั่วเหลืองเป็นสารลดแรงตึงผิวในสภาวะที่มีกลีเซอรอลสามารถเพิ่มการกระจายตัวเป็นเนื้อเดียวกันและเพิ่มความคงตัวของหยดน้ำมันใน FFD และสามารถปรับปรุงสมบัติการต้านทานไอน้ำของฟิล์มเจลาตินโดยไม่มีผลเปลี่ยนแปลงสมบัติเชิงกล

เมื่อเตรียมฟิล์มเจลาตินด้วยเลซิตินจากถั่วเหลืองและระดับน้ำมันปาล์มที่คัดเลือก (ร้อยละ 50 และ 75 ตามลำดับ) ในสภาวะที่มีกลีเซอรอลระดับต่างๆ (ร้อยละ 0, 10, 20 และ 30 ต่อปริมาณโปรตีน) พบว่าการเพิ่มระดับของกลีเซอรอลส่งผลให้ฟิล์มมีค่า WVP ค่า EAB และค่าการส่องผ่านแสงเพิ่มขึ้น แต่มีค่า TS ลดลง ($p < 0.05$) การเพิ่มระดับของน้ำมันปาล์มส่งผลให้ฟิล์มมีค่า L^* และ a^* ลดลง แต่ค่า b^* และ ΔE^* เพิ่มขึ้น ($p < 0.05$) จากสเปกตรัมอินฟราเรด แสดงให้เห็นว่าอันตรกิริยาระหว่างโปรตีนในโครงสร้างของฟิล์มลดลงเมื่อมีการเติมน้ำมันปาล์ม ฟิล์มที่เติมน้ำมันปาล์มมีอุณหภูมิ

การเปลี่ยนสถานะคล้ายแก้วและอุณหภูมิการเสื่อมสภาพโดยความร้อนต่ำกว่าฟิล์มชุดควบคุม การเติมน้ำมันปาล์มร้อยละ 75 และกลีเซอรอลร้อยละ 10 สามารถปรับปรุงสมบัติความต้านทานไอน้ำของฟิล์มเจลาตินจากปลาโดยไม่มีผลเปลี่ยนแปลงสมบัติเชิงกล

เมื่อนำ FFD (โปรตีนร้อยละ 3.5 น้ำมันปาล์มร้อยละ 75 และกลีเซอรอลร้อยละ 10) ที่เติมเลซิตินจากถั่วเหลือง ปริมาณต่างๆ (ร้อยละ 50 และ 75 ของปริมาณน้ำมันปาล์ม) มาทำการไมโครฟลูอิดไดซ์ด้วยแรงดันระดับต่างๆ (1, 2 และ 3 ปอนด์ต่อตารางนิ้ว) และจำนวนรอบต่างๆ (2 และ 4 รอบ) พบว่า FFD ที่เติมเลซิตินจากถั่วเหลืองร้อยละ 50 มีหยดน้ำมันขนาดเล็กและมีความคงตัวสูงระหว่างการเก็บรักษา ฟิล์มที่เติมเลซิตินจากถั่วเหลืองร้อยละ 50 มีค่า WVP ต่ำกว่า รวมถึงมีค่า TS และ EAB สูงกว่าฟิล์มที่เติมเลซิตินจากถั่วเหลืองร้อยละ 75 ($p < 0.05$) ฟิล์มจาก FFD ที่ไมโครฟลูอิดไดซ์ด้วยแรงดัน 3 ปอนด์ต่อตารางนิ้ว จำนวน 2 รอบ มีค่า WVP ต่ำสุดและ TS และ EAB สูงสุด การเพิ่มปริมาณเลซิตินจากถั่วเหลือง ส่งผลให้ฟิล์มมีค่า L^* และค่าการส่องผ่านแสงลดลง ในขณะที่ค่า b^* และ ΔE^* เพิ่มขึ้น ($p < 0.05$) ฟิล์มจาก FFD ที่ผ่านกระบวนการไมโครฟลูอิดไดเซชันมีผิวหน้าและโครงสร้างภาคตัดขวางเรียบ ฟิล์มจาก FFD ที่เติมเลซิตินจากถั่วเหลืองร้อยละ 50 และผ่านกระบวนการไมโครฟลูอิดไดเซชัน มีความคงตัวต่อความร้อนสูง ดังนั้นสมบัติของฟิล์มเจลาตินอิมัลชันสามารถปรับปรุงโดยการใช้เลซิตินจากถั่วเหลืองร้อยละ 50 เป็นสารลดแรงตึงผิวร่วมกับกระบวนการไมโครฟลูอิดไดเซชัน

จากการศึกษาการใช้ฟิล์มเจลาตินจากหนังปลาที่ไม่เติม (GF) และเติมน้ำมันปาล์ม (EF) สำหรับการเก็บรักษาข้าวเกรียบกุ้งทอดเปรียบเทียบกับฟิล์มไนลอน/พอลิเอธิลีนความหนาแน่นต่ำเชิงเส้น (Nylon/LLDPE) พบว่าตลอดระยะเวลาการเก็บรักษาที่อุณหภูมิ 28 ± 0.5 องศาเซลเซียส ความชื้นสัมพัทธ์เท่ากับร้อยละ 65 ± 5 ปริมาณความชื้นและค่าแอคติวิตี้ของน้ำ (a_w) ของข้าวเกรียบกุ้งที่เก็บรักษาด้วยฟิล์มทุกชนิดเพิ่มขึ้น ($p < 0.05$) ตัวอย่างที่เก็บรักษาด้วยฟิล์ม Nylon/LLDPE มีปริมาณความชื้นและ a_w ต่ำสุด ($p < 0.05$) โดยทั่วไปตัวอย่างที่เก็บรักษาด้วยฟิล์ม EF มีปริมาณความชื้นต่ำกว่า ($p < 0.05$) ตัวอย่างที่เก็บรักษาด้วยฟิล์ม GF ในช่วง 12 วันแรก ของการเก็บรักษาในระหว่าง 15 วัน ของการเก็บรักษาพบว่าข้าวเกรียบกุ้งที่เก็บรักษาด้วยฟิล์ม Nylon/LLDPE มีค่า PV และ TBARS ตลอดจนปริมาณสารระเหย ยกเว้น n-nonanal ต่ำกว่าข้าวเกรียบกุ้งที่เก็บรักษาด้วยฟิล์ม GF และ EF ตัวอย่างข้าวเกรียบกุ้งทั้งหมดมีค่าความกรอบ (crispiness) ต่ำลง แต่ค่าความเหนียว (toughness) เพิ่มขึ้นระหว่าง 15 วัน ของการเก็บรักษา อย่างไรก็ตามตัวอย่างที่เก็บรักษาด้วยฟิล์ม Nylon/LLDPE มีการเปลี่ยนแปลงเล็กน้อย โดยรวมฟิล์มเจลาตินมีสมบัติการป้องกันก๊าซ

ออกซิเจนอย่างดีเยี่ยม การเติมน้ำมันในฟิล์มเจลาตินสามารถลดค่า WVP แต่เพิ่มค่าการซึมผ่านของก๊าซออกซิเจน ดังนั้นจึงยังมีความจำเป็นที่จะต้องปรับปรุงสมบัติการขวางกั้นของฟิล์มเจลาตินต่อไป

Thesis Title Development of Microfluidized Emulsion Film Based on Gelatin and Palm Oil

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Major Program Food Science and Technology

Academic Year 2015

ABSTRACT

Gelatin films generally exhibit the poor water vapor barrier property. The use of hydrophobic substances such as palm oil, which is abundant and available in Thailand, can be a means to lower water vapor migration. To disperse oil in film-forming dispersion (FFD), surfactant along with appropriate homogenization is required. The influences of different palm oil levels (25, 50, 75 and 100%, based on protein) and surfactant types (Tween-20 and Soy lecithin) in the absence or presence of glycerol (30%, based on protein) on properties of FFD and gelatin films were investigated. Similar oil droplet size (d_{32} and d_{43}) of FFD containing soy lecithin were observed, regardless of palm oil levels used ($p > 0.05$). After 12 h of storage, the slight increases in d_{32} and d_{43} were noticeable in all FFD samples. Lower water vapor permeability (WVP) and tensile strength (TS) but higher elongation at break (EAB) were obtained as palm oil levels increased ($p < 0.05$), regardless of glycerol and surfactant used. Films without glycerol had lower WVP and EAB with higher TS than those containing 30% glycerol ($p < 0.05$). No differences in WVP and mechanical properties were found between films containing both surfactants ($p > 0.05$). The incorporation of 50 or 75% palm oil using soy lecithin as a surfactant in the presence of 30% glycerol could increase homogeneity and stability of oil droplets in FFD and also improved water vapor barrier property of gelatin films without marked changes in mechanical properties.

When soy lecithin and the selected palm oil levels (50 and 75% , respectively) with different levels of glycerol (0, 10, 20 and 30%, w/v) were used for preparation of fish gelatin film, the increases in WVP, EAB and light transmittance along with decrease in TS were noticeable when levels of glycerol were increased ($p < 0.05$). Decreases in L^*- and a^*-values with coincidental increases in b^*- and ΔE^*-

values were observed in emulsified films when amount of palm oil incorporated increased ($p < 0.05$). Fourier transforms infrared (FTIR) spectra suggested that the protein-protein interaction in film matrix was decreased when palm oil was incorporated. Films added with palm oil had lower glass transition and degradation temperatures than control films. The addition of 75% palm oil and 10% glycerol could improve water vapor barrier property of fish skin gelatin films without the drastic alteration of mechanical properties.

As FFD (3.5% protein, 75% palm oil and 10% glycerol) containing different levels of soy lecithin (50 and 75%, based on palm oil) were microfluidized with different pressures (1, 2 and 3 kpsi) and pass numbers (2 and 4 passes), the microfluidized FFD containing 50% soy lecithin showed the smaller oil droplet size and the emulsion was more stable during storage. Films containing 50% soy lecithin had lower WVP with higher TS and EAB than those containing 75% soy lecithin ($p < 0.05$). The lowest WVP and highest TS and EAB were found for films from FFD microfluidized at 3 kpsi for 2 passes. Decreases in L^* value and light transmittance with coincidental increases in b^*- and ΔE^* values were observed in films when the amount of soy lecithin incorporated increased ($p < 0.05$). The smooth surface and compact cross-section were observed in films from microfluidized FFD. Film from microfluidized FFD containing 50% soy lecithin showed higher thermal stability. Thus, the emulsion gelatin film with the improved properties could be prepared from FFD using 50% soy lecithin as a surfactant with the aid of microfluidization.

When the fried shrimp cracker was packaged with fish gelatin film incorporated without (GF) and with palm oil (EF), in comparison with nylon/linear low-density polyethylene (Nylon/LLDPE), the moisture content and water activity of shrimp cracker packaged with all films increased during storage ($p < 0.05$). The lowest moisture content and water activity were found in the sample packaged with Nylon/LLDPE film throughout the storage ($p < 0.05$). Sample packaged with EF generally had lower moisture content than that covered with GF during the first 12 days of storage ($p < 0.05$). During 15 days of storage, shrimp cracker packaged with Nylon/LLDPE film generally had the lower PV and TBARS value as well as volatile compounds, except for n-nonanal, than those stored in fish gelatin films, regardless of

oil incorporation. The decreases in crispiness and increases in the toughness occurred for all samples during 15 days of storage. Nevertheless, the lower changes were observed in the sample packaged with Nylon/LLDPE film. Overall, gelatin film showed the excellent oxygen barrier property. The incorporation of oil into gelatin film could lower WVP but negatively increased oxygen permeability of resulting film. Thus, the further improvement of barrier properties of gelatin film is still required.

ACKNOWLEDGEMENT

Firstly, I would like to express my deep appreciation and sincere gratitude to my advisor, Prof. Dr. Soottawat Benjakul of the Department of Food Technology, Faculty of Agro-industry, Prince of Songkla University, for his initiation, kindness, mentorship and excellent guidance during my study. I feel very honor to work under his supervision.

I gratefully acknowledge my co-advisor, Asst. Prof. Dr. Thummanoon Prodpran of the Department of Material Product Technology, Faculty of Agro-industry, Prince of Songkla University, who has contributed to the improvement of my science knowledge and research capability. He has provided recommendation and encouragement throughout my graduate program.

I am also very grateful to my examining committee, Asst. Prof. Dr. Manee Vittayanont of the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University and Assoc. Prof. Dr. Rungsinee Sothornvit of the Department of Food Engineering, Faculty of Engineering at Kamphaengsaen, Kasetsart University, for the valuable time devoted to my thesis and their kindness and helpful suggestions.

I would like to express my deepest appreciation to my beloved family for their love, unrelenting support and inspiration to move always on the right direction, which helped my dreams come true.

I would like to express my special appreciation and thanks to all Thai and International friends of Seafood Chemistry and Biochemistry Laboratory (2205) for their kindness, unfailing support and invaluable suggestions.

Finally, this study could not be successful without the financial support from the Graduate School, Prince of Songkla University.

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CHAPTER 1
INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Packaging materials are widely used to protect the product from surroundings, retard deterioration, extend shelf-life, maintain the quality of foods, and distribute foods and products conveniently. Petroleum-based materials are commonly used and have many advantages. However, these materials are not biodegraded easily and generate a global pollution (Zhang and Mittal, 2010). Biopolymer films have gained interest in their use as edible food packaging. Edible films can be defined as thin continuous layer of biopolymer materials which can be applied as a coating on food, used as a wrap or made into pouch to hold the food or to protect it against external factors (Kester and Fennema, 1986; Krochta, 1997a). Proteins have been extensively used for the development of biodegradable films due to their abundance and good film-forming ability. Proteins are heteropolymers containing a variety of amino acids, which can undergo a wide range of interactions and chemical reactions (Stevens, 1990). Among the proteins, gelatin possesses an excellent film-forming property, and is one of the first materials applied to edible coatings and films (McHugh and Krochta, 1994b). Physical and structural properties of gelatin are mainly influenced by the molecular weight distribution and amino acid composition that play a vital role in the rheological and barrier properties of the resulting films (Gómez-Estaca *et al.*, 2009b). Gelatin yields transparent, colorless and highly extensible films (Núñez-Flores *et al.*, 2012). Nevertheless, the gelatin films present a poor water resistance and are almost completely soluble in water (Sobral *et al.*, 2001). This could limit their applications in food products with high moisture content, because the films may swell, partially dissolve or disintegrate upon contact with the wet surface (Núñez-Flores *et al.*, 2012). The incorporation of hydrophobic substances such as lipid, fatty acid and wax has been implemented to improve water barrier property of gelatin film (Limpisophon *et al.*, 2010; Prodpran *et al.*, 2007; Soazo *et al.*, 2011). Those substances are not dissolved in aqueous phase, in which film-forming dispersion or emulsion must be prepared before making the films. The homogeneity of oil droplets and stability of emulsion in the film-

forming dispersion (FFD) play a significant role in properties of emulsion-based films. Small particles and high emulsion stability during the film drying give rise to a homogeneous distribution of the lipid particles in the film, which in turn contributes to the efficient control of water vapor migration (Debeaufort *et al.*, 1993).

To disperse oil in film-forming aqueous phase, surfactant along with appropriate homogenization is required. Surfactants or emulsifiers are a substance that reduces surface tension between oil and water, thereby enhancing emulsification and increasing emulsion stability (Dalgleish, 1997). Tween-20 and soy lecithin have been reported as the effective surfactants for stabilization of protein-based emulsion films (Prodpran *et al.*, 2007; Tongnuanchan *et al.*, 2014). Furthermore, various homogenization techniques have been used to reduce lipid droplets in the film-forming emulsion (Urban *et al.*, 2006). Microfluidization is one of potential homogenization techniques to obtain fine and more uniform oil droplet dispersion as well as more emulsion stability, compared to typical homogenization. Along with appropriate use of surfactant, microfluidization under proper condition may provoke the stable state of two-phase emulsion system with homogeneity of oil droplet distribution in the film-forming dispersion and the emulsion film. This may enhance the properties of emulsion-based film especially the water vapor barrier property.

Palm oil is the natural hydrophobic substance obtained from the mesocarp of palm seed. Throughout the world, 90% of palm oil is used for edible purposes (e.g., margarine, deep fat frying, shortening, ice cream, cocoa butter substitutes in chocolate); the remaining 10% is used for soap and oleochemical manufacturing (fatty acids, methyl esters, fatty nitrogenous derivatives, surfactants and detergents) (Edem, 2002). Due to its abundance and cheap price, palm oil could be used as promising hydrophobic substance to improve water vapor barrier property of gelatin film.

As a consequence, smart films with varying property can be produced, especially for shelf-life extension of foods. Consumer demands for more natural foods and environmental protection lead to the development of new packaging materials. Biodegradable and edible packaging is emerging for food industry. Packaging from film incorporated with natural compound, e.g. palm oil is able to retard deterioration caused by chemical reaction, especially lipid oxidation. Film be also able to protect the

moisture adsorption of dried food products. Therefore, foods can be stored for an extended time. Gelatin from fish processing byproduct also can be better used, thereby increasing the value of byproducts from fish processing industry.

1.2 Review of literature

1.2.1 Biodegradable and edible film

1.2.1.1 Characteristics of biodegradable and edible film

Packaging materials are widely used to protect the product from surroundings, retard food product deterioration, extend shelf-life, keep the quality of foods, and distribute food conveniently. Petroleum-based materials are commonly used and have many advantages. However, these materials are not easy to biodegrade and generate a global pollution (Zhang and Mittal, 2010). Thus, biodegradable film has been an alternative packaging to those synthetic packaging material (Kester and Fennema, 1986). Biodegradable or compostable packaging is preferable to recyclable packaging because recyclable packaging, though better than non-recyclable packaging, still requires external energy to be provided to bring about the recycling process. Nevertheless, biodegradable or compostable packaging is difficult to be recycled (Guilbert *et al.*, 1997).

Due to the convenience, edible packaging has gained attention. Edible packaging material must meet requirements related with their transport properties (mainly water vapor, carbon dioxide and oxygen permeabilities), mechanical properties (especially their resistance to stretching and rupture), optical properties (mainly related with their opacity and color) and flavor (in most cases, flavorless coatings are needed) (Cerqueira *et al.*, 2011). Edible films can be used to extend the shelf-life by reducing moisture and solute migration, gas exchange, respiration and oxidative reaction rates (Han and Gennadios, 2005). The most beneficial characteristics of edible films are their edibility and inherent biodegradability (Guilbert *et al.*, 1996; Krochta and De Mulder-Johnston, 1997). To maintain their edibility, all film components (i.e., biopolymers, plasticizers, and other additives) should be food-grade ingredients, and all process facilities and equipment should be acceptable for food processing (Guilbert *et al.*,

1996). With regard to biodegradability, all components should be biodegradable and environmentally safe (Witt *et al.*, 1996).

1.2.1.2 Protein-based films

Proteins are heteropolymers containing different 20 amino acids. They are macromolecules with specific amino acid sequences and there are limitless number of sequential arrangements with a wide range of interactions and chemical reactions (Pommet *et al.*, 2003; Stevens, 1990). All structures of proteins can be easily modified by heat, pressure, irradiation, mechanical treatment, acids, alkalines, metal ions, salts, chemical hydrolysis, enzymatic treatment and chemical cross-linking (Han and Gennadios, 2005; Krochta and De Mulder-Johnston, 1997). The most distinctive characteristics of proteins, compared to other film-forming materials, are conformational denaturation, electrostatic charges, and amphiphilic nature. Many factors can affect the conformation, charge density and hydrophilic-hydrophobic balance of proteins, thereby influencing the physical and mechanical properties of prepared films and coatings (Gontard *et al.*, 1996; Park *et al.*, 1996). In addition, properties of protein-based films depend on various factors such as the source of protein, pH of protein solution, heating temperature, plasticizers, film thickness, preparation conditions, formation process and additives incorporated into the film-forming solutions (Benjakul *et al.*, 2008; Paulo *et al.*, 2005).

Protein-based films show the impressive gas barrier properties and mechanical properties, compared with those prepared from polysaccharides and fat-based films (Cao *et al.*, 2007). However, the poor water vapor resistance limits their application. Improvement of protein film properties could be attained by modifying the properties of protein by chemical and enzymatic methods (Kester and Fennema, 1986; Krochta, 1997a). Additionally, several approaches such as enzymatic modifications (De Carvalho and Grosso, 2004; Staroszczyk *et al.*, 2012), incorporation of appropriate plasticizers (Vanin *et al.*, 2005) and hydrophobic materials (Limpan *et al.*, 2010; Prodpran *et al.*, 2007), etc. have been implemented.

1.2.1.3 Plasticizers

Plasticizers are required for edible film, especially for protein-based films. Protein films are often brittle and stiff due to extensive interactions between polymer molecules (Krochta and De Mulder-Johnston, 1997). Plasticizers are low molecular weight agents incorporated into the polymeric film-forming materials, which decrease the glass transition temperature of the polymers. They are able to position themselves between polymer molecules and to interfere with the polymer-polymer interaction to increase flexibility and process ability (Guilbert *et al.*, 1995; Krochta, 2002). Plasticizers increase the free volume of polymer structures or the molecular mobility of polymer molecules (Sothornvit and Krochta, 2000). Plasticizers decrease the ratio of crystalline region to the amorphous region and lower the glass transition temperature (Guilbert *et al.*, 1997; Krochta, 2002). The addition of plasticizers affects not only the elastic modulus and other mechanical properties, but also the resistance of edible films and coatings to permeation of vapors and gases (Sothornvit and Krochta, 2000; Sothornvit and Krochta, 2001). Most plasticizers are very hydrophilic and hygroscopic. Therefore, they can attract water molecules and form a large hydrodynamic plasticizer-water complex. For protein and polysaccharide edible films, plasticizers disrupt inter- and intra-molecular hydrogen bonds, increase the distance between polymer molecules, and reduce the proportion of crystalline to amorphous region (Krochta, 2002). Water molecules in the films function as plasticizers. Water is actually a very good plasticizer, but it can easily be lost by dehydration at a low relative humidity (Guilbert *et al.*, 1995). Therefore, the addition of hydrophilic chemical plasticizers to films can reduce water loss through dehydration, increase the amount of bound water, and maintain a high water activity. Sothornvit and Krochta (2001) suggested that several factors affect plasticizing efficiency of plasticizers, including size and shape of plasticizer molecules, number of oxygen atoms and their spatial distance within the structure of the plasticizers, and water-binding capacity. Besides the effect of hydrogen bonding, repulsive forces between molecules of the same charge or between polar and non-polar polymers can increase the distance between polymers, thus achieving the function of plasticization in the case of charged polymeric film structures (Sothornvit and Krochta, 2001). Therefore, compared to neutral polymer

films (e.g. starch films), the flexibility of charged polymer films (e.g. soy protein, carboxymethyl cellulose or alginate films) may be affected more significantly by altering pH and salt addition at the same water activity level (Sothornvit and Krochta, 2001). Several plasticizers with different properties have been used in protein-based films. Types and levels of plasticizers directly determine the properties of films. Jongjareonrak *et al.* (2006a) reported that both sorbital and mannitol are hexabasic alcohols, and they are isomeric compounds. Sorbitol had been extensively used as plasticizer for gelatin-based films. Tensile strength (TS) and elongation at break (EAB) of gelatin-starch blended films plasticized with sorbitol were higher than those of films plasticized with glycerol (Fakhoury *et al.*, 2012). Plasticizer causes no apparent tendency to re-crystallization in the film structure, but alter other physical properties, such as flexibility, interactions between the macromolecule chains and susceptibility to humidity (Bergo and Sobral, 2007). Films containing glycerol presented greater water absorption in comparison to films containing ethylene glycol, diethylene glycol and polypropylene glycol, which was attributed to the higher hydrophilic nature of the glycerol (Bergo *et al.*, 2013; Vanin *et al.*, 2005). It was found that the plasticizer concentration has more prominent effects than the plasticizer type (Bergo *et al.*, 2013). Rivero *et al.* (2010) prepared gelatin films using glycerol as plasticizer (0–100% based on protein mass) and established the relationships between glycerol content and film properties. WVP exhibited a minimum value for films containing 20 g glycerol/100 g gelatin, while flexibility increased from 2.2% to 180.9% and T_g shifted from 137.5 to 21.3°C, for films without glycerol and films plasticized with 80 g glycerol/100 g gelatin, respectively. Nur Hanani *et al.* (2013) also reported that the increasing glycerol contents (0.2 to 1.1% of protein) increased the EAB, oxygen permeability (OP), solubility in water and seal strength values of gelatin films, but decreased TS values. Fourier transform infrared (FTIR) spectroscopic results indicated that increasing glycerol concentration increased the amplitude of peak situated around 1032 cm^{-1}, corresponding to glycerol.

1.2.2 Film-forming mechanisms and processes

1.2.2.1 Film-forming mechanism

Film is essentially a dried and extensively interacting polymer network of a three-dimensional gel structure. Despite the film-forming process, whether it is wet casting or dry casting, film-forming materials should form a spatially rearranged gel structure with all incorporated film-forming agents, such as biopolymers, plasticizers, other additives, and solvents in the case of wet casting (Felton, 2013; Rhim and Ng, 2007). Biopolymers as film-forming materials are generally dissolved or dispersed to produce film-forming solutions. Further drying of the hydrogels eliminates excess solvents from the gel structure. Whey protein emulsion films are produced from whey-protein gels by dehydration after heat-set or cold-set gel formation (Soazo *et al.*, 2011). The film-forming mechanism during the drying process may differ from the wet-gelation mechanism, though wet gelation is the initial stage of the film-forming process. There could be a critical stage of a transition from a wet gel to a dry film, which relates to a phase transition from a polymer-in-water (or other solvents) system to a water-in-polymer system (Han and Gennadios, 2005).

Protein-based film can be formed in three steps (Figure 1) (Marquié and Guilbert, 2002):

(1) Break intermolecular bonds (non-covalent and covalent bonds) that stabilize polymers in their native forms by using chemical or physical rupturing agents (by solubilization or thermal treatment). Polymer chains become mobile.

(2) Arrange and orient mobile polymer chains in the desired shape.

(3) Allow the formation of new intermolecular bonds and interactions to stabilize the three-dimensional network. The shape obtained in step 2 is maintained by eliminating agents used in step 1 (e.g., solvent removal or cooling).

Based on these three steps, solvent process is based on dispersing and solubilizing the proteins in various solvents and then casting, spraying or dipping, followed by drying. This process has been extensively studied and applied to produce films from various proteins (Cuq *et al.*, 1995).

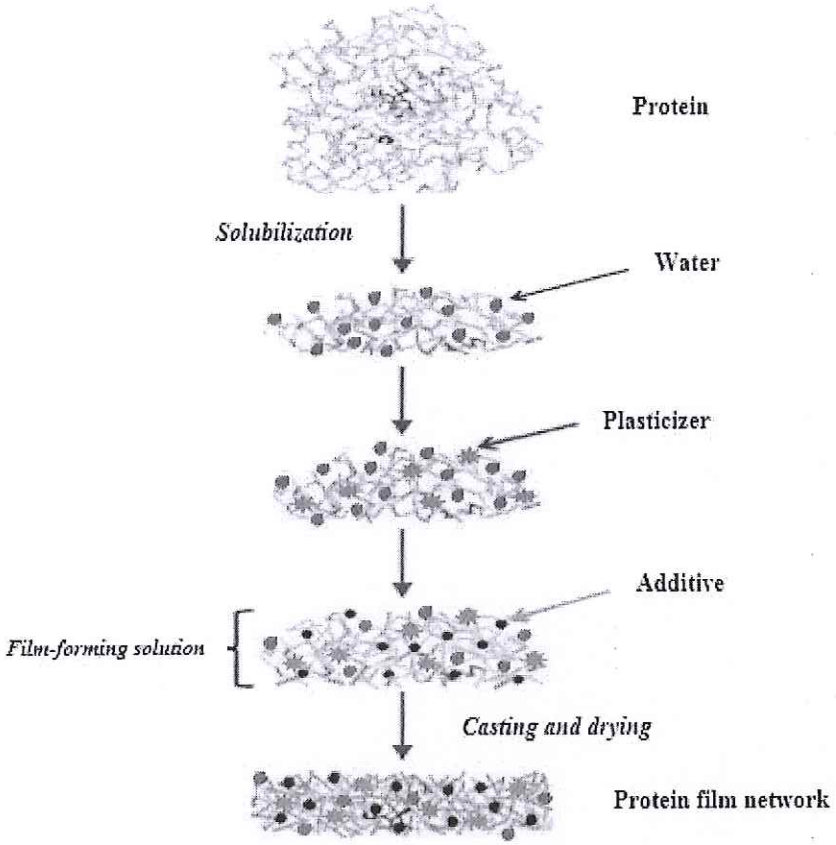

Figure 1. Mechanism of protein film formation
Source: Adapted from Marquié and Guilbert (2002)

Potential chemical and physical approaches have been implemented for the modification of film-forming mechanisms by altering film-forming raw materials, varying film-forming processing conditions, and applying treatments on formed films (Figure 2). Potential chemical methods of modifying the film-forming mechanisms of protein-based films include pH changes, salt addition, heat denaturation, solvent changes, chemical modification of the side chains of peptides, cross-linking, and hydrolysis of peptides (Kowalczyk and Baraniak, 2011; Were *et al.*, 1999; Yildirim and Hettiarachchy, 1997), irradiation of peptides (Lacroix and Ouattara, 2000), and the addition of foreign proteins (Denavi *et al.*, 2009; Mecitoğlu *et al.*, 2006).

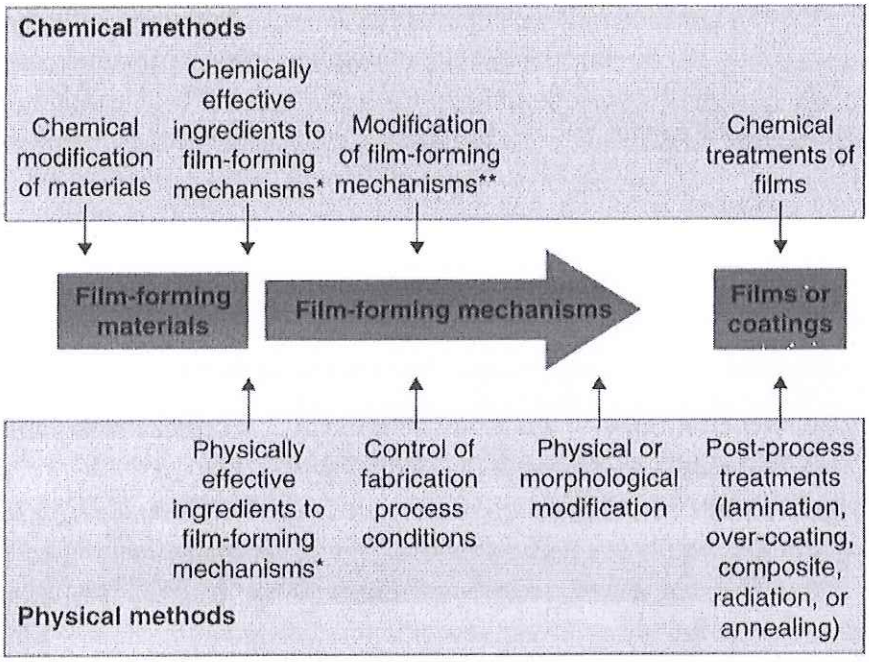

Figure 2. Chemical and physical modifying methods for preparation of film and coatings. * indicates the addition of chemically or physically active ingredients, which may enhance or interfere with the film-forming mechanisms. ** includes any chemical cross-linking, chemical substitution of side chains to create hydrophobic interactions or electrostatic interactions, and other extra mechanisms caused by chemical modifications.

Source: Han and Gennadios (2005)

Physical modifications of edible films and coatings include lamination, formation of composites, addition of particles or emulsions, perforation, over-coating, annealing heat curing (Gennadios *et al.*, 1996; Miller *et al.*, 1997), orientation, radiation (Gennadios *et al.*, 1998; Micard *et al.*, 2000), and ultrasound treatment (Banerjee *et al.*, 1996). Physicochemical properties of proteins determine the behavior of proteins during preparation, processing, storage and consumption. These properties are not only important to facilitate processing, but also to determine the quality of the final product (Ralston and Osswald, 2008).

1.2.2.2 Film formation processes

There are two categories of film formation processes: dry and wet (Guilbert *et al.*, 1997) (Figure 3). For the dry process, film production does not use

liquid solvents, such as water or alcohol. Heat is applied to the film-forming materials to increase the temperature to above the melting point of the film-forming materials, to cause them to flow. Molten casting, extrusion, and heat pressing are good examples of dry processes (Han and Gennadios, 2005). Therefore, the thermoplastic properties of the film-forming materials should be identified in order to design film-manufacturing processes. It is necessary to determine the effects of plasticizers and any other additives on the thermo plasticity of the film-forming materials (Guilbert *et al.*, 1997; Krochta, 2002).

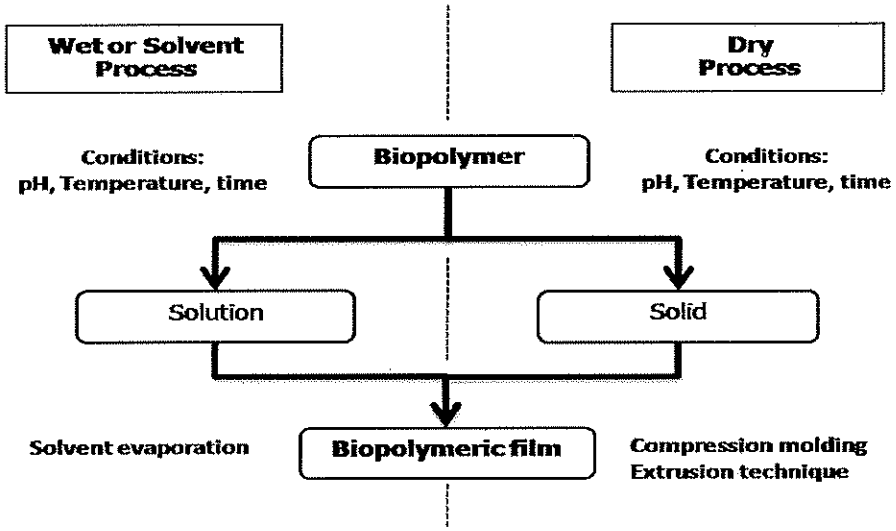

Figure 3. Processing methods: wet (or solvent) and dry process.
Source: Adapted from Guerrero *et al.* (2010)

For the wet process, the solvents are used for the dispersion of film-forming materials, followed by drying to remove the solvent and form a film structure. The selection of solvents is one of the most important factors. Since the film-forming solution should be edible and biodegradable, only water, ethanol, and their mixtures are appropriate as solvents (Krochta, 2002). All the ingredients of film-forming materials should be dissolved or homogeneously dispersed in the solvents to produce film-forming solutions (Han and Floros, 1997; Han and Gennadios, 2005). The film-forming solution should be applied to flat surfaces using a sprayer, spreader or dipping roller, and dried to eliminate the solvent, forming a film structure. Several protein-based films from different sources, e.g. fish gelatin (Tongnuanchan *et al.*, 2012), fish muscle protein (Oujifard *et al.*, 2013; Prodpran *et al.*, 2012), porcine plasma protein (Nuthong *et al.*,

2009), etc. were prepared by casting methods, in which film-forming solutions were firstly prepared prior to casting.

Macroscopic phase separation of incompatible ingredients from the film-forming solution is not generally desirable unless the phase separation is intentionally designed for the formation of a bi-layer film structure (Han and Gennadios, 2005). To produce a homogeneous film structure avoiding macroscopic phase separation, various emulsifiers can be added to the film-forming solution (Andreuccetti *et al.*, 2009; Krochta, 2002; Tongnuanchan *et al.*, 2014). The solvent compatibility of ingredients is very important to develop homogeneous edible film and coating systems carrying active agents. All ingredients, including active agents as well as biopolymers and plasticizers, should be homogeneously dissolved in the solvent to produce film-forming solutions. Most film-forming solutions possess much higher surface tension than the surface energy of dried films, since they contain excessive amounts of water or ethanol (Han and Gennadios, 2005). During the solvent drying process, the film-forming solution is concentrated and its surface energy is decreased due to the loss of solvent (Guerrero *et al.*, 2010; Marquié and Guilbert, 2002).

1.2.3 Gelatin films

Gelatin is a soluble protein compound obtained by partial hydrolysis of collagen, the main fibrous protein constituent in bones, cartilages and skins. In general, the source, age of animal, and type of collagen are all intrinsic factors influencing the properties of the gelatins (Johnston-Banks, 1990). Gelatin possesses an excellent film-forming property, and is one of the first materials applied to edible coatings and films (Gennadios *et al.*, 1994). Edible films have been prepared from gelatin of different sources including bigeye snapper and brown stripe red snapper (Jongjareonrak *et al.*, 2006a) baltic cod (Kołodziejska and Piotrowska, 2007), tilapia (Tongnuanchan *et al.*, 2012), tuna (Gómez-Estaca *et al.*, 2009b), blue shark (Limpisophon *et al.*, 2009), unicorn leatherjacket (Ahmad *et al.*, 2012), cuttlefish (Hoque *et al.*, 2010; Jridi *et al.*, 2013), and squid (Nagarajan *et al.*, 2012).

Amino acid compositions of gelatin from varying fish species are different and film composition as well as processes can be varied. Therefore, properties of resulting films are different (Table 1). In general, the physical properties of gelatin

are mainly governed by the source and the extracting conditions (Jongjareonrak *et al.*, 2006a; Nagarajan *et al.*, 2012). Molecular properties, especially chain length and amino acid composition, have been shown to determine properties of gelatin films. Hoque *et al.* (2011) reported that shorter gelatin molecules generated by enzyme hydrolysis (Alcalase) yielded the film with the lower junction zone or shorter strands via weak bonds during film formation. This led to the lower mechanical properties and thermal stability of the resulting films. Hydrolysis process increased hydrophilic groups which can interact with water molecules, leading to the increased WVP of the film. Furthermore, the structure analyses of edible films prepared by enzyme hydrolysis (pepsin) revealed the disruption of the smooth, compact and homogenous structure of films (Jridi *et al.*, 2013). Therefore, the chain length of gelatin molecules and plasticizer concentration were crucial factors governing the properties of gelatin-based films from cuttlefish skin (Hoque *et al.*, 2011). Nagarajan *et al.* (2013) studied the properties of gelatin films from splendid squid (*Loligo formosana*) skin bleached with hydrogen peroxide (H_2O_2) at various concentrations (0–8% w/v). TS and WVP of films decreased, but elongation at break (EAB) increased as the concentration of H_2O_2 increased. The shorter chain peptides found in gelatin caused by fragmentation induced by H_2O_2 at high levels might be associated with lower intermolecular interaction in the film matrix. It has been reported that various proteins could undergo oxidation (Liu and Xiong, 2000). Cleavage of the glutamyl side chain and proline residue of the protein in the presence of high concentration of H_2O_2 via excessive amounts of the $HO^{\bullet}$ radical was reported (Stadtman, 2001).

Protein content, type and concentration of plasticizers have been reported to affect the properties of gelatin based films (Vanin *et al.*, 2005). TS of gelatin film from shark (*Prionace glauca*) skin was affected by the protein concentration (1, 2 and 3%) of FFS (Limpisophon *et al.*, 2009). TS of film made from a 2% protein FFS was the highest. EAB and WVP of film increased with increasing protein concentration. Addition of glycerol improved flexibility and enhanced UV barrier property at 280 nm. Transparency at the visible range and WVP increased with increasing glycerol.

Table 1. Properties of gelatin-based films from different fish species

Fish Species	Protein Conc.	Plasticizer Conc.	Thickness (mm)	Mechanical properties			WVP ($x10^{-10}$ g/m s Pa)	References
				Elastic modulus (MPa)	TS (MPa)	EAB (%)		
Alaska pollock (*Theragra chalcogramma*) [1]	5% (w/v) of FFS	-	-	2091-1991	45.9-50.1	3.23-3.44	0.73-0.86[a]	Chiou *et al.* (2008)
Alaska pink salmon (*Oncorhynchus gorbuscha*) [2]	5% (w/v) of FFS	-	-	2245-2043	49.7-60.0	3.36-3.8	0.85-1.08[a]	Chiou *et al.* (2008)
Atlantic halibut (*Hippoglossus hippoglossus*) [3]	2% (w/v) of FFS	Sorbitol, 30% (w/w) of protein	0.080 ± 0.006	0.071±0.010	3.8 ± 0.8	294.5 ± 47.8	12.0 ± 2.2 [d]	Carvalho *et al.* (2008)
Atlantic halibut (*Hippoglossus hippoglossus*) [4]	2% (w/v) of FFS	Sorbitol, 30% (w/w) of protein	0.079 ± 0.006	0.906±0.107	11.1 ± 2.6	170.3 ± 36.4	13.0 ± 2.2 [d]	Carvalho *et al.* (2008)

Table 1. Properties of gelatin-based films from different fish species (cont.)

Fish Species	Protein Conc.	Plasticizer Conc.	Thickness (mm)	Mechanical properties			WVP ($x10^{-10}$ g/m s Pa)	References
				Elastic modulus (MPa)	TS (MPa)	EAB (%)		
Bigeye snapper (*Priacanthus tayenus*)[5]	2% (w/v) of FFS	Glycerol, 50% (w/w) of protein	0.029-0.030	-	10.04 – 11.43	12.11 – 25.98	0.89 – 1.28	Rattaya *et al.* (2009)
Blue shark (*Prionace glauca*)	1-3% (w/v) of FFS	Glycerol, 50% (w/w) of protein	0.011-0.043	-	12.58 – 27.29	61.13 – 74.17	0.4 – 1.12	Limpisophon *et al.* (2009)
Cod (*Godus morhua*)	4% (w/v) of FFS	Glycerol and sorbitol, 0.75 and 0.75 g/g polymer	0.047-0.086	-	2.83 - 8.25 N (Breaking force)	10 - 100 (Puncture deformation)	1.75 - 3.86 [b]	Denavi *et al.* (2009)
Cuttlefish (*Sepia pharaonis*)[6]	3% (w/v) of FFS	Glycerol, 25% (w/w)of protein	0.037-0.041	-	4.99 - 9.66	15.56 – 51.89	0.92 – 1.30	Hoque *et al.* (2010)

Table 1. Properties of gelatin-based films from different fish species (cont.)

Fish Species	Protein Conc.	Plasticizer Conc.	Thickness (mm)	Mechanical properties			WVP ($x10^{-10}$ g/m s Pa)	References
				Elastic modulus (MPa)	TS (MPa)	EAB (%)		
Giant squid (*Dosidicus gigas*) [7]	4% (w/v) of FFS	Glycerol and sorbitol, 0.15 and 0.15 g/g gelatin	-	-	1.57 - 10.51 N (Puncture force)	8.35 – 17.60 (Puncture deformation)	2.19-3.3	Giménez *et al.* (2009a)
Giant squid (*Dosidicus gigas*) [8]	4% (w/v) of FFS	Glycerol and sorbitol, 0.15 and 0.15 g/g gelatin	-	-	4.94 N (Puncture force)	46 (Puncture deformation)	1.89 [b]	Giménez *et al.* (2009b)
Giant squid (*Dosidicus gigas*) [9]	4% (w/v) of FFS	Glycerol and sorbitol, 0.15 and 0.15 g/g gelatin	-	-	2.63 N (Puncture force)	34.7 (Puncture deformation)	1.78 [b]	Giménez *et al.* (2009b)
Grouper (*Epinephelus chlorostigma*)	3% (w/v) of FFS	Sorbitol, 30% (w/w) of protein	0.076	48	9.25	37.48	975 [c]	Jeya Shakila *et al.* (2012)

Table 1. Properties of gelatin-based films from different fish species (cont.)

Fish Species	Protein Conc.	Plasticizer Conc.	Thickness (mm)	Mechanical properties			WVP ($x10^{-10}$ g/m s Pa)	References
				Elastic modulus (MPa)	TS (MPa)	EAB (%)		
Red snapper (*Lutjanus campechanus*)	3% (w/v) of FFS	Sorbitol, 30% (w/w) of protein	0.065	-	8.53	59.50	1040 [c]	Jeya Shakila *et al.* (2012)
Sole (*Solea spp.*) [10]	4% (w/v) of FFS	Glycerol and sorbitol, 0.15 and 0.15 g/g gelatin	-	-	11.4 – 28.5 N (Breaking force)	18.1 - 16.8 (Breaking Deformation)	1.66 – 1.77 [d]	Gómez-Estaca *et al.* (2009a)
Tuna (*Thunnus tynnus*) [11]	2% (w/v) of FFS	Glycerol, 0.25 g/g protein	0.098 – 0.10	-	2.75 – 5.91 N (Puncture force)	3.56 – 13.77 (Puncture deformation)	1.83 - 2.87 [b]	Gómez-Guillén *et al.* (2007)

Table 1. Properties of gelatin-based films from different fish species (cont.)

Fish Species	Protein Conc.	Plasticizer Conc.	Thickness (mm)	Mechanical properties			WVP ($x10^{-10}$ g/m s Pa)	References
				Elastic modulus (MPa)	TS (MPa)	EAB (%)		
Unicorn leatherjacket [aa]	3% (w/v) of FFS	Glycerol, 0-25% (w/w) of protein	0.042-0.048	-	23.75-36.34	3.06-8.76	1.21-1.94	Ahmad *et al.* (2012)
Warm-water tilapia	4, 6, and 8% (w/v) of FFS	Glycerol, 40% (w/w) of protein	39.25, 50.40 and 63.50	-	3.42, 3.47 and 5.85	53.05, 56.07 and 100.91	55.20, 78.10 and 110.55 [e]	Nur Hanani *et al.* (2012)

* FFS= film-forming solution; [a] WVP unit (g mm/m^2 h kPa); [b] WVP unit (10^{-8} g mm/h cm Pa); [c] WVP unit (g/m^2/day at 90% RH at 25 °C); [d] WVP unit (10^8 g mm/h cm^2 Pa); [e] WVP unit (g mm/kPa d m^2); [1] and [2] FFS prepared by adding 0.25, 0.50 and 0.75 (w/w) glutaraldehyde; [3] Films from gelatin concentrated by evaporation at 60 °C before spray drying; [4] Films prepared with different ratios of SPI:gelatin 0, 25, 50, 75, 100% (w/w);[5] FFS with 6% seaweed extract at pH 6 and 9;[6] FFS with different heating temperature (40 – 90 °C); [7] FFS was replaced with hydrolyzed gelatin (0 - 10%); [8]Film from gelatin extracted with distilled water at 60 °C/18 h); [9] Film from the second gelatin extraction of collagenous residues at 60 °C/18 h; [10] FFS added with borage extract at a ratio 1:1 (dissolved gelatin: borage extract); [11] FFS added with murta extracts; [aa] FFS added with bergamot oil.

TS of gelatin film from the skin of brownstripe red snapper (*Lutjanus vitta*) and bigeye snapper (*Priacanthus macracanthus*) decreased with increasing glycerol concentration from 25 to 75% (Jongjareonrak *et al.*, 2006a). The proteins network becomes less dense, and more permeable with the plasticizer incorporation. The increase in free volume of the system also raises the solvent mobility, thereby increasing the water diffusion in the matrix of the film (Cuq *et al.*, 1997). Moreover, at the same plasticizer concentration, fish skin gelatin from the two different species plasticized with glycerol (Gly) showed the greatest EAB whereas ethylene glycol (EG) plasticized film showed the highest TS (Jongjareonrak *et al.*, 2006b). Cao *et al.* (2009) also studied the effects of different kinds of plasticizer including oligosaccharide-sucrose, some organic acids and polyethylene glycol (PEG) with different molecular weight (300, 400, 600, 800, 1500, 4000, 10000, 20000). It was found that PEG of lower molecular weights exhibited better plasticizing effects for gelatin film and such films had better visual properties.

Heat treatment at appropriate temperature (70 °C) brought about the stretching or unfolding of gelatin molecule, in which higher inter-chain interaction could be formed via hydrogen bond or hydrophobic interaction and the improved mechanical property was obtained. With the excessive heating, gelatin degradation occurred and the corresponding film showed the increased EAB but lower TS. Thus, heat treatment of film-forming solution directly had the impact on the properties of film from cuttlefish skin gelatin (Hoque *et al.*, 2010).

Drying condition, e.g. temperature and time also show the impact on properties of films. Gelatin films dried at different temperatures had varying physical properties (Chiou *et al.*, 2009). Gelatin chains in a solution dried below gelation temperature can form triple helical structures before complete evaporation of water. The dried film, usually termed cold-cast gelatin film, can then retain these helical structures, depending on moisture content. In contrast, gelatin chains in a solution dried above gelation temperature remain as random coils during the drying process (Bradbury and Martin, 1952). The dried film, usually termed hot-cast gelatin film, then retain this amorphous structure (Chiou *et al.*, 2009). Fish gelatin solutions, especially those derived from cold-water species, have much lower gelation temperatures than mammalian gelatin solutions. This is mainly due to the fact that fish gelatin has the lower concentrations of proline and hydroxyproline (Bradbury and Martin, 1952;

Kozlov and Burdygina, 1983). Fish gelatin films made from Alaska pollock (*Theragra chalcogramma*) and Alaska pink salmon (*Oncorhynchus gorbuscha*) were dried at 4 °C, 23 °C, 40 °C, and 60 °C. Cold-cast gelatin films (dried below gelation temperature at 4 °C) had higher tensile strength, percent elongation values and water sorption isotherms than hot-cast (dried above gelation temperature at the higher temperatures) gelatin films. Water vapor permeability of cold-cast gelatin films was 2-3 fold higher than that of hot-cast gelatin films (Chiou *et al.*, 2009).

Casting methods are time consuming and are not appropriate for commercial production. A large scale production method like extrusion is imperative for industrial production (Wang and Padua, 2003). Most extrusion processes are for polymer films and are often followed by compression molding to reduce the thickness of the extruded sheets (Fishman *et al.*, 2004; Liu *et al.*, 2008; Selling *et al.*, 2009). Extrusion can be implemented as a continuous unit operation with control of temperature, size, shape, and moisture (Gómez-Guillén *et al.*, 2009). Krishna *et al.* (2012) reported that WVP of extruded gelatin films was higher than that of solution-cast films while the glass transition temperatures (T_g) of the extruded films were generally lower than those of solution-cast films.

1.2.4 Emulsion and emulsion-based film

1.2.4.1 Emulsion system

Emulsion is defined as a system which consists of two immiscible liquids (usually oil and water). One of the liquids disperse as small spherical droplets (dispersed phase) in the other (continuous phase). The process of converting two immiscible liquids into an emulsion is known as *homogenization*, and a mechanical device designed to carry out this process is called a *homogenizer* (Walstra, 1993). The creation of an emulsion directly from two separate liquids will be defined as *primary* homogenization, whereas the reduction in size of the droplets in an already existing emulsion will be defined as *secondary* homogenization (McClements, 2005) (Figure 4). The separate oil and water phases are converted to a coarse emulsion that contains fairly large droplets using one type of homogenizer (e.g., a high-speed blender), and then the size of the droplets is reduced using another type of homogenizer (e.g., a high-

pressure valve homogenizer). Emulsion can be categorized into three main systems including coarse or macroemulsion, microemulsion and nanoemulsion (Burguera and Burguera, 2012). Coarse emulsion has cloudy appearance. The droplet size is typically between 0.5 and 50 μm. The appearance of microemulsions is optically transparent. For a mini- or nanoemulsion, the appearance may be translucent (0.05-0.2 μm) or turbid (up to 0.5 μm), depending upon the droplet size and the refractive index difference between the droplets and the continuous phase (Burguera and Burguera, 2012).

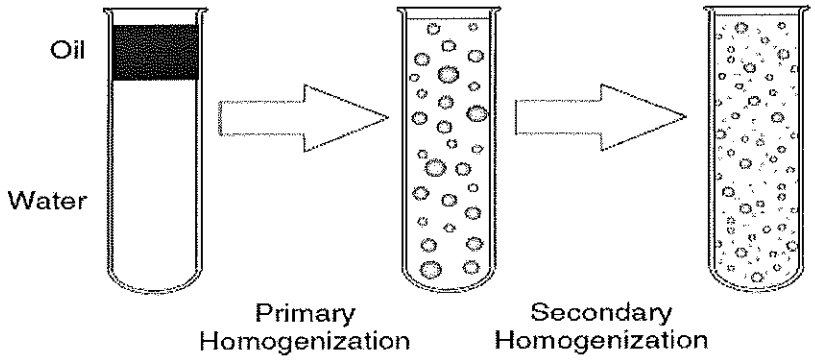

Figure 4. Primary and secondary homogenization.
Source: McClements (2005)

Lipid particle size is determined as the droplet size distribution measured by laser light scattering (McClements, 2005; McHugh and Krochta, 1994a), volume-surface mean diameter (d_{32}) and weight mean diameter (d_{43}). d_{32} is widely used to express the volume-surface mean diameter, which is related to the average surface area of droplets exposed to the continuous phase per unit volume of emulsion. d_{43} is another method of expressing the volume-length diameter of a polydisperse emulsion, which is the sum of the volume ratio of droplets in each size-class multiplied by the mid-point diameter of the size-class. d_{43} is more sensitive to the presence of large particles in an emulsion than d_{32}, hence it is often more sensitive to phenomenon such as flocculation (McClements, 2005).

In addition, emulsifier clearly has a major impact on the size of the droplets produced (Qian and McClements, 2011). Small-molecule surfactants (like SDS and Tween) are more effective at making small droplets than biopolymers (caseinate and β-lactoglobulin) under similar homogenization conditions because they adsorb to the droplet surfaces more rapidly (Qian and McClements, 2011). At

sufficiently low concentrations, surfactants or emulsifiers exist as monomers in solution because the entropy of mixing overweighs the attractive forces operating between the molecules (Holmberg *et al.*, 2004). Nevertheless, as their concentration is increased they can spontaneously aggregate into a variety of thermodynamically stable structures known as "association colloids", for example, micelles, bilayers, vesicles and reverse micelles (McClements, 2005).

1.2.4.2 Emulsion-based film

The protein-based films have excellent barrier properties toward oxygen, carbon dioxide and volatile compounds (Limpan *et al.*, 2010). However, protein-based films have poor water vapor barrier property due to its hydrophilicity in nature, thereby limiting its use as potential packaging (Gennadios *et al.*, 1997; Gómez-Guillén *et al.*, 2009). To tackle this problem, the hydrophobic substances such as oil, fatty acid and wax have been incorporated into protein-based film to improve water vapor barrier property and take advantage of the special functional characteristics of compounds added (Limpisophon *et al.*, 2010; Soazo *et al.*, 2011; Tongnuanchan *et al.*, 2012). To obtain protein-lipid composite films, a lipid can be incorporated into a protein matrix either by the formation of bilayer films or dispersing a lipid in the protein aqueous solution to obtain an emulsified film. Bilayer films tend to develop pinholes or cracks and exhibit non-uniform surface and cohesion characteristics (Quezada Gallo *et al.*, 2000). The emulsified films that exhibit good water vapor barrier capacity were governed by several parameters such as preparation techniques used (Fabra *et al.*, 2011; Pérez-Gago and Krochta, 2001). Emulsified films require only a single step and exhibit good mechanical properties. However, they have low water vapor barrier property due to the fact that water vapor can migrate through the continuous hydrophilic matrix but the dispersed lipid phase only increases the tortuosity factor for mass transfer in the matrix (Fabra *et al.*, 2011; Kamper and Fennema, 1984; Quezada Gallo *et al.*, 2000). A decrease of lipid particle sizes has been well correlated with the reduction of water vapor permeability (Pérez-Gago and Krochta, 2001). Small particles and high emulsion stability during the film drying give rise to a homogeneous distribution of the lipid particles in the film, which in turn contributes to a more efficient control of water transfer (Debeaufort *et al.*, 1993). Various homogenization techniques and surfactants

have been used for preparation of emulsion-based films (Tongnuanchan *et al.*, 2014; Urban *et al.*, 2006).

1.2.4.2.1 Dispersion techniques used for emulsion-based film preparation

Emulsion-based film can only be formed by the emulsion technique, which involves dispersion of the lipid into either the polysaccharide or protein film-formation solution to make a stable emulsion before casting a film (Pérez-Gago and Krochta, 2005). To reduce lipid droplet size of film-forming emulsion, mechanical devices such as rotor-stator homogenizers, high-pressure homogenizers, microfluidizers, etc. are used. This equipment generates the intense disruptive forces that breakup the lipid and water phases and lead to the formation of tiny lipid droplets before casting film (Qian and McClements, 2011).

1.2.4.2.1.1 Homogenization

In general, rotor-stator homogenizer is used to reduce the lipid droplet size in film-forming emulsion. Many film-forming materials were prepared with rotor-stator homogenizer (Table 2). Speed and time of homogenization have the influence on the reduction of lipid droplet size in film-forming emulsion. Fabra *et al.* (2011) reported that the homogenization of sodium caseinate incorporated with oleic acid at 9,500 rpm for 3 min reduced d_{32} and d_{43} values of oleic acid to 22.8 and 37 μm, respectively. d_{32} and d_{43} values of olive oil droplet in bovine gelatin film were decreased to 3.090 and 13.99 μm, respectively, when homogenized at 10,000 rpm for 4 min (Ma *et al.*, 2012b). Moreover, two steps of homogenization could generate the smaller droplet size than one step. d_{32} and d_{43} values of oleic acid were reduced to 0.747 and 0.99 μm with homogenization at 13,500 rpm for 1 min in the first step and at 20,500 rpm for 3 min in the second step (Fabra *et al.*, 2011). In addition, increasing speed of homogenization from 8,000 to 24,000 rpm improved the properties of gelatin–stearic acid emulsion film. TS and EAB were increased from 11.03 to 13.62 MPa and from 74.35 to 108.41%, respectively. In contrast, WVP was decreased from 1.04×10^{-10} to 0.70×10^{-10} g m^{-1} Pa^{-1} s^{-1} (Limpisophon *et al.*, 2010).

Table 2. Dispersing techniques applied in emulsion-based film preparation.

Dispersing techniques	Film-forming material	References
Rotor-stator homogenizer	Fish gelatin-citrus essential oils, fish gelatin-root essential oils, fish gelatin-basil and citronella essential oils, kefiran-oleic acid, blue shark skin gelatin-stearic and oleic acid, bovine gelatin-*Zataria multiflora* essential oil, hake protein-thyme oil, sunflower protein-clove essential oil, sodium caseinate-stearic acid, sodium caseinate-oleic and stearic acid, sodium caseinate-maize germ oil bodies, bovine gelatin-olive oil	Tongnuanchan *et al.* (2012); Tongnuanchan *et al.* (2013); Ghasemlou *et al.* (2011); Limpisophon *et al.* (2010); Kavoosi *et al.* (2014); Pires *et al.* (2011); Salgado *et al.* (2013); Rezvani *et al.* (2013); Fabra *et al.* (2011); Matsakidou *et al.* (2013); Ma *et al.* (2012b).
Microfluidizer	Bovine gelatin-olive oil, sodium caseinate-oleic and stearic acid, chitosan-basil and thyme essential oil, chitosan–lemon essential oil	(Ma *et al.*, 2012a); (Fabra *et al.*, 2011); (Bonilla *et al.*, 2012); (Perdones *et al.*, 2012).

1.2.4.2.1.2 Microfluidization

The rotor-stator homogenizer provides a lower energy input and so produces the largest particles, tended to develop pinholes or cracks and exhibited non-uniform surface. The resulting films probably had formed the bilayer structure and exhibited excellent water barrier ability, but poor mechanical resistance (Park *et al.*, 1996; Quezada Gallo *et al.*, 2000). High-energy approach is capable of generating intense disruptive forces that breakup the oil and water phases. This leads to the formation of tiny oil droplets. Those devices include high-pressure valve homogenizers, microfluidizers and sonicator (Gutiérrez *et al.*, 2008; Leong *et al.*, 2009; Velikov and Pelan, 2008; Wooster *et al.*, 2008).

Microfluidization technique utilizes the shearing forces of high-pressure homogenization as well as the severe stress of head-on collision to create a finer particle size emulsion (Keane *et al.*, 2000; Strawbridge *et al.*, 1995). Diagram of microfluidization process is illustrated in Figure 5.

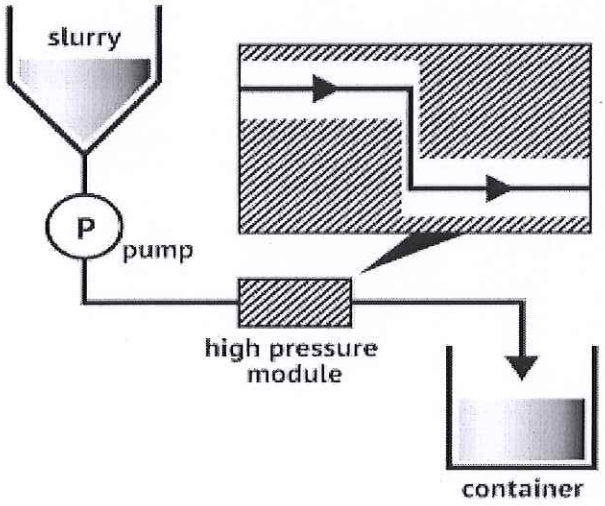

Figure 5. Schematic diagram of Microfluidization process
Source: Kim *et al.* (2015)

Microfluidizers can produce pressures in the range of 1000 bar or higher. Although the use of such pressures leads to finer emulsions, the average particle size is only reduced slightly (Perrier-Cornet *et al.*, 2005). This type of homogenizer usually consists of a fluid inlet, some kind of pumping device, and an interaction chamber containing two jets of crude emulsion from two opposite channels

collide with one another (Olson *et al.*, 2004; Schultz *et al.*, 2004). Emulsions were produced in two stages. Coarse emulsions were obtained with a high-speed blender or a rotor-stator homogenizer. Fine emulsions can be produced using microfluidization by passing a coarse emulsion through an interaction chamber using a high-pressure pumping device that is capable of pressurizing the emulsion in house compressed air (Jafari *et al.*, 2007). The interaction chamber consists of two flow channels. They cause two streams of the coarse emulsion to impinge on each other at high velocity, thus creating a very high shearing action that provides an exceptionally fine emulsion (Jafari *et al.*, 2007; Schultz *et al.*, 2004). Particle size distribution produced by microfluidizers tends to be narrower and smaller that those produced by other homogenization devices (Jafari *et al.*, 2007; Perrier-Cornet *et al.*, 2005; Wooster *et al.*, 2008). Emulsified films prepared using microfluidization have been reported (Table 2). The parameters affecting emulsion formation included homogenization pressure, number of passes, emulsifier type and concentration (Bonilla *et al.*, 2012; Salvia-Trujillo *et al.*, 2015). The increase in these factors decreased the emulsion droplet size. Ma *et al.* (2012a) reported that the individual increase in homogenization pressure or number of passes promoted a significant decrease in average diameter of olive oil in gelatin-based film. When homogenization pressure was increased from 69 to 138 MPa for single pass, the d_{32} values of olive droplet decreased from 0.570 to 0.433 μm. Similarly, the d_{32} value was decreased from 0.570 to 0.396 μm with increasing number of passes from 1 to 3 cycles. The microfluidized lemongrass oil-loaded nanoemulsions exhibited a dramatic reduction of their average droplet size, down to 5.5±0.3 nm, in comparison with the values of the coarse emulsion (prepared with rotor-stator homogenizer), with the average size of 1120±230 nm (Salvia-Trujillo *et al.*, 2015). Fabra *et al.* (2011) prepared sodium caseinate-based films containing oleic acid by using a rotor-stator and a microfluidizer. When microfluidizer was used at 103.39 MPa for 3 cycle, d_{32} and d_{43} values of film-forming emulsion were 0.236 and 0.402 μm, respectively, which were smaller than those homogenization with rotor-stator (d_{32} = 22.8 μm; d_{43} = 37 μm). Thus, filmogenic dispersions with narrower particle size distributions promoted better mechanical and water barrier properties of the emulsified films. Ma *et al.* (2012a) reported that properties of films prepared from different homogenization techniques (rotor-stator homogenizer at 10,000 for 4 min or microfluidizer at 138 MPa for 3 cycles)

were different. TS and EAB of the 5% (w/w) gelatin containing 12.5% olive oil films increased from 17.1 MPa (rotor-stator homogenizer) to 23.2 MPa (microfluidizer) and 39.5% to 106.7%, respectively, while WVP was decreased from 5.6×10^{-10} g/m s Pa to 3.7×10^{-10} g/m s Pa. In addition, the microfluidized gelatin–olive oil films exhibited the excellent barrier ability to UV light. The transparency values and surface irregularity of the films were decreased when lipid droplets in the film-forming dispersion (FFD) and films decreased (Ma *et al.*, 2012a).

1.2.4.2.2 Surfactants

Surfactants or emulsifiers are defined as a substance that reduces surface tension between oil and water, thereby enhancing emulsification and increasing emulsion stability (Dalgleish, 1997). Emulsion films generally require the proper surfactant in order to provoke the stable state of two-phase emulsion system with homogeneity of oil droplet distribution and to modify surface energy to control the adhesion and wettability of the film surface (Hsu and Nacu, 2003; Krochta, 2002). Although many biopolymers possess certain levels of emulsifying capacity, it is necessary to incorporate emulsifiers into FFD to produce lipid-emulsion films. Some proteins have sufficient emulsifying capacity due to their amphiphilic structure (Krochta, 1997b). Different surfactants were used to stabilize protein-based emulsion film. Prodpran *et al.* (2007) prepared fish muscle protein-based film containing palm oil, in which Tween-20 at 15% (w/w) of oil was used as surfactant. Tongnuanchan *et al.* (2012) also used Tween-20 at 25% (w/w) of oil as surfactant in fish skin gelatin film incorporated with citrus essential oils. Tween-80 was used as surfactants for whey protein concentrate film containing cinnamon essential oil (Bahram *et al.*, 2014). Tanaka *et al.* (2001) prepared fish protein-lipid emulsion films using lecithin at 10% as surfactant. Soy lecithin has been reported as more effective surfactant to prepare gelatin-basil or citronella oil composite films, compared to those using Tween 20 and Tween 80 (Tongnuanchan *et al.*, 2014). Furthermore, Limpisophon *et al.* (2010) reported the use of sucrose stearate as surfactant for preparation of gelatin film incorporated with stearic and oleic acids.

The addition of more hydrophobic compounds inside film formulation induces the development of a heterogeneous structure with the presence of

discontinuous areas, producing lower tensile strength (Bravin *et al.*, 2004). Lower tensile strength was also observed for biodegradable films, due to the incorporation of increasing amounts of lipids and / or surfactants (Chen *et al.*, 2009; Ziani *et al.*, 2008). For films prepared with potato starch, the uses of surfactants (Tween 20, Span 80 and lecithin) without the addition of a plasticizer led to slightly weaker and less flexible films with lower tensile strength, as compared to those containing glycerol in combination with surfactant (Rodríguez *et al.*, 2006). Andreuccetti *et al.* (2011) reported that films containing yucca extract showed higher tensile strength and moisture contents but less elongation and water vapor permeability, compared to films containing lecithin. At the high amount of surfactants used, tensile strength decreased by 78.4% for films added with lecithin and by 62% for those added with yucca extract, compared to gelatin-based film without surfactant. Moreover, WVP was significantly reduced in films added with hydrophobic substances (Chen *et al.*, 2010).

1.2.4.2.3 Lipids

Fatty acids, oils or waxes have been used to augment the hydrophobicity of the films and reduce the WVP and water solubility (Pérez-Mateos *et al.*, 2009). Budi Santosa and Padua (1999) reported that TS of zein sheets decreased and EAB increased when oleic acid was added. Bertan *et al.* (2005) evaluated the effect of stearic acid, palmitic acid and mixed fatty acids on the properties of gelatin films. The addition of stearic acid resulted in a greater reduction in WVP of the gelatin films, compared with palmitic acid. The addition of the blend (stearic / palmitic, 1:1) significantly decreased the WVP of the films as compared to those without lipid addition or containing palmitic acid, but was similar to the film containing stearic acid. Amount of oil directly determines properties of films. Jongjareonrak *et al.* (2006c) blended bigeye snapper and brownstripe red snapper-skin gelatins with fatty acids (FAs: palmitic acid and stearic acid) or the sucrose esters (FASEs) of those FAs. The resulting composite films showed the appreciable reduction in WVP. FAs lowered the tensile strength, while FASEs progressively increased the tensile strength. A marked increase in breaking elongation was also recorded when either FAs or FASEs were added to the films at a proportion of 25%. When cod-skin gelatin emulsified film containing different sunflower oil concentration (0, 0.3, 0.6, and 1% w/w) was characterized, the maximum breaking

strength decreased by 30–60% and WVP was decreased, depending on the amount of oil added (Pérez-Mateos *et al.*, 2009). With higher oil concentration in the film, the lower the protein fraction in the water-soluble matter was obtained. Gelatin–oil interactions in the film resulted in protein insolubilization. Lipid–protein interactions (hydrogen bonds, ester formation) as well as early oil oxidation observed by FTIR were related to alterations in the structure of the composite gelatin-oil films (Pérez-Mateos *et al.*, 2009). Atarés *et al.* (2010) also found that the use of cinnamon or ginger essential oils at the low ratio used (less than 1:0.1 protein to lipid ratio) in the sodium caseinate films, had no impact on mechanical properties and only slightly reduced water vapor permeability. Limpisophon *et al.* (2010) reported that increasing fatty acid (stearic and oleic) concentration significantly decreased WVP of edible films based on blue shark (*Prionace glauca*) skin gelatin. Incorporation of root essential oils from ginger, turmeric and plai markedly decreased TS and WVP with the concomitant increase in EAB of fish skin gelatin films, particularly with increasing levels (25%, 50% and 100%, based on protein content) (Tongnuanchan *et al.*, 2013). Water barrier property was effectively improved when film was incorporated with all types of essential oils. However, essential oils incorporated decreased the transparency of films. Film incorporated with root essential oil at high level (50%) exhibited a bilayer morphological microstructure; however, incorporation of essential oil at an excessive level (100%) resulted in some oil exudates on the film surface (Tongnuanchan *et al.*, 2013).

1.2.5 Applications of protein-based film

Edible films provide many benefits in terms of convenience (Gennadios *et al.*, 1994). Quality maintenance and enhancement are also very significant function of edible film (Krochta, 1997b). They can retard surface dehydration, moisture absorption, oxidation of ingredients, aroma loss, frying oil absorption, ripening/aging and microbial deterioration of food products (Krochta, 1997a). Edible films and coatings can be used to preserve the quality of several food commodities. The oxygen barrier properties of film can prevent oxidation or lipid ingredients, colorants and flavors of food products such as nuts, confectionary, fried products and colored produce (Baldwin *et al.*, 1997). High-fat meat and fish products, such as sausages, jerky and

fillets, can be protected from oxidation by protein-based films (Artharn *et al.*, 2009). According to Artharn *et al.* (2009), fish muscle protein-based films were used to cover dried fish powder. The samples covered with the film containing 25% palm oil and 40% chitosan could prevent lipid oxidation, as evidenced by the lower thiobarbituric acid reactive substances and yellowness than other samples during the extended storage up to 21 days. Salgado *et al.* (2013) determined the lipid oxidation of fish patties wrapped with sunflower protein films enriched with clove essential oil during 13 days of storage at 2 °C. The rate of malonaldehyde production was lower in patties wrapped with clove containing films during the first 3 days of storage, indicating a noticeable delay in hydroperoxide (primary lipid oxidation products) degradation mediated by the clove essential oil components. This allowed TBARS remaining at the lowest values during storage. Nowzari *et al.* (2013) also reported that rainbow trout (*Oncorhynchus mykiss*) fillets covered with chitosan (1%, w/v) - gelatin (3%, w/v) film retained their good quality characteristics during refrigerated storage (4 ± 1 °C) for 16 days, as indicated by lower peroxide value and TBARS values than control samples at the end of the storage. The oxygen-barrier property is useful for retarding the respiration rate of fresh produce (Baldwin *et al.*, 1995). Moisture barrier properties of edible film and coatings can protect foods from dehydration. Moisture loss is the most critical quality degradation factor of fresh produce (Kester and Fennema, 1986). The moisture barrier property can also be utilized to prevent moisture migration between heterogeneous food product ingredients (Han and Gennadios, 2005).

Due to their hydrophilic nature, films primarily composed of proteins are highly sensitive to moisture and show poor water vapor barrier properties (Guilbert *et al.*, 1996). Protein-based films absorbed water from foods and slowly swelled during storage (Wu *et al.*, 2000). Film swelling is believed to be due to the conformational changes involving structural relaxation (Slade *et al.*, 1989). These may have contributed to the observed higher relative moisture loss in the wrapped samples. Patties wrapped with wheat gluten and soy protein films had higher relative moisture loss than those with coatings and the polyvinyl chloride-wrapped patties and became dry on the surface during the storage (Wu *et al.*, 2000). The coating would allow for the movement of water vapor across the film, thus preventing water condensation and microbial spoilage of the Ricotta during storage of 21 days (Di Pierro *et al.*, 2011).

1.3 Objectives

1. To study the effects of palm oil concentration on the properties of fish skin gelatin-based film.

2. To investigate the effects of microfluidization pressures and passes on properties of film-forming dispersion (FFD) and fish skin gelatin-based film incorporated with palm oil.

3. To elucidate the effects of types and concentrations of surfactants on the properties of FFD and fish skin gelatin-based film incorporated with palm oil.

4. To use emulsion-based gelatin film for shelf-life extension of shrimp cracker.

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CHAPTER 2
EMULSION STABILITY AND PROPERTIES OF FISH GELATIN-BASED FILMS AS AFFECTED BY PALM OIL AND SURFACTANTS

2.1 Abstract

Gelatin films exhibit the poor water vapor barrier property. The use of hydrophobic substances such as palm oil, which is abundant and available in Thailand, can be a means to lower water vapor migration. To disperse oil in film-forming dispersion (FFD), surfactant along with appropriate homogenization is required. The influences of palm oil levels and surfactant types in the absence or presence of glycerol on FFD and gelatin films were investigated. Similar oil droplet size (d_{32} and d_{43}) of FFD containing soy lecithin were observed, regardless of palm oil levels used ($p > 0.05$). FFD having Tween-20 had larger droplet size as the levels of oil increased ($p < 0.05$). After 12 h of storage, the slight increases in d_{32} and d_{43} were noticeable in all FFD samples. When the films were determined, lower water vapor permeability (WVP) and tensile strength (TS) but higher elongation at break (EAB) were obtained as palm oil levels increased ($p < 0.05$), regardless of glycerol and surfactant used. Films without glycerol had lower WVP and EAB with higher TS than those containing 30% glycerol ($p < 0.05$). No differences in WVP and mechanical properties were found between films containing both surfactants ($p > 0.05$). The incorporation of 50% or 75% palm oil using soy lecithin in the presence of 30% glycerol could enhance homogeneity and stability of oil droplets in FFD and also improved water vapor barrier property of gelatin films without marked physical changes.

2.2 Introduction

Packaging materials are widely used to protect the product from surroundings, retard deterioration, extend shelf-life, maintain the quality of foods, and distribute foods and products conveniently. Petroleum-based materials are commonly used and have many advantages. However, these materials are not biodegraded easily and generate a global pollution (Zhang and Mittal, 2010). Biodegradable film prepared from various biopolymers has been attended as an alternative packaging to those

synthetic packaging materials (Kester and Fennema, 1986; Krochta, 1997). Among these biopolymers, proteins have been extensively used for the development of biodegradable films due to their abundance and good film-forming ability. Proteins are heteropolymers containing a variety of amino acids, which can undergo a wide range of interactions and chemical reactions (Stevens, 1990). Among the proteins, gelatin possesses an excellent film-forming property, and is one of the first materials applied to edible coatings and films (McHugh and Krochta, 1994). Nevertheless, the gelatin films present a poor water vapor barrier ability (Sobral *et al.*, 2001). This could limit their applications in food products with high moisture content, because the films may swell, partially dissolve or disintegrate upon contact with the wet surface (Núñez-Flores *et al.*, 2012). The incorporation of hydrophobic substances such as lipid, fatty acid and wax has been implemented to improve water barrier property of gelatin film (Limpisophon *et al.*, 2010; Prodpran *et al.*, 2007; Soazo *et al.*, 2011). Those substances are not dissolved in aqueous phase, in which film-forming dispersion or emulsion must be prepared before making the films. The homogeneity of oil droplets and stability of emulsion in the film-forming dispersion (FFD) play a significant role in properties of emulsion-based films. Small particles and high emulsion stability during the film drying give rise to a homogeneous distribution of the lipid particles in the film, which in turn contributes to the efficient control of water vapor migration (Debeaufort *et al.*, 1993). To disperse oil in film-forming aqueous phase, surfactant along with appropriate homogenization is required. Surfactants or emulsifiers are a substance that reduces surface tension between oil and water, thereby enhancing emulsification and increasing emulsion stability (Dalgleish, 1997). Tween-20 and soy lecithin have been reported as the effective surfactants for stabilization of protein-based emulsion films (Prodpran *et al.*, 2007; Tongnuanchan *et al.*, 2014). Furthermore, microfluidization is one of potential homogenization techniques to obtain fine and more uniform oil droplet dispersion as well as more emulsion stability, compared to typical homogenization.

Palm oil is the natural hydrophobic substance obtained from the mesocarp of palm seed. Throughout the world, 90% of palm oil is used for edible purposes (e.g., margarine, deep fat frying, shortening, ice cream, cocoa butter substitutes in chocolate); the remaining 10% is used for soap and oleochemical manufacturing (fatty acids, methyl esters, fatty nitrogenous derivatives, surfactants and

detergents) (Edem, 2002). Due to its abundance and cheap price, palm oil could be used as promising hydrophobic substance to improve water vapor barrier property of gelatin film. Nevertheless, rare information regarding the use of palm oil and processing parameters on properties of fish gelatin-based film has been reported. Thus, this study aimed to investigate the influence of palm oils levels and surfactant types on stability of FFD and properties of resulting fish skin gelatin-based emulsion films.

2.3 Materials and methods

2.3.1 Chemicals and gelatin

Glycerol, Tween-20, soy lecithin, Nile blue A and sodium dodecyl sulfate (SDS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All chemicals are analytical grade. Fish gelatin produced from tilapia skin (~ 240 blooms) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Palm oil was obtained from *OLEEN Company Limited* (Bangkok, Thailand).

2.3.2 Preparation of film-forming dispersion (FFD)

Gelatin (3.5 g) was dissolved in 90 mL of distilled water and then heated at 70 °C for 30 min. Palm oils previously mixed with Tween-20 or soy lecithin at 50% palm oil were added into gelatin solution to obtain the final concentrations of 25, 50, 75 and 100% protein in the absence and presence of glycerol (30% w/w, based on protein content). The volume was adjusted to 100 mL using distilled water. The mixtures were homogenized at a speed of 22,000 rpm for 3 min using a homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The coarse emulsions were passed through a Microfluidics homogenizer (Model HC-5000, Microfluidizer, Newton, MA, USA) at 2,000 psi for 2 passes. Fine emulsion termed 'film-forming dispersion: FFD' was subjected to analyses and also used for film preparation.

2.3.3 Characterization and stability of FFD

FFD samples were stored at room temperature (28-30 °C) for 0 and 12 h. At time designated, FFD were taken for analyses.

2.3.3.1 Oil droplet size

Particle size distribution of oil droplets in FFD was determined using a ZetaPALs zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA). Prior to analysis, FFDs were diluted with 1% (w/v) sodium dodecyl sulfate (SDS) solution in order to dissociate flocculated droplets. The surface-weighted mean (d_{32}) and the volume-weighted mean particle diameter (d_{43}) of the oil droplets were calculated by equations (1) and (2), respectively (Fabra *et al.*, 2011).

$$d_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}$$

$$d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$$

where n_i and d_i are the number of droplets of a determined size range and the droplet diameter, respectively.

2.3.3.2 Flocculation and coalescence

To determine flocculation factor (F_f) and coalescence index (C_i), FFDs were diluted with distilled water in the presence and absence of 1% (w/v) SDS. F_f and C_i were calculated using the following equations (Palazolo *et al.*, 2005):

$$F_f = \frac{d_{43\text{-SDS}}}{d_{43+\text{SDS}}}$$

$$C_i = \frac{(d_{43+\text{SDS, t}} - d_{43+\text{SDS, in}}) \times 100}{d_{43+\text{SDS, in}}}$$

where $d_{43+\text{SDS}}$ and $d_{43\text{-SDS}}$ are the volume-weighted mean particle diameter of the emulsion droplets in the presence and absence of 1% (w/v) SDS, respectively. $d_{43+\text{SDS, in}}$ is initial value of the volume-weighted mean particle diameter of the emulsion droplets in the presence of 1% (w/v) SDS; $d_{43+\text{SDS, t}}$ is the value of the volume-weighted mean particle diameter of the emulsion droplets in the presence of 1% (w/v) SDS at the designated storage time.

2.3.3.3 Confocal laser scanning microscopy (CLSM)

To visualize oil droplets in FFD, palm oil was added with Nile blue A (1 mg 30 g palm oil) prior to FFD preparation. After homogenization, the FFD was

determined for morphology using a confocal laser scanning microscope (CLSM) (Olympus, FV300, Tokyo, Japan).

2.3.4 Characterization of gelatin films containing palm oils at various levels as affected by surfactants

2.3.4.1 Preparation of film

To prepare the film, the dissolved air in the FFD was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim Germany) for 30 min at room temperature. FFD (4 mL) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown for 12 h at room temperature (28-30 °C) prior to further drying at 25 °C and 50 ± 5% RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and subjected to analyses. Control films without palm oils were prepared from film-forming solution in the absence and presence of 30% glycerol (based on protein).

2.3.4.2 Determinations of film properties

2.3.4.2.1 Film thickness

The thickness of film was measured using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Five random locations around each film of ten film samples were used for average thickness determination.

2.3.4.2.2 Mechanical properties

Prior to testing, films were conditioned for 48 h at 25 °C and 50 ± 5% RH. Tensile strength (TS) and elongation at break (EAB) were determined as described by Iwata *et al.* (2000) with a slight modification using the Universal Testing Machine (Lloyd Instrument, Hampshire, UK) equipped with tensile load cell of 100 N. Ten samples (2 × 5 cm^2) with initial grip length of 3 cm were used for testing. Cross-head speed was set at 30 mm/min.

2.3.4.2.3 Water vapor permeability (WVP)

WVP was measured using a modified ASTM method (ASTM., 1989) as described by Shiku *et al.* (2004). The films were sealed on an aluminium permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and a rubber gasket to hold the films in place. The cups were placed in a desiccator containing the distilled water at 30 °C. The cups were weighed at 1-h intervals over a 10-h period. WVP of the film was calculated as follows:

$$WVP \text{ (g/m s Pa)} = \frac{wl}{At(P_2-P_1)}$$

where w is the weight gain of the cup (g); l is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); $P_2 - P_1$ is the vapor pressure difference across the film (4242.31 Pa at 30 °C).

2.3.4.2.4 Color

Film samples were subjected to color measurement using a CIE colorimeter (Hunter associates laboratory, Inc., Reston, VA, USA). D_{65} (day light) and a measure cell with opening of 30 mm was used. The color of the films was expressed as L^* -value (lightness), a^* -value (redness/greenness) and b^* -value (yellowness/blueness). Total difference of color (ΔE^*) was calculated as follows (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences between the color parameter of the samples and those of the white standard ($L^* = 93.61$, $a^* = -0.97$, $b^* = 0.44$).

2.3.4.2.5 Light transmittance and transparency value

The light transmittance of films was measured at the ultraviolet and visible range (200-800 nm) using a UV-vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) as described by Shiku *et al.* (2004). The transparency value of film was calculated using the following equation (Han and Floros, 1997):

$$\text{Transparency value} = \frac{-logT_{600}}{x}$$

where T_{600} is the fractional transmittance at 600 nm and x is the film thickness (mm). The greater transparency value represents the lower transparency of film.

2.3.4.2.6 Scanning electron microscopy (SEM)

Morphology of surface and cross-section of film samples was visualized using a scanning electron microscope (SEM) (Quanta 400, FEI, Eindhoven, the Netherlands). For cross-section, samples were fractured under liquid nitrogen prior to visualization. Then, the samples were mounted on bronze stub and sputtered with gold (Sputter coater SPI-Module, PA, USA) in order to make the sample conductive. The photographs were taken at an acceleration voltage of 15 kV.

2.3.5 Statistical analysis

Completely randomized design (CRD) was used throughout the study. All experiments were run in triplicate with different three lots of films. Data were subjected to analysis of variance (ANOVA), and mean comparisons were carried out by Duncan's multiple range test. For pair comparison, T-test was used (Steel and Torrie, 1960). Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL, USA).

2.4 Results and discussion

2.4.1 Effect of palm oil levels and surfactant types on characteristics and stability of film-forming dispersion in the absences or presence of glycerol

2.4.1.1 Droplet size distribution

Oil droplet sizes of FFD containing palm oil at levels of 25-100% (w/w, based on protein content) using Tween-20 and soy lecithin as surfactants in the absence and presence of 30% glycerol expressed as d_{32} and d_{43} are shown in Table 3. When soy lecithin was used in FFD, both d_{32} and d_{43} of FFD incorporated with palm oil at different levels were similar after emulsification (storage time = 0) ($p > 0.05$), regardless of glycerol contents. In contrast, FFD containing Tween-20 had the continuous increase in both d_{32} and d_{43} when palm oil levels were increased ($p < 0.05$). The d_{32} is inversely

related to specific surface area. The smaller d_{32} means the higher specific surface area (Hebishy *et al.*, 2013). The d_{43} can be used as the index of coalescence and flocculation. The increase in d_{43} reflects the assembly of individual droplets into larger flocs (Intarasirisawat *et al.*, 2014). It was noted that d_{32} and d_{43} of FFD containing soy lecithin were lower than those of FFD with Tween-20 ($p < 0.05$). Soy lecithin more likely migrated and absorbed at the surface of the newly formed oil droplets faster and effectively than Tween-20. Moreover, soy lecithin and Tween-20 are different in nature in which soy lecithin is ionic surfactant while Tween-20 is non-ionic surfactant. In the present study, ionic surfactant (soy lecithin) could migrate and occupy the oil–water interface more effectively compared to non-ionic surfactant (Tween-20). As a result, it could lower the surface tension and formed the strong outer layers around oil droplets (Dickinson, 2009). Soy lecithin consists of a hydrophilic head group and two lipophilic tail groups (low HLB number), whereas Tween-20 is a non-ionic surfactant with large polyol head groups and has a high HLB number. The adsorption and orientation at the oil–water interface are affected mostly by its hydrophobicity (Zayas, 1997). Soy lecithin, with a higher hydrophobicity as indicated by lower HLB number, more likely showed higher interfacial tension-lowering activity than Tween-20. With higher palm oil levels in FFD containing Tween-20, shearing force applied per oil volume became less and efficacy of Tween-20 to occupy at interface might be insufficient. This led to the larger oil droplet size.

Overall, the increases in both d_{32} and d_{43} were noticeable in all FFD samples after 12 h of storage. This indicated the instability of the emulsion, in which collapse of the emulsion by the coalescence mechanism as well as the Ostwald ripening phenomenon or assembly of individual droplets by flocculation might occur. With extended storage time, oil droplets more likely aligned themselves closely, leading to flocculation and creaming. Those phenomena could foster the coalescence of emulsion. At 12 h of storage, d_{32} and d_{43} of all FFD containing Tween-20 were higher than those of all FFD containing soy lecithin ($p < 0.05$). A higher percentage of oil in the emulsions resulted in a larger mean droplet diameter for the same homogenization condition (Floury *et al.*, 2000). This result indicated that soy lecithin showed higher efficiency in the migration or participating at oil-water interface and thus stabilizing oil droplet in FFD than Tween-20. The result was in accordance with Tongnuanchan *et al.* (2013a)

Table 3. Oil droplet size and emulsion stability of film-forming dispersion as affected by palm oil levels, types of surfactant and glycerol level.

Glycerol (%)	Surfactant types	Palm oils (%)	Storage time (h)	d_{32} (μm)	d_{43} (μm)	F_f	C_i (%)
0	Tween-20	25	0	$0.331 \pm 0.026^{*e,1}$	$0.365 \pm 0.014^{e,1}$	$2.230 \pm 0.091^{d,1}$	-
			12	$0.434 \pm 0.053^{d,1}$	$0.479 \pm 0.024^{d,1}$	$2.362 \pm 0.125^{d,1}$	$8.64 \pm 1.35^{b,1}$
		50	0	$0.437 \pm 0.049^{d,1}$	$0.408 \pm 0.027^{d,1}$	$2.373 \pm 0.179^{d,1}$	-
			12	$0.620 \pm 0.070^{bc,1}$	$0.691 \pm 0.108^{b,1}$	$3.069 \pm 0.166^{bc,1}$	$10.17 \pm 0.46^{b,1}$
		75	0	$0.459 \pm 0.045^{d,1}$	$0.474 \pm 0.043^{d,1}$	$2.447 \pm 0.337^{d,1}$	-
			12	$0.759 \pm 0.049^{b,1}$	$0.767 \pm 0.049^{b,1}$	$3.383 \pm 0.120^{ab,1}$	$16.52 \pm 3.25^{a,1}$
		100	0	$0.648 \pm 0.084^{c,1}$	$0.722 \pm 0.057^{b,1}$	$2.948 \pm 0.277^{c,1}$	-
			12	$1.003 \pm 0.045^{a,1}$	$1.014 \pm 0.046^{a,1}$	$3.529 \pm 0.121^{a,1}$	$17.30 \pm 0.13^{a,1}$
	Soy lecithin	25	0	$0.124 \pm 0.018^{d,2}$	$0.171 \pm 0.024^{e,2}$	$1.296 \pm 0.018^{c,2}$	-
			12	$0.185 \pm 0.010^{ab,2}$	$0.231 \pm 0.010^{b,2}$	$1.392 \pm 0.020^{ab,2}$	$5.76 \pm 2.51^{c,2}$
		50	0	$0.134 \pm 0.012^{cd,2}$	$0.179 \pm 0.004^{ce,2}$	$1.304 \pm 0.068^{bc,2}$	-
			12	$0.182 \pm 0.028^{ab,2}$	$0.239 \pm 0.018^{b,2}$	$1.594 \pm 0.183^{a,2}$	$8.19 \pm 1.47^{b,2}$
		75	0	$0.134 \pm 0.020^{cd,2}$	$0.185 \pm 0.005^{ce,2}$	$1.322 \pm 0.083^{bc,2}$	-
			12	$0.195 \pm 0.046^{ab,2}$	$0.241 \pm 0.004^{b,2}$	$1.598 \pm 0.096^{a,2}$	$8.80 \pm 0.22^{b,2}$
		100	0	$0.148 \pm 0.002^{cd,2}$	$0.193 \pm 0.010^{ce,2}$	$1.331 \pm 0.035^{bc,2}$	-
			12	$0.222 \pm 0.034^{a,2}$	$0.266 \pm 0.016^{a,2}$	$1.630 \pm 0.088^{a,2}$	$12.82 \pm 1.66^{a,2}$

Different superscript letters in the same column under the same glycerol content and surfactant type indicate significant differences ($p < 0.05$). Different superscript numbers in the same column under the same glycerol content, palm oil level and storage time indicate significant differences ($p < 0.05$). F_f : Flocculation factor; C_i : Coalescence index. * Mean ± SD (n = 3).

Table 3 (Cont.). Oil droplet size and emulsion stability of film-forming dispersion as affected by palm oil levels, types of surfactant and glycerol level.

Glycerol (%)	Surfactant types	Palm oils (%)	Storage time (h)	d_{32} (μm)	d_{43} (μm)	F_f	C_i (%)
30	Tween-20	25	0	$0.332 \pm 0.028^{e,1}$	$0.340 \pm 0.034^{f,1}$	$2.241 \pm 0.210^{d,1}$	-
			12	$0.445 \pm 0.004^{d,1}$	$0.448 \pm 0.043^{e,1}$	$2.267 \pm 0.266^{d,1}$	$7.67 \pm 1.87^{b,1}$
		50	0	$0.456 \pm 0.056^{d,1}$	$0.462 \pm 0.056^{e,1}$	$2.475 \pm 0.121^{cd,1}$	-
			12	$0.574 \pm 0.057^{c,1}$	$0.605 \pm 0.042^{d,1}$	$2.597 \pm 0.266^{bcd,1}$	$10.32 \pm 2.84^{b,1}$
		75	0	$0.635 \pm 0.024^{c,1}$	$0.676 \pm 0.018^{c,1}$	$2.846 \pm 0.180^{bc,1}$	-
			12	$0.692 \pm 0.023^{b,1}$	$0.746 \pm 0.029^{b,1}$	$3.265 \pm 0.312^{a,1}$	$16.65 \pm 1.00^{a,1}$
		100	0	$0.763 \pm 0.056^{b,1}$	$0.771 \pm 0.057^{b,1}$	$2.956 \pm 0.061^{ab,1}$	-
			12	$1.019 \pm 0.069^{a,1}$	$1.107 \pm 0.033^{a,1}$	$3.323 \pm 0.173^{a,1}$	$17.08 \pm 0.30^{a,1}$
	Soy lecithin	25	0	$0.135 \pm 0.025^{c,2}$	$0.185 \pm 0.014^{ce,2}$	$1.290 \pm 0.116^{c,2}$	-
			12	$0.180 \pm 0.020^{ab,2}$	$0.222 \pm 0.007^{b,2}$	$1.397 \pm 0.045^{bc,2}$	$6.58 \pm 0.28^{c,2}$
		50	0	$0.151 \pm 0.015^{bc,2}$	$0.194 \pm 0.010^{ce,2}$	$1.361 \pm 0.141^{abc,2}$	-
			12	$0.181 \pm 0.011^{a,2}$	$0.227 \pm 0.005^{b,2}$	$1.421 \pm 0.008^{abc,2}$	$8.12 \pm 0.54^{b,2}$
		75	0	$0.157 \pm 0.016^{c,2}$	$0.197 \pm 0.011^{ce,2}$	$1.359 \pm 0.052^{abc,2}$	-
			12	$0.194 \pm 0.017^{ab,2}$	$0.234 \pm 0.005^{b,2}$	$1.446 \pm 0.058^{ab,2}$	$9.17 \pm 0.30^{b,2}$
		100	0	$0.162 \pm 0.012^{bc,2}$	$0.203 \pm 0.009^{c,2}$	$1.397 \pm 0.042^{abc,2}$	-
			12	$0.209 \pm 0.017^{a,2}$	$0.258 \pm 0.006^{a,2}$	$1.492 \pm 0.025^{a,2}$	$14.68 \pm 1.43^{a,2}$

Different superscript letters in the same column under the same glycerol content and surfactant type indicate significant differences ($p < 0.05$). Different superscript numbers in the same column under the same glycerol content, palm oil level and storage time indicate significant differences ($p < 0.05$). F_f : Flocculation factor; C_i : Coalescence index. * Mean ± SD (n = 3).

who reported that soy lecithin was an effective surfactant in FFD containing different leaf essential oils. Thus, palm oil level and surfactant type determined oil droplet size which might affect the distribution of droplets throughout the film.

For flocculation factor (F_f) and coalescence index (C_i), FFD samples containing soy lecithin showed the lower F_f and C_i than those comprising Tween-20 when the same glycerol contents and palm oil levels were used ($p < 0.05$). After emulsification, similar F_f of all FFD containing soy lecithin were observed when palm oil at different levels was incorporated ($p > 0.05$), regardless of glycerol contents. This was generally in accordance with similarity of d_{32} and d_{43} in these samples. For FFD containing Tween-20, no differences in F_f were found in FFD incorporated with 25, 50 and 75% palm oils after emulsification ($p > 0.05$). However, higher F_f was obtained for FFD with 100% palm oil. During 12 h of storage, the increases in F_f were observed for all FFD samples, compared to those found at 0 h (after emulsification). The results coincided with the increases in d_{32} and d_{43} in these samples after storage. It was suggested that the assembly of individual droplets by flocculation took place in the emulsion. For coalescence, the increases in coalescence index (C_i) were generally observed for all FFD samples after 12 h of storage ($p < 0.05$). Coalescence is the index for instability of the emulsion. The increase in C_i was attributed to the collapse of the oil droplets as evidenced by the higher d_{43} (Fredrick *et al.*, 2010). The highest C_i was noticeable in FFD containing high level of palm oil (100%), especially for FFD containing Tween-20 as a surfactant. Higher coalescence of droplets in FFD containing palm oil at high level was presumably caused by the closer assembly of dispersed oil droplets when higher amount of oil was present. This could enhance the coalescence of oil droplets in FFD. Thus, the palm oil levels and surfactant types had the marked impact on stability of emulsion in FFD.

2.4.1.2 Confocal scanning laser microscopy (CSLM)

CLSM images of FFD containing Tween-20 and soy lecithin incorporated with 100% palm oil in the absence or presence of glycerol after emulsification were illustrated in Figure 6. FFD using Tween-20 as a surfactant had the larger oil droplet, compared to those stabilized by soy lecithin, regardless of glycerol contents. The result was in agreement with the larger d_{32} and d_{43} of FFD containing

Tween-20 (Table 3). This CLSM image indicated that FFD containing soy lecithin had very small oil droplet which could not be observed by CLSM at this magnification (200×). However, the larger oil droplet size was obviously observed in this FFD after 12 h of storage (data not shown). Soy lecithin was therefore shown to prevent creaming or oil-phase separation more effectively than Tween-20. For FFS (without palm oil), no oil droplets were detected. CLSM results reconfirmed the role of surfactant in oil

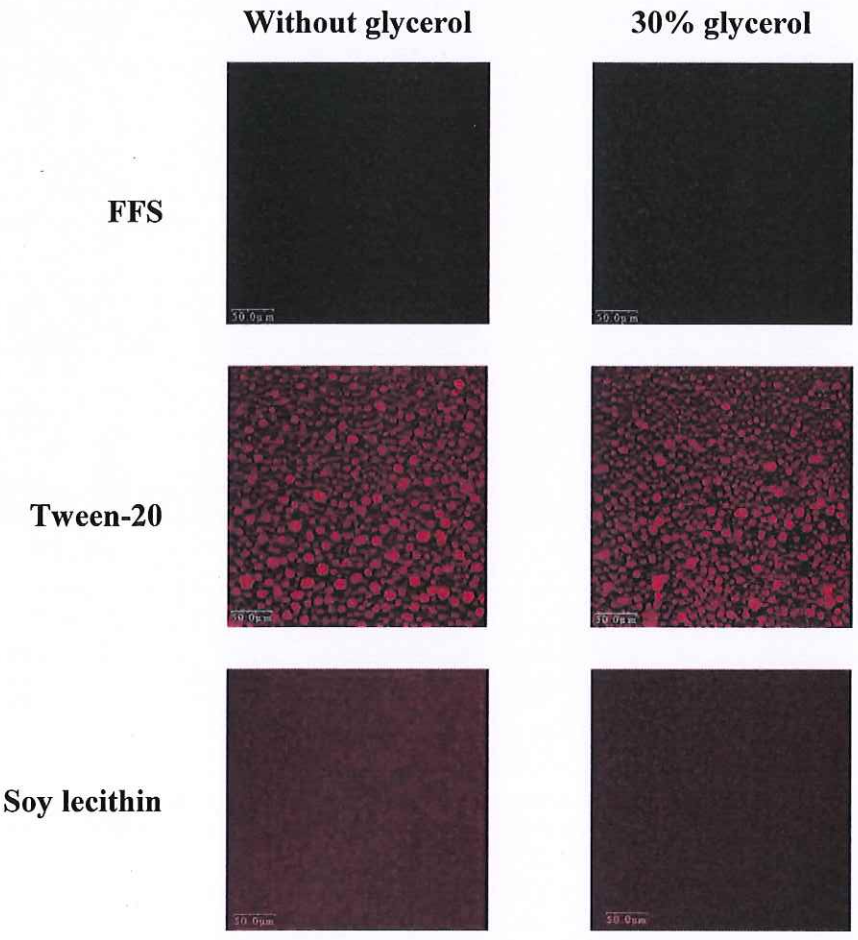

Figure 6. CLSM micrograph of film-forming dispersion containing 100% of palm oil without and with 30% glycerol in the presence of different surfactants. Magnification; 200×. FFS: film-forming solution (without palm oil).

2.4.2 Effect of palm oil levels and surfactant types on characteristics of gelatin films in the absence and presence of glycerol

2.4.2.1 Thickness

Film thickness of fish skin gelatin films incorporated with palm oil was higher than the control film (without incorporated palm oil), irrespective of surfactant types and glycerol contents ($p < 0.05$) (Table 4). At the same glycerol content and surfactant type, the continuous increases in film thickness were obtained when palm oil levels were increased from 25% to 100% ($p < 0.05$). The highest thickness was found in films added with 100% palm oil ($p < 0.05$). In the presence of oil droplets at a larger amount, the interaction between peptide chains could be reduced. As a result, the less compact network with protruded structure was developed. The thickness of films containing 30% glycerol was higher than films without glycerol ($p < 0.05$) when the same level of palm oil and surfactant were used. Moreover, the films prepared using different surfactants had varying thickness. Films containing soy lecithin had the lower thickness than those with Tween-20, regardless of palm oil levels and glycerol content ($p < 0.05$). The smaller oil droplets in FFD (Table 3, Figure 6) using soy lecithin as a surfactant were related well with the thinner corresponding films, compared with films having Tween-20. Small oil droplets could be distributed more uniformity throughout film matrix. As a result, the protein network with homogenous dispersion of oil droplets was formed, leading to the higher compactness of film. Thus, palm oil level, surfactants and glycerol level directly affected free volume and internally structural organization of film, which in turn determined thickness of films based on gelatin.

2.4.2.2 Mechanical properties

Mechanical properties expressed as tensile strength (TS) and elongation at break (EAB) of films incorporated with palm oil at various levels, with different types of surfactants are shown in Table 4. Addition of palm oil generally resulted in the lower TS, but higher EAB of resulting films, regardless of surfactants type and glycerol content ($p < 0.05$). Such changes were more pronounced with increasing palm oil levels,

Table 4. Properties of fish skin gelatin films incorporated with palm oil at various levels and different surfactants in the absence and presence of glycerol.

Glycerol (%)	Surfactant types	Palm oils (%)	Thickness (mm)	TS (MPa)	EAB (%)	WVP ($\times 10^{-12}$ g/m s Pa)
0	Without surfactant	Without palm oil	$0.047 \pm 0.002^{i,2}$	$76.90 \pm 2.19^{a,1}$	$7.12 \pm 1.17^{e,2}$	$21.53 \pm 0.29^{*a,2}$
	Tween-20	25	$0.061 \pm 0.002^{g,2}$	$59.40 \pm 3.77^{b,1}$	$14.45 \pm 0.57^{d,2}$	$13.17 \pm 0.21^{b,2}$
		50	$0.083 \pm 0.001^{e,2}$	$41.97 \pm 1.23^{c,1}$	$23.48 \pm 1.70^{c,2}$	$8.31 \pm 0.38^{c,2}$
		75	$0.094 \pm 0.001^{c,2}$	$32.18 \pm 1.91^{d,1}$	$30.88 \pm 2.05^{b,2}$	$6.17 \pm 0.66^{d,2}$
		100	$0.110 \pm 0.002^{a,2}$	$26.34 \pm 2.47^{e,1}$	$37.18 \pm 3.13^{a,2}$	$5.07 \pm 0.17^{e,2}$
	Soy lecithin	25	$0.058 \pm 0.002^{h,2}$	$57.74 \pm 3.20^{b,1}$	$14.76 \pm 2.98^{d,2}$	$12.92 \pm 0.34^{b,2}$
		50	$0.080 \pm 0.001^{f,2}$	$39.13 \pm 2.74^{c,1}$	$24.52 \pm 1.20^{c,2}$	$7.96 \pm 0.48^{c,2}$
		75	$0.089 \pm 0.002^{d,2}$	$30.69 \pm 1.00^{d,1}$	$31.66 \pm 2.42^{b,2}$	$5.99 \pm 0.24^{d,2}$
		100	$0.106 \pm 0.001^{b,2}$	$24.52 \pm 1.27^{e,1}$	$37.10 \pm 2.83^{a,2}$	$4.86 \pm 0.33^{e,2}$
30	Without surfactant	Without palm oil	$0.054 \pm 0.003^{h,1}$	$26.95 \pm 1.41^{a,2}$	$37.34 \pm 1.85^{g,1}$	$24.52 \pm 0.51^{a,1}$
	Tween-20	25	$0.076 \pm 0.005^{f,1}$	$23.01 \pm 1.44^{b,2}$	$97.86 \pm 2.74^{f,1}$	$14.02 \pm 0.50^{b,1}$
		50	$0.093 \pm 0.005^{d,1}$	$20.27 \pm 0.57^{c,2}$	$109.83 \pm 3.76^{e,1}$	$9.95 \pm 0.66^{c,1}$
		75	$0.109 \pm 0.003^{c,1}$	$15.05 \pm 1.23^{d,2}$	$116.46 \pm 1.56^{cd,1}$	$8.30 \pm 0.46^{d,1}$
		100	$0.133 \pm 0.005^{a,1}$	$10.53 \pm 1.58^{e,2}$	$124.01 \pm 3.39^{ab,1}$	$6.41 \pm 0.59^{e,1}$
	Soy lecithin	25	$0.066 \pm 0.002^{g,1}$	$22.87 \pm 2.17^{b,2}$	$99.71 \pm 3.27^{f,1}$	$13.68 \pm 0.31^{b,1}$
		50	$0.085 \pm 0.003^{e,1}$	$19.49 \pm 1.90^{c,2}$	$113.97 \pm 3.42^{de,1}$	$9.92 \pm 0.61^{c,1}$
		75	$0.099 \pm 0.001^{d,1}$	$14.46 \pm 0.62^{d,2}$	$119.99 \pm 1.20^{bc,1}$	$7.62 \pm 0.40^{d,1}$
		100	$0.124 \pm 0.003^{b,1}$	$8.92 \pm 0.74^{e,2}$	$126.35 \pm 4.80^{a,1}$	$6.37 \pm 0.30^{e,1}$

Different superscript letters in the same column under the same glycerol content indicate significant differences ($p < 0.05$).
Different superscript numbers in the same column under the same palm oil level and surfactant type indicate significant differences ($p < 0.05$). * Mean ± SD ($n = 3$).

when the same glycerol content and surfactant type were used. Among all films, the lowest TS, but the highest EAB were found in the film incorporated with 100% palm oil ($p < 0.05$). Addition of lipid or oil in protein-based or polysaccharide-based films may hinder polymer chain-to-chain interactions and provide flexible domains within the film (Limpisophon *et al.*, 2010). As a consequence, the decrease in rigidity with the concomitant increase in extensibility/elasticity of film was gained. In general, films containing 30% glycerol had lower TS along with higher EAB than those without glycerol ($p < 0.05$), regardless of surfactants and palm oils. Hoque *et al.* (2011) reported that increasing glycerol content enhanced the flexibility of gelatin film from cuttlefish skin. Glycerol with small size and good compatibility with protein could distribute in the matrix of film uniformly, in which the interaction between protein chains could be suppressed (Gontard *et al.*, 1993). Palm oil particularly at higher levels was able to hinder the interaction between protein chains in combination with glycerol. This could enhance the elasticity and flexibility of films more effectively as indicated by the higher increase in EAB with lowered TS. However, no differences in both TS and EAB between films containing Tween-20 and soy lecithin were noticeable ($p > 0.05$) when the same levels of glycerol was used. It is suggested that oil droplet size, in the range observed in emulsion at the same level of glycerol used, had no marked impact on protein–protein interaction in the film matrix. Therefore both palm oil and glycerol levels had a direct influence on the mechanical properties of the resulting films.

2.4.2.3 Water vapor permeability (WVP)

WVP of fish skin gelatin films incorporated with palm oil at levels of 25-100% (w/w, based on protein) and different surfactants (50% (w/w) based on palm oil) in the absence and presence of 30% glycerol is shown in Table 4. Films without glycerol had lower WVP than did those containing glycerol ($p < 0.05$). Glycerol as hydrophilic plasticizer could increase free volume and chain movement as well as water vapor absorptivity. This could allow water vapor to absorb and diffuse through film structure (Cerqueira *et al.*, 2012; Tongnuanchan *et al.*, 2012). WVP of films incorporated with palm oil decreased ($p < 0.05$), especially with increasing amount of palm oil ($p < 0.05$), regardless of glycerol contents and surfactant types. The addition of hydrophobic substances such as palm oil could increase the hydrophobicity of films,

thereby enhancing the barrier property of film toward the water vapor migration through the film. This result was in agreement with Tongnuanchan *et al.* (2013b) who reported that roots essential oil (ginger, turmeric and plai) incorporated into fish skin gelatin film decreased WVP of resulting films, especially with increasing essential oil amount. Limpisophon *et al.* (2010) also reported that the increasing stearic acid from 0 to 100% of protein concentration in the film-forming solution decreased water vapor permeability of gelatin-fatty acid emulsion films. It was noted that there were no differences in WVP between films containing both Tween-20 and soy lecithin ($p > 0.05$), when palm oil and glycerol at the same level were present. This result indicated that the efficiency in preventing water vapor migration of films containing Tween-20 and soy lecithin was not different. Soy lecithin consists of hydrophilic head groups with the lipophilic tail groups (McClements, 2005). This effectively showed the stabilizing effect and provided the small oil droplets in FFDs (Table 3). Despite the oil droplets with small size with coincidentally large surface area, the use of soy lecithin could lower hydrophobicity of films. Hydrophilic charged domain of soy lecithin preferably bound with water molecule, leading to the increased WVP of films. For Tween-20, this surfactant is the non-ionic surfactants with large polyol head groups and has high HLB numbers (McClements, 2005). Nevertheless, large oil droplets of FFD containing Tween-20 might decrease the tortuosity factor for the transfer of water molecules. Thus, WVP of gelatin films mainly depended on the level of palm oil and partially governed by the nature of surfactants used. The incorporation of high palm oil level ($\geq 50\%$) yielded the film with improved water vapor barrier property.

2.4.2.4 Color, light transmittance and film transparency

The color of gelatin films incorporated with palm oil at various levels in the absence or presence of glycerol, in which Tween-20 and soy lecithin used as surfactants, is shown in Table 5. Overall, the palm oil levels and surfactant types exhibited the profound impact on the color of films. L^*-values continuously decreased, while the b^*- and ΔE^*-values increased ($p < 0.05$) as palm oil levels increased from 25 to 100%. The result suggested that the coloring components e.g. pigment in palm oil most likely caused the color changes of gelatin film (Hopkins *et al.*, 2015; Tongnuanchan *et al.*, 2013b). Surfactants were also found to show the impact on the color of resulting films. Films using soy lecithin as a surfactant had the lower L^*-value

Table 5. Color of fish skin gelatin films incorporated with palm oil at various levels and different surfactants in the absence and presence of glycerol.

Glycerol (%)	Surfactant types	Palm oils (%)	L^*	a^*	b^*	ΔE^*
0	Without surfactant	Without palm oil	$90.42 \pm 0.033^{*a,1}$	$-0.96 \pm 0.017^{a,1}$	$0.61 \pm 0.043^{i,1}$	$3.20 \pm 0.032^{i,1}$
	Tween-20	25	$90.26 \pm 0.035^{b,1}$	$-1.09 \pm 0.022^{b,1}$	$1.40 \pm 0.020^{h,1}$	$3.49 \pm 0.029^{h,1}$
		50	$90.11 \pm 0.017^{c,1}$	$-1.25 \pm 0.017^{c,1}$	$1.56 \pm 0.048^{g,1}$	$3.69 \pm 0.028^{g,1}$
		75	$90.06 \pm 0.033^{d,1}$	$-1.31 \pm 0.037^{d,1}$	$1.84 \pm 0.033^{f,1}$	$3.83 \pm 0.023^{f,1}$
		100	$89.76 \pm 0.028^{e,1}$	$-1.43 \pm 0.062^{e,1}$	$2.16 \pm 0.098^{e,1}$	$4.25 \pm 0.071^{e,1}$
	Soy lecithin	25	$88.54 \pm 0.014^{f,1}$	$-1.44 \pm 0.029^{e,1}$	$4.46 \pm 0.026^{d,1}$	$6.48 \pm 0.015^{d,1}$
		50	$88.10 \pm 0.022^{g,1}$	$-1.52 \pm 0.013^{f,1}$	$7.53 \pm 0.041^{c,1}$	$9.00 \pm 0.036^{c,1}$
		75	$87.51 \pm 0.044^{h,1}$	$-1.79 \pm 0.010^{g,1}$	$9.60 \pm 0.085^{b,1}$	$11.04 \pm 0.056^{b,1}$
		100	$87.06 \pm 0.046^{i,1}$	$-1.87 \pm 0.010^{h,1}$	$13.59 \pm 0.043^{a,1}$	$14.72 \pm 0.022^{a,1}$
30	Without surfactant	Without palm oil	$90.47 \pm 0.018^{a,1}$	$-0.98 \pm 0.017^{a,1}$	$0.67 \pm 0.027^{i,1}$	$3.39 \pm 0.020^{i,1}$
	Tween-20	25	$90.27 \pm 0.028^{b,1}$	$-1.11 \pm 0.029^{b,1}$	$1.40 \pm 0.015^{h,1}$	$3.48 \pm 0.026^{h,1}$
		50	$90.12 \pm 0.018^{c,1}$	$-1.18 \pm 0.026^{c,1}$	$1.61 \pm 0.034^{g,1}$	$3.69 \pm 0.012^{g,1}$
		75	$90.02 \pm 0.038^{d,1}$	$-1.31 \pm 0.032^{d,1}$	$1.82 \pm 0.070^{f,1}$	$3.86 \pm 0.054^{f,1}$
		100	$89.78 \pm 0.026^{e,1}$	$-1.54 \pm 0.032^{e,1}$	$2.15 \pm 0.047^{e,1}$	$4.24 \pm 0.032^{e,1}$
	Soy lecithin	25	$88.55 \pm 0.013^{f,1}$	$-1.41 \pm 0.022^{d,1}$	$4.46 \pm 0.038^{d,1}$	$6.48 \pm 0.028^{d,1}$
		50	$88.11 \pm 0.013^{g,1}$	$-1.52 \pm 0.013^{e,1}$	$7.52 \pm 0.039^{c,1}$	$8.98 \pm 0.027^{c,1}$
		75	$87.49 \pm 0.029^{h,1}$	$-1.78 \pm 0.030^{f,1}$	$9.54 \pm 0.073^{b,1}$	$11.00 \pm 0.074^{b,1}$
		100	$87.05 \pm 0.043^{i,1}$	$-1.86 \pm 0.034^{g,1}$	$13.62 \pm 0.053^{a,1}$	$14.75 \pm 0.036^{a,1}$

Different superscript letters in the same column under the same glycerol content indicate significant differences ($p < 0.05$). Different superscript numbers in the same column under the same palm oil level and surfactant type indicate significant differences ($p < 0.05$). * Mean ± SD ($n = 3$).

with the concomitantly higher b^*- and ΔE^*-values than those containing Tween-20 ($p < 0.05$). The result indicated the higher yellowness in films prepared using soy lecithin as surfactant in comparison with those having Tween-20. Brownish yellow color of soy lecithin mainly contributed to the lower L^*-value and higher b^*-value of resulting film. This result was in agreement with Tongnuanchan *et al.* (2013a) who reported that the color of fish skin gelatin film incorporated with leaf essential oil tended to be yellowish when soy lecithin was used as a surfactant.

Light transmission at selected wavelengths from 200 to 800 nm in UV and visible ranges and transparency value of films from fish skin gelatin incorporated with palm oil at different levels (25-100%) and Tween-20 or soy lecithin as surfactants in the absence or presence of 30% glycerol are shown in Table 6. All gelatin films had the excellent barrier property against UV light at 200 and 280 (Table 6), especially when higher levels of palm oil were used. Protein based films had greater UV light barrier capacity owing to their high amount of aromatic amino acids that absorb UV light (Hamaguchi *et al.*, 2007). This result was in accordance with those of gelatin films from skins of tilapia (Tongnuanchan *et al.*, 2013b), bigeye snapper and brownstripe red snapper (Jongjareonrak *et al.*, 2006). Lower transmission of visible light in the range of 350-800 nm was observed in all emulsion films, compared with control film. The highest barrier property toward light transmission was obtained for films incorporated with 100% palm oil. This was more likely due to the increase in opaqueness of films containing palm oils, in which oil droplets were distributed throughout the films (Ma *et al.*, 2012). When soy lecithin was used as surfactant, higher light transmittance at wavelengths of 350-800 nm was observed, compared with film containing Tween-20. The larger oil droplets in FFD containing Tween-20 most likely rendered the film with more opaqueness. Those large oil droplets could prevent the transmission of light through the film more effectively. This was coincidental with the higher turbidity of film with Tween-20 as a surfactant. For transparency values, the lower transparency value indicated that the film was more transparent. Films with all palm oil levels had the higher transparency value than the control films ($p < 0.05$) (Table 6). This result suggested that the incorporation of palm oil yielded less transparent films. Oil droplets localized in the film matrix lowered the transparency of gelatin film, more likely due to

Table 6. Light transmittance and transparency value of fish skin gelatin films incorporated with palm oil at various levels and different surfactants in the absence and presence of glycerol.

Glycerol (%)	Surfactant types	Palm oils (%)	Light transmittance (%) at different wavelength (nm)								Transparency value
			200	280	350	400	500	600	700	800	
0	Without surfactant	Without palm oil	0.04	43.73	82.19	84.67	86.99	88.09	89.11	89.57	$1.23 \pm 0.004^{*i,1}$
	Tween-20	25	0.01	3.65	19.34	27.04	38.72	47.62	54.78	60.60	$5.33 \pm 0.005^{a,2}$
		50	0.01	0.99	10.76	18.12	30.67	40.54	48.47	55.05	$4.86 \pm 0.003^{b,2}$
		75	0.01	0.77	8.87	15.24	26.46	35.56	42.93	49.05	$4.79 \pm 0.005^{c,2}$
		100	0.00	0.45	6.61	12.04	22.24	31.12	38.56	44.82	$4.60 \pm 0.004^{d,2}$
	Soy lecithin	25	0.00	5.27	33.26	46.78	62.79	69.79	73.40	78.13	$2.58 \pm 0.001^{e,1}$
		50	0.01	0.90	21.31	34.59	51.89	66.29	69.09	73.86	$2.56 \pm 0.001^{f,1}$
		75	0.00	0.19	10.85	31.80	49.01	62.06	67.63	70.93	$2.36 \pm 0.003^{g,1}$
		100	0.00	0.12	5.28	22.28	45.02	58.71	62.52	68.35	$2.01 \pm 0.031^{h,1}$
30	Without surfactant	Without palm oil	0.03	40.59	77.14	80.75	83.84	85.50	86.69	87.56	$1.29 \pm 0.001^{i,1}$
	Tween-20	25	0.00	2.08	10.15	14.78	24.46	33.70	41.16	47.23	$5.55 \pm 0.014^{a,1}$
		50	0.01	0.85	7.77	13.55	23.57	32.00	39.86	46.94	$5.30 \pm 0.008^{b,1}$
		75	0.00	0.39	5.04	9.73	19.57	28.58	36.33	42.89	$4.82 \pm 0.010^{c,1}$
		100	0.00	0.20	2.75	5.38	11.63	18.43	25.18	31.61	$4.78 \pm 0.012^{d,1}$
	Soy lecithin	25	0.02	8.81	37.51	48.44	60.36	71.88	75.83	79.77	$2.21 \pm 0.002^{e,2}$
		50	0.02	3.50	28.44	43.29	59.64	67.71	74.12	78.35	$2.05 \pm 0.001^{f,2}$
		75	0.02	2.07	23.72	39.22	57.43	65.61	69.30	71.84	$1.91 \pm 0.001^{g,2}$
		100	0.02	0.39	11.14	33.26	51.46	61.56	65.87	69.79	$1.83 \pm 0.002^{h,2}$

Different superscript letters in the same column under the same glycerol content indicate significant differences ($p < 0.05$).
Different superscript numbers in the same column under the same palm oil level and surfactant type indicate significant differences ($p < 0.05$). * Mean ± SD ($n = 3$).

the light scattering effect (Tongnuanchan *et al.*, 2012). In general, films with soy lecithin showed the lower transparency value than those using Tween-20 ($p < 0.05$), indicating that the formers were more transparent than the latter. In the presence of glycerol (30%), films had lower transparency value, when palm oil at the same level and the same surfactant were used. Glycerol could insert themselves among the protein chains, rendering the less compactness of resulting films. This could result in the higher transmission of light through the films. Thus, the incorporation of different palm oil levels and surfactants resulted in the varying color, light transmittance and transparency of emulsified films.

2.4.2.5 Film morphology

SEM micrographs of the surface and freeze-fractured cross-section of films from fish skin gelatin incorporated with 100% palm oil, in which Tween-20 and soy lecithin were used in the absence and presence of 30% glycerol, are illustrated in Figure 7. The control film (without incorporated palm oils) had the smooth and continuous surface for both glycerol levels. Smooth surface was obtained for film containing soy lecithin, whereas those involving Tween-20 had the small pin holes. This result indicated that FFD using soy lecithin as a surfactant had the stable emulsion system and no collapse of emulsion occurred during casting and drying. For cross-section, the control film containing 0% glycerol had the higher compact structure, compared with others. The cross-section of films became rougher when palm oil was added. Film with Tween-20 showed the rougher network than that containing soy lecithin. The protein-protein disruption in the film matrix arose from droplets of palm oils could enhance roughness of film cross-section (Tongnuanchan *et al.*, 2013b). Those oil droplets, particularly with the larger size, were localized inside the film network and interrupted protein-protein chain interaction. This more likely brought about the discontinuous network as evidenced by the increase irregular network. For film containing soy lecithin, small oil droplets were localized uniformly and did not show the marked hindering effect on protein-protein interaction. However, glycerol had no effect on the microstructure of gelatin-based film added with palm oil. The film microstructure might be associated with mechanical properties and WVP of films incorporated with palm oils.

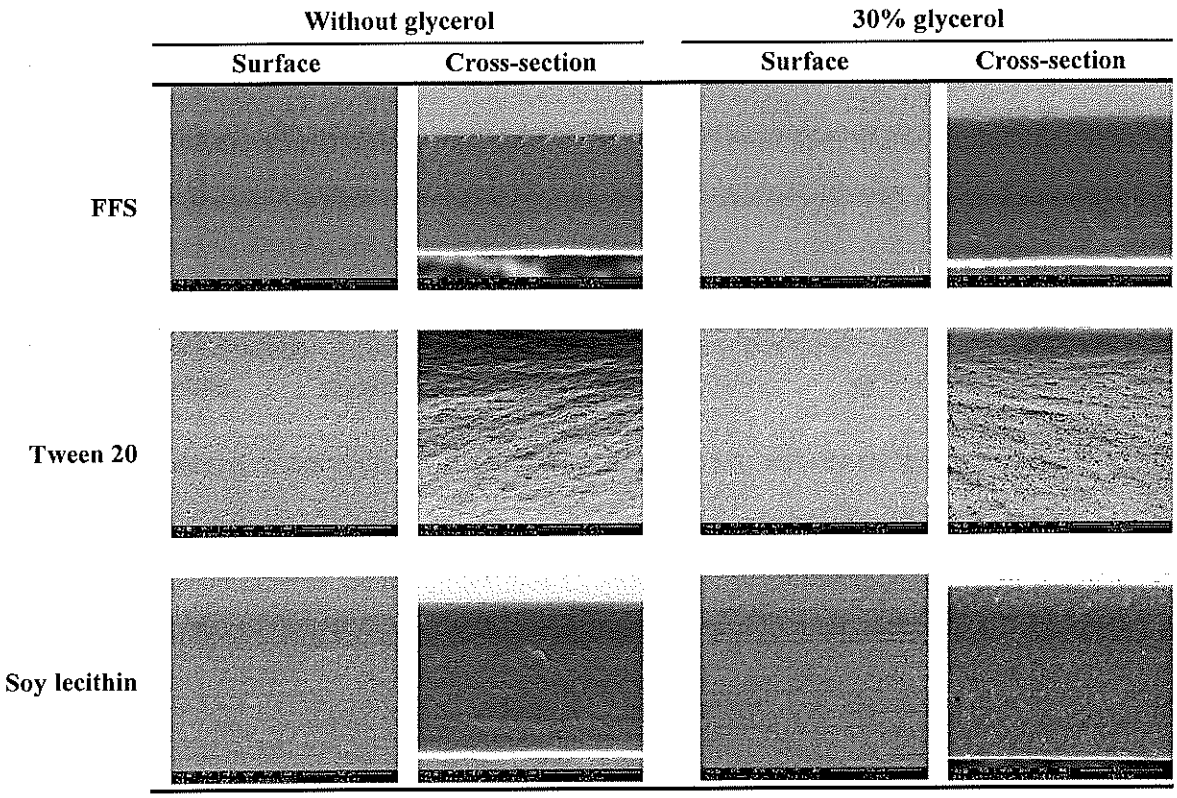

Figure 7. SEM micrographs of surface (magnification: 500×) and cross-section (magnification: 1800×) of fish skin gelatin film containing 100% of palm oil without and with 30% glycerol in the presence of different surfactants. FFS: film-forming solution (without palm oil).

2.5 Conclusions

Incorporation of palm oil, type of surfactants and glycerol affected the stability of FFD and properties of gelatin films. FFD containing soy lecithin showed the small oil droplet size and the emulsion was more stable during storage. Films showed the increased water vapor barrier property and elasticity as palm oil levels increased. Films without glycerol had lower WVP and EAB with higher TS than those containing 30% glycerol. Decreases in L^* value and light transmittance with coincidental increases in b^*- and ΔE^* values were observed in films, especially when the amount of palm oil incorporated increased. The yellowish color and smooth surface were observed in films using soy lecithin as a surfactant. Thus, the incorporation of 50 or 75% palm oil using soy lecithin as a surfactant in the presence of 30% glycerol could

enhance stability of emulsion in FFD, which directly determined properties and morphology of film.

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CHAPTER 3
INFLUENCE OF PALM OIL AND GLYCEROL ON PROPERTIES OF FISH SKIN GELATIN-BASED FILMS

3.1 Abstract

Properties of fish skin gelatin film incorporated with palm oil at 50% and 75% (w/w, based on protein content) as affected by glycerol at 0-30% (w/w, based on protein content) were investigated. Increases in water vapor permeability and elongation at break along with decrease in tensile strength were noticeable when levels of glycerol were increased ($p < 0.05$). Decreases in L^*- and a^*-values with coincidental increases in b^*- and ΔE^*-values were observed in emulsified films when amount of palm oil incorporated increased ($p < 0.05$). Light transmittance of all films increased as glycerol levels were increased ($p < 0.05$). FTIR results suggested that the protein-protein interaction in film matrix was decreased when palm oil was incorporated. Films added with palm oil had lower glass transition and degradation temperatures than control films. The addition of 75% palm oil and 10% glycerol could improve water vapor barrier property of fish skin gelatin films without the drastic alteration of mechanical properties.

3.2 Introduction

Biopolymer films have gained interest in their use as edible food packaging. Edible films can be defined as thin continuous layer of biopolymer materials which can be applied as a coating on food, used as a wrap or made into pouch to hold the food or to protect it against external factors (Krochta and De Mulder-Johnston, 1997). Proteins from various sources have been used as materials for biodegradable film because of their relative abundance and good film-forming ability (Hamaguchi *et al.*, 2007). Gelatin is one of proteins possessing an excellent film-forming property and can be used for edible coatings and film making (Gennadios *et al.*, 1994). Nevertheless, the gelatin films have a poor water vapor barrier ability (Sobral *et al.*, 2001). Hydrophobic substances such as lipid have been incorporated to improve water barrier property of gelatin film (Limpisophon *et al.*, 2010). Those substances are not dissolved

in aqueous phase, in which film-forming dispersion or emulsion must be prepared before making the films. The homogeneity of oil droplets and stability of emulsion in the film-forming dispersion (FFD) during film drying give rise to a homogeneous distribution of the lipid droplets in the film, which in turn contributes to the efficient control of water vapor migration (Debeaufort *et al.*, 1993). To disperse oil in film-forming aqueous phase, the appropriate surfactant and homogenization condition are required. Microfluidization is a potential technique to bring about the fine and more uniform oil droplets as well as more emulsion stability, compared to typical homogenization.

Palm oil is the natural hydrophobic substance obtained from the mesocarp of palm seed. Throughout the world, 90% of palm oil is used for human consumption (Edem, 2002). Due to its abundance and cheap price, palm oil could be used as a promising hydrophobic substance to improve water vapor barrier property of gelatin film. Recently, the incorporation of palm oil in combination with 30% glycerol using lecithin as surfactant in film-forming dispersion could reduce the water vapor transfer through the film to some degree (Tongnuanchan *et al.*, 2015). The uses of reduced amount of hydrophilic glycerol might be a means to lower hydrophilicity of resulting film. However, no information regarding the impact of glycerol in combination with palm oil on properties of gelatin films exists. Thus, this study aimed to investigate the influence of glycerol amounts on mechanical, physical and thermal properties as well as molecular interaction of fish skin gelatin films incorporated with palm oil at different levels.

3.3 Materials and methods

3.3.1 Chemicals and gelatin

Glycerol and soy lecithin were purchased from Sigma–Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade. Tilapia skin gelatin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Palm oil was obtained from *OLEEN Company Limited* (Bangkok, Thailand).

3.3.2 Preparation of film

Gelatin powder was mixed with distilled water and heated at 70 °C for 30 min to obtain the protein concentration of 3.5% (w/v). Glycerol at 0, 10, 20 and 30% (w/w, based on protein content) was used as a plasticizer. Palm oil previously mixed with soy lecithin at 50% (w/w, based on palm oil content) was added into gelatin solution at levels of 50% and 75% (w/w, based on protein content). The mixtures were emulsified using a rotor-stator homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia) at 22,000 rpm for 3 min and a Microfluidics homogenizer (Model HC-5000, Microfluidizer, Newton, MA, USA) at 2,000 psi for 2 passes. Film-forming dispersion (FFD) was subjected to the removal of dissolved air by a vacuum pump (Diaphragm vacuum pump, Wertheim Germany) for 30 min at room temperature (28-30 °C). FFD (4 mL) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown for 12 h at room temperature prior to further drying at 25 °C and 50 ± 5% RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). Control films (without palm oil) were prepared from film-forming solution in the absence and presence of glycerol (30%). All films were manually peeled off and subjected to analyses.

3.3.3 Analyses of film

3.3.3.1 Film thickness

The thickness of film was measured using a digital micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Five random locations around each film of ten film samples were used for average thickness determination.

3.3.3.2 Mechanical properties

Prior to testing, films were conditioned for 48 h at 25 °C and 50 ± 5% RH. Tensile strength (TS) and elongation at break (EAB) were determined as described by Iwata *et al.* (2000) with a slight modification using the Universal Testing Machine

(Lloyd Instrument, Hampshire, UK). Ten samples (2×5 cm^2) with initial grip length of 3 cm were used for testing. Cross-head speed was set at 30 mm/min.

3.3.3.3 Water vapor permeability (WVP)

WVP was measured using a modified ASTM method (ASTM, 1989) as described by Shiku *et al.* (2004). The films were sealed on an aluminium permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and a rubber gasket to hold the films in place. The cups were placed in a desiccator containing the distilled water at 30 °C. The cups were weighed at 1-h intervals over a 10-h period. WVP of the film was calculated as follows:

$$WVP \text{ (g/m s Pa)} = \frac{wl}{At(P_2-P_1)}$$

where w is the weight gain of the cup (g); l is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); P_2-P_1 is the vapor pressure difference across the film (4242 Pa at 30 °C).

3.3.3.4 Color

Film samples were subjected to color measurement using a CIE colorimeter (Hunter associates laboratory, Inc., Reston, VA, USA). D_{65} (day light) and a measure cell with opening of 30 mm was used. The color of the films was expressed as L^*-value (lightness), a^*-value (redness/greenness) and b^*-value (yellowness/blueness). Total color difference (ΔE^*) was calculated as follows (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences between the color parameter of the samples and those of the white standard ($L^* = 93.63$, $a^* = -0.95$ and $b^* = 0.46$).

3.3.3.5 Light transmittance and transparency value

The light transmittance of films was measured in the range of 200-800 nm using a UV-vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) as described by Shiku *et al.* (2004) The transparency value of film was calculated using the following equation (Han and Floros, 1997):

$$\text{Transparency value} = \frac{-\log T_{600}}{x}$$

where T_{600} is the fractional transmittance at 600 nm and x is the film thickness (mm). The greater transparency value represents the lower transparency of film.

3.3.3.6 Differential scanning calorimetry

Thermal transitions of films were scanned using a differential scanning calorimeter (DSC) (Perkin Elmer, Model DSC-7, Norwalk, CT, USA) as described by Tongnuanchan *et al.* (2015) Temperature calibration was performed using the indium thermogram. Film samples (2-5 mg) were accurately weighed into aluminium pans, hermetically sealed, and scanned over the temperature range of -20 to 150 °C, with a heating rate of 5 °C/min. Liquid nitrogen was used as cooling medium and the system was equilibrated at -20 °C for 5 min prior to the scan. An empty aluminium pan was used as the reference. A second scan was also performed in the same manner, followed by quench-cooling of the sample after completing the first scan. Glass transition temperature (T_g), melting transition temperature (T_{max}) and the melting enthalpy (ΔH) were determined.

3.3.3.7 Thermo-gravimetric analysis (TGA)

Films were scanned using a thermo-gravimetric analyser (TGA7, PerkinElmer, Norwalk, CT, USA) from 25 to 800°C at a rate of 10 °C/min (Nuthong *et al.*, 2009). Thermal degradation temperature (T_d) and weight loss of film samples were determined.

3.3.3.8 Attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy

Films were scanned with a Bruker Model Equinox 55 FTIR spectrometer (Bruker Co., Ettlingen, Germany) equipped with a horisontal ATR Trough plate crystal cell (45° ZnSe; 80 mm long, 10 mm wide and 4 mm thick) (PIKE Technology Inc., Madison, WI, USA) at 25 °C as described by Nuthong *et al.* (2009)

Films were placed onto the crystal cell and the cell were clamped into the mount of FTIR spectrometer. The spectra in range of 650-4000 cm^{-1} were determined.

3.3.4 Statistical analysis

Completely randomised design (CRD) was used throughout the study. All experiments were run in triplicate with different three lots of films. Data were subjected to analysis of variance (ANOVA), and mean comparisons were carried out by the Duncan's multiple range test (Steel *et al.*, 1980). Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL, USA).

3.4 Results and discussion

3.4.1 Thickness

Thickness of fish skin gelatin films containing glycerol at various levels (0-30% w/w, based on protein) in the presence of palm oil at levels of 50% and 75% (w/w, based on protein content) is shown in Table 7. For the control films, that containing 30% glycerol had higher thickness than that without glycerol addition ($p < 0.05$). Emulsified films generally had the higher film thickness than the control films, regardless of glycerol levels ($p < 0.05$). The thickness of film incorporated with 75% palm oil was higher than that of film containing 50% palm oil, when the same glycerol level was used ($p < 0.05$). When the same level of palm oil was incorporated, the continuous increases in film thickness were found when glycerol levels were increased from 0 to 30% ($p < 0.05$). The highest film thickness was obtained in film containing 75% palm oil in the presence of 20% or 30% glycerol ($p < 0.05$). Addition of glycerol, which is a small hydrophilic molecule, into the film as a plasticizer reduced the protein-protein interaction (Gontard *et al.*, 1993). As a result, the less compactness was found as evidenced by the increased film thickness in the film containing a larger amount of glycerol. The result was in agreement with that reported by Limpisophon *et al.* (2010) Moreover, oil droplets also lowered the interaction between peptide chains. Oil droplets could distribute and insert between the protein chains, thereby lowering the compactness of film network (Tongnuanchan *et al.*, 2013). Thus, palm oil and glycerol

Table 7. Thickness, mechanical properties and water vapor permeability of fish skin gelatin films incorporated without and with palm oil and glycerol at various levels.

Palm oils (%)	**Glycerols** (%)	**Thickness** (mm)	**TS** (MPa)	**EAB** (%)	**WVP** ($\times 10^{-12}$ g/m s Pa)
Without palm oil	0	0.046 ± 0.002^{h}	77.96 ± 1.56^{a}	7.47 ± 0.90^{j}	21.21 ± 0.57^{b}
	30	0.054 ± 0.004^{g}	26.04 ± 1.59^{ef}	38.17 ± 2.37^{g}	22.9 ± 0.69^{a}
50	0	0.077 ± 0.002^{f}	41.95 ± 3.89^{b}	23.45 ± 4.01^{i}	7.96 ± 0.19^{e}
	10	0.079 ± 0.002^{ef}	35.55 ± 3.74^{c}	47.46 ± 5.57^{f}	8.51 ± 0.04^{d}
	20	0.082 ± 0.002^{de}	26.63 ± 1.85^{e}	70.20 ± 6.10^{d}	8.89 ± 0.12^{d}
	30	0.085 ± 0.003^{d}	19.50 ± 2.32^{g}	108.64 ± 7.19^{b}	10.56 ± 0.22^{c}
75	0	0.088 ± 0.001^{c}	35.80 ± 1.38^{c}	31.63 ± 1.63^{h}	5.87 ± 0.28^{g}
	10	0.090 ± 0.002^{bc}	30.77 ± 1.42^{d}	57.51 ± 4.07^{e}	6.65 ± 0.15^{f}
	20	0.092 ± 0.001^{ab}	23.35 ± 1.29^{f}	80.35 ± 5.55^{c}	7.06 ± 0.08^{f}
	30	0.095 ± 0.002^{a}	14.12 ± 2.38^{h}	116.25 ± 8.10^{a}	7.92 ± 0.11^{e}

Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

TS, tensile strength; EAB, elongation at break; WVP, water vapor permeability. * Mean ± SD (n = 3).

levels directly affected free volume of film, which in turn determined thickness of gelatin films.

3.4.2 Mechanical properties

Tensile strength (TS) and elongation at break (EAB) of gelatin films containing glycerol (0-30% w/w, based on protein content) and palm oil (50% and 75% w/w, based on protein content) are shown in Table 7. For the control films (without palm oil), the lower TS and higher EAB were found for the control film containing 30% glycerol ($p < 0.05$). At either 0% or 30% glycerol, the emulsified films showed the lower TS and higher EAB than those without palm oil ($p < 0.05$). At the same palm oil level used, the decreases in TS with coincidental increases in EAB were observed when the level of glycerol increased ($p < 0.05$). The film incorporated with 75% palm oil showed the lower TS, but higher EAB than those containing 50% palm oil ($p < 0.05$), regardless of glycerol levels. Addition of lipid or oil in protein-based or polysaccharide-based films may hinder polymer chain-to-chain interactions and provide flexible domains within the film (Limpisophon *et al.*, 2010). As a consequence, the decrease in rigidity with the concomitant increase in extensibility/elasticity of film was gained. With the addition of 75% palm oil, TS of film decreased from 35.80 to 14.12 MPa ($p < 0.05$) with increasing glycerol levels from 0 to 30%. In contrast, EAB of film increased from 31.63% to 116.25%. Limpisophon *et al.* (2009) reported that increasing glycerol content enhanced the flexibility of gelatin film from blue shark (*Prionace glauca*) skin. Glycerol with small size could distribute in the aqueous phase uniformly, in which the interaction between protein chains could be suppressed. Palm oil particularly at higher levels in conjunction with glycerol was able to lower the interaction between protein chains in film matrix. This could increase the elasticity and flexibility of films as evidenced by the higher EAB along with lower TS. Therefore, the mechanical properties of resulting films were influenced by both palm oil and glycerol.

3.4.3 Water vapor permeability (WVP)

WVP of fish skin gelatin films containing palm oil and glycerol at various levels is shown in Table 7. Generally, high WVP was observed in the control

films (without palm oil). The control film with 30% glycerol showed higher WVP than that without glycerol ($p < 0.05$). For emulsified films, WVP increased with increasing glycerol contents ($p < 0.05$). The film incorporated with 75% palm oil showed the lower WVP than those containing 50% palm oil ($p < 0.05$), regardless of glycerol levels. Addition of hydrophobic substances such as palm oil could increase the hydrophobicity of films, thereby reducing the water vapor adsorption and also migration through the film. This result was in agreement with Tongnuanchan *et al.* (2013) who reported that roots essential oil (ginger, turmeric and plai) incorporated into fish skin gelatin film decreased WVP of resulting films, especially with increasing essential oil amount. Limpisophon *et al.* (2009) also reported that the increasing stearic acid from 0 to 100% in the film-forming solution decreased WVP of gelatin-fatty acid emulsified films. It was noted that the addition of palm oil into gelatin film in combination with reduced amount of glycerol could decrease WVP of gelatin film effectively. The incorporation of high palm oil level (75%) with low glycerol level (0% and 10%) yielded the film with high water vapor barrier property.

3.4.4 Color, light transmittance and film transparency

The color of gelatin films containing glycerol (0-30% w/w, based on protein content) and palm oil (50% and 75% w/w, based on protein content) is shown in Table 8. In general, the addition of palm oil resulted in the changes in color of gelatin films. Film incorporated with palm oil showed the lower L^*- and a^*-values along with higher b^* and ΔE^*-values than the control films (without palm oil) ($p < 0.05$). Those changes were more pronounced with increasing amount of palm oil added. In general, films added with palm oil became more yellowish as indicated by the increased b^*-value. This result suggested that the coloring components in palm oil most likely contributed to the color of gelatin film (Hopkins *et al.*, 2015; Tongnuanchan *et al.*, 2013). No differences in color between films containing different levels of glycerol were found ($p > 0.05$), when the palm oil at the same level was used. Therefore, the level of palm oil noticeably affected the color of gelatin film.

Light transmission at the selected wavelengths from 200 to 800 nm in UV and visible ranges and transparency value of films from fish skin gelatin containing palm oil at levels of 50% and 75% in the presence of glycerol at various levels is shown

Table 8. Color of fish skin gelatin films incorporated without and with palm oil and glycerol at various levels.

Palm oils (%)	**Glycerols (%)**	L^*	a^*	b^*	ΔE^*
Without palm oil	0	90.42 ± 0.33*[a]	-0.96 ± 0.04 [a]	0.62 ± 0.07 [a]	3.20 ± 0.32 [a]
	30	90.41 ± 0.22 [a]	-0.95 ± 0.04 [a]	0.64 ± 0.02 [a]	3.21 ± 0.21 [a]
50	0	88.35 ± 0.10 [b]	-1.54 ± 0.02 [b]	7.52 ± 0.36 [b]	8.84 ± 0.29 [b]
	10	88.35 ± 0.22 [b]	-1.54 ± 0.03 [b]	7.52 ± 0.39 [b]	8.83 ± 0.41 [b]
	20	88.34 ± 0.17 [b]	-1.55 ± 0.01 [b]	7.53 ± 0.17 [b]	8.85 ± 0.15 [b]
	30	88.33 ± 0.25 [b]	-1.53 ± 0.03 [b]	7.53 ± 0.37 [b]	8.85 ± 0.19 [b]
75	0	87.17 ± 0.06 [c]	-1.81 ± 0.02 [c]	9.68 ± 0.19 [c]	11.29 ± 0.18 [c]
	10	87.16 ± 0.24 [c]	-1.81 ± 0.02 [c]	9.68 ± 0.19 [c]	11.30 ± 0.21 [c]
	20	87.16 ± 0.36 [c]	-1.80 ± 0.02 [c]	9.68 ± 0.13 [c]	11.30 ± 0.18 [c]
	30	87.15 ± 0.24 [c]	-1.82 ± 0.02 [c]	9.68 ± 0.19 [c]	11.30 ± 0.20 [c]

Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

* Mean ± SD (n = 3).

Table 9. Light transmittance and transparency value of fish skin gelatin films incorporated without and with palm oil and glycerol at various levels.

Palm oils (%)	Glycerols (%)	Light transmittance (%) at different wavelength (nm)								Transparency value
		200	280	350	400	500	600	700	800	
Without palm oil	0	0.02	37.14	73.39	77.74	81.74	83.96	85.48	86.64	1.40 ± 0.001 *h
	30	0.03	42.07	83.13	85.14	87.39	88.56	89.23	89.70	1.15 ± 0.002 g
50	0	0.00	0.82	21.69	35.54	53.68	63.05	71.49	76.17	2.53 ± 0.029 a
	10	0.00	1.03	22.83	36.75	55.05	64.96	72.89	76.28	2.36 ± 0.027 b
	20	0.00	1.23	23.58	37.39	55.58	65.25	73.17	77.67	2.29 ± 0.017 c
	30	0.00	1.32	25.92	40.37	58.30	66.53	74.92	79.13	2.08 ± 0.004 e
75	0	0.00	0.31	16.08	29.65	48.52	62.29	68.07	73.41	2.36 ± 0.011 b
	10	0.00	0.45	17.20	30.86	49.51	62.99	68.50	73.66	2.26 ± 0.017 d
	20	0.00	0.78	20.69	35.41	53.88	64.42	71.40	75.89	2.10 ± 0.014 e
	30	0.00	0.87	21.09	35.70	53.88	64.95	71.08	75.51	1.88 ± 0.003 f

Different lowercase letters in the same column indicate significant differences ($p < 0.05$). * Mean ± SD ($n = 3$).

in Table 9. All gelatin films had the excellent barrier property against UV light at 200 and 280, especially when palm oil was incorporated. Protein-based films had the excellent UV light barrier capacity owing to their high amount of aromatic amino acids that absorb UV light (Hamaguchi *et al.*, 2007). Similar result was found for gelatin films from skins of tilapia (Tongnuanchan *et al.*, 2013). All emulsified films showed the lower transmission of visible light in the range of 350-800 nm, compared with control film (without palm oil). The higher barrier property toward light transmission was obtained for films incorporated with 75% palm oil in comparison with the films containing 50% palm oil, regardless of glycerol level. This was more likely associated with the increase in opaqueness of films containing palm oils. Oil droplets distributed throughout the films might hinder light transmission through the films (Ma *et al.*, 2012). It was noted that transmission increased when glycerol content increased. Glycerol more likely reduced the compactness of film matrix, thereby facilitating the light transmission of films. For transparency values, the lower transparency value indicated that the film was more transparent. Films added with palm oil had the higher transparency value than the control films ($p < 0.05$) (Table 9). This result suggested that the incorporation of palm oil yielded less transparent films. Oil droplets localised in the film matrix had light scattering effect, thus lowering the transparency of gelatin film (Tongnuanchan *et al.*, 2012). With increasing glycerol levels (0-30%), transparency value decreased, indicating the increased transparency of films. Thus, levels of both palm oil and glycerol played an essential role in color, light transmittance and transparency of emulsified gelatin films.

3.4.5 Differential scanning calorimetry (DSC)

DSC thermograms of the 1st and 2nd-heating scans of selected emulsified film (containing 10% glycerol incorporated with 75% palm oil) and the control film (containing 30% glycerol without palm oil), showing the highest WVP, are illustrated in Figure 8A and B, respectively. Glass transition temperature (T_g), melting transition temperature (T_{max}) and the melting enthalpy (ΔH) of both film samples are summarized in Table 10. From thermogram of the 1st heating scan (from -20 to 150°C), the control film showed a step-like transitions, indicating the glass transition temperature (T_g), and an endothermic melting transition (T_{max}). The glass transition is associated with

molecular segmental motion of disordered (amorphous phase) structure which undergoes from a brittle glassy solid state to a rubbery or highly viscous state, whereas the melting transition of the protein film indicated the temperature causing a disruption of ordered or aggregated structure (Tang *et al.*, 2009). T_g of the control gelatin film was found at temperature of 71.27 °C, which was more likely associated with T_g of plasticized gelatin-rich phase. Gelatin-based films had various T_g, depending upon gelatin sources, compositions of film and process used (Tongnuanchan *et al.*, 2015). T_g of gelatin film was decreased from 71.27 °C (control film) to 62.76 °C, when palm oil at the levels of 75% was incorporated. It was suggested that the addition of palm oil more likely impeded protein-protein interaction in film matrix, thereby increasing the mobility of gelatin chain. The losses of cohesive structure integrity were found in film network when oil droplets were present (Tongnuanchan *et al.*, 2014). This result was in agreement with the decreased strength and increased EAB (Table 7) of emulsified gelatin film.

For endothermic/melting transition, the control film showed endothermic peak with T_{max} of 127.85 °C. This endothermic transition obtained after the glass transition was possibly associated with the helix-coil transition of gelatin (Vanin *et al.*, 2005) as well as the disruption of other kinds of ordered or aggregated molecular structure. Gelatin chains could undergo partial renaturation during film formation process (Hoque *et al.*, 2011b). However, melting/ordered-phase transition peak disappeared when palm oil was incorporated. It was noted that the addition of palm oil might disrupt the protein-protein chain interaction in the resulting film. When palm oil droplets were dispersed in the gelatin film matrix, the weaker film structure was developed, particularly with a larger amount of oil added. The weaker film structure had the lower thermal stability, which required a lower enthalpy for destroying the inter-molecular interaction. Moreover, the thermograms of films incorporated with 75% palm oil exhibited another endothermic transition peak (T_{max1}), observed at temperature of -0.82 °C. As expected, no endothermic transition peak at this range of temperature was found in the control film. This endothermic transition was most likely attributed to the melting transition of palm oil droplets dispersed in the film network. This result was in agreement with Tongnuanchan *et al.* (2015) who reported that fish

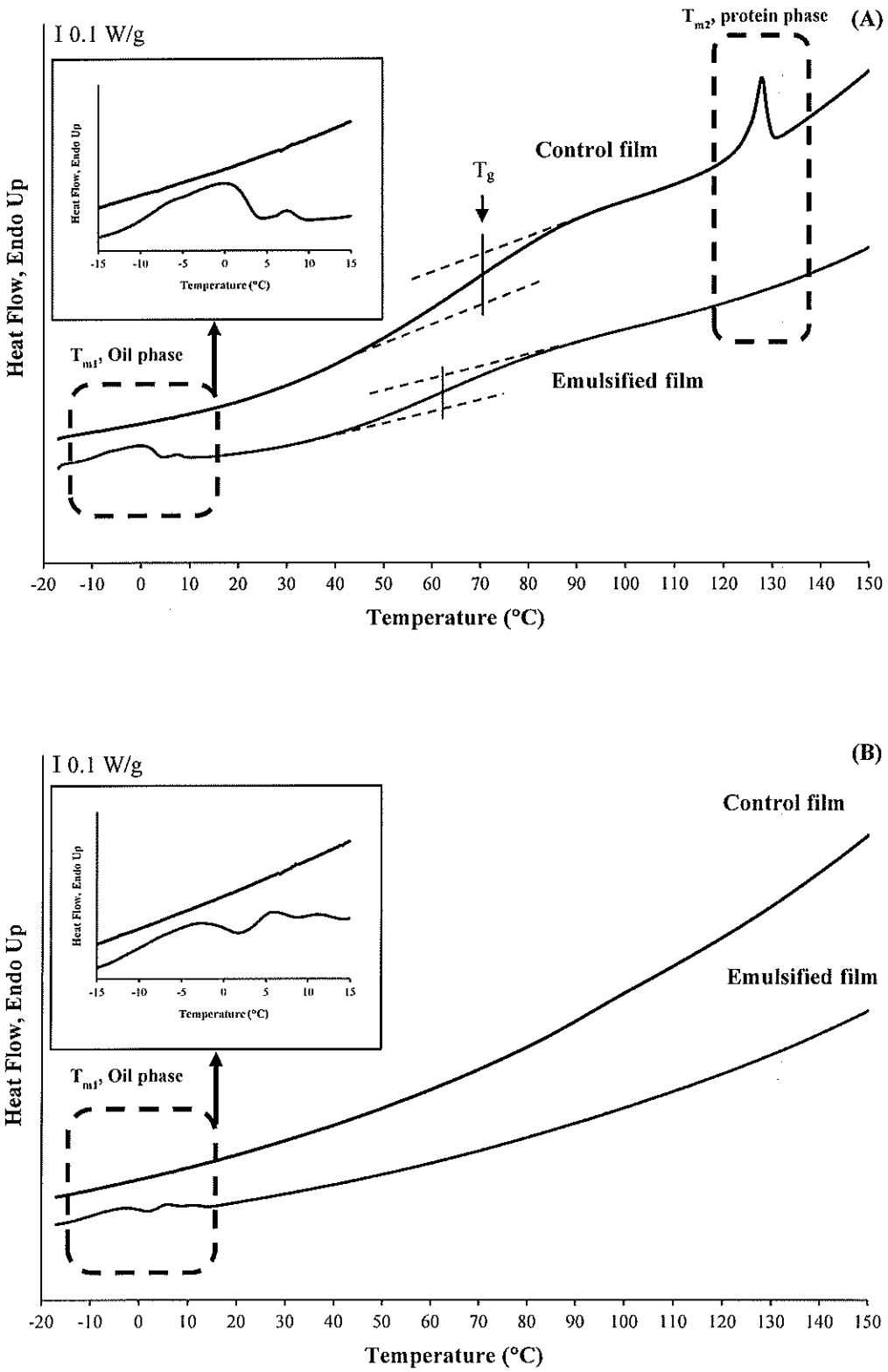

Figure 8. DSC thermograms of 1st-heating scan (A) and 2nd-heating scan (B) of the control gelatin film and film incorporated with 75% palm oil and 10% glycerol.

Table 10. Glass transition temperature (T_g), melting/order-phase transition temperature (T_{max}) and enthalpy (ΔH) of the control fish skin gelatin film and film incorporated with 75% palm oil and 10% glycerol.

Palm oils (%)	**Glycerols (%)**	**1st-Heating**								**Glass transition**
		Melting/Order-phase transition								
		Oil phase				**Protein phase**				
		T_{onset} (°C)	**$T_{peak, max}$ (°C)**	**T_{end} (°C)**	**ΔH (J/g)**	**T_{onset} (°C)**	**$T_{peak, max}$ (°C)**	**T_{end} (°C)**	**ΔH (J/g)**	**T_g (°C)**
0	30	-	-	-	-	124.95	127.85	129.62	12.80	71.27
75	10	-12.42	-0.82	3.70	7.62	-	-	-	-	62.76
		2nd-Heating								
0	30	-	-	-	-	-	-	-	-	-
75	10	-13.97	-3.90	1.44	3.55	-	-	-	-	-

gelatin film incorporated with palm oil at levels of 25-100% exhibited the endothermic peak in the range of -2.32 to 0.52 °C which was due to the melting peak of palm oil.

From the thermograms of the 2nd-heating scan (Figure 8B), the endothermic peak, which was related to gelatin-rich phase transition (T_{max2}), was not observed for both film samples. It was suggested that gelatin was not able to rearrange themselves into ordered structure upon quench cooling during DSC scan. These results suggested that gelatin molecules were completely disrupted along with the loss of adsorbed water after the first-heating scan. Furthermore, the endothermic melting peak was observed for films incorporated with palm oil, but it was not found for the control film. This endothermic transition was correlated with the melting transition of palm oil as previously described. Thus, the incorporation of palm oil directly affected the thermal behavior of gelatin film.

3.4.6 Thermo-gravimetric analysis (TGA)

The degradation temperatures (T_d), weight loss (Δw) and residue of selected emulsified films (containing 10% glycerol and 75% palm oil) and control film (containing 30% glycerol without palm oil) are presented in Table 11. The control film exhibited three stages of weight loss, while the emulsified film had four stages of weight loss. For both films, the first stage weight loss (Δw_1 = 5.76-6.10%) was observed over the temperature (T_{d1}) ranging from 54.50 to 56.36 °C. The weight loss at this temperature range was more likely associated with the loss of free and bound water absorbed in the film. Emulsified film had lower weight loss than the control film. This might be due to the lower amount of water in film matrix, associated with higher hydrophobicity of film containing palm oil (Tongnuanchan *et al.*, 2014). The second stage of weight loss of films appeared approximately at the onset temperature of 211.19-213.70 °C (T_{d2}) with Δw_2 of 4.11-19.50%. Weight loss at this stage was mostly owing to the loss of low molecular weight protein fraction and glycerol as well as structural bound water (Hoque *et al.*, 2011a). It was noted that Δw_2 of films incorporated with palm oil was lower than those of control film. This might be due to the lower proportion of glycerol in film matrix. However, this range of temperature was higher than the boiling point of glycerol (182 °C) (Guerrero *et al.*, 2011). Some kinds of

Table 11. Thermal degradation temperatures (T_d, °C) and weight loss (Δw, %) of the control fish skin gelatin film and film incorporated with 75% palm oil and 10% glycerol.

Palm oils (%)	**Glycerols** (%)	Δ_1		Δ_2		Δ_3		Δ_4		**Residual** (%)
		$T_{d1,onset}$	Δw_1	$T_{d2,onset}$	Δw_2	$T_{d3,onset}$	Δw_3	$T_{d4,onset}$	Δw_4	
0	30	54.50	6.10	211.19	19.50	295.62	55.83	-	-	18.57
75	10	56.36	5.76	213.70	4.11	286.80	37.35	361.84	36.98	15.80

Δ_1, Δ_2, Δ_3 and Δ_4 denote the first, second, third and fourth stage weight loss, respectively, of film during heating scan.

interaction such as hydrogen bond were plausibly formed between protein fractions and glycerol (Guerrero *et al.*, 2011). For the third stage of weight loss, Δw_3 of 37.35-55.83% and T_{d3} of 286.80-295.62 °C were observed for both films. This stage of weight loss was most likely associated with the degradation or decomposition of larger size or highly interacted protein fractions. In general, the T_{d3} for films incorporated with palm oil was lower than the control film. The results suggested that film incorporated with palm oil showed the lower heat resistance than the control film. Incorporation of palm oil yielded a weaker film network due to the lower inter/intra-molecular protein interaction in film matrix. This led to the lower heat resistance of resulting films. Moreover, T_{d3} was higher than the smoke point of palm oil (~235 °C) (Guzman *et al.*, 2010). The loss of volatile compounds or low molecular weight free fatty acids in palm oil could occur at this temperature. For the fourth stage of weight loss, Δw_4 of 36.98% with T_{d4} of 361.84 °C was obtained for emulsified films, suggesting the loss of high thermal stable components possibly gelatin molecules closely associated with soy lecithin. However, the fourth stage of weight loss (Δw_4) was undetectable for the control film. In general, gelatin films incorporated with palm oil had lower residue mass from thermal degradation, compared with the control film. This confirmed that films containing palm oil had the weaker interaction between protein molecules in film network than the control film as evidenced by the lowered TS (Table 7). As a consequence, the residue or char in films added with palm oil was found at a lower extent. Thus, the incorporation of palm oil could loosen film network, leading to higher degree of thermal degradation.

3.4.7 Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra of control film and the selected emulsified film are illustrated in Figure 9. Generally, the control film and emulsified film showed the similar major peaks but the amplitudes of peaks varied as influenced by both glycerol and palm oil incorporated. The band situated at the wavenumber of 1035-1038 cm^{-1} was found in both film samples, corresponding to the OH group, mainly from glycerol added as a plasticizer (Bergo and Sobral, 2007). It was noted that the amplitude of the OH group of control film was higher than that found in film incorporated with palm oil.

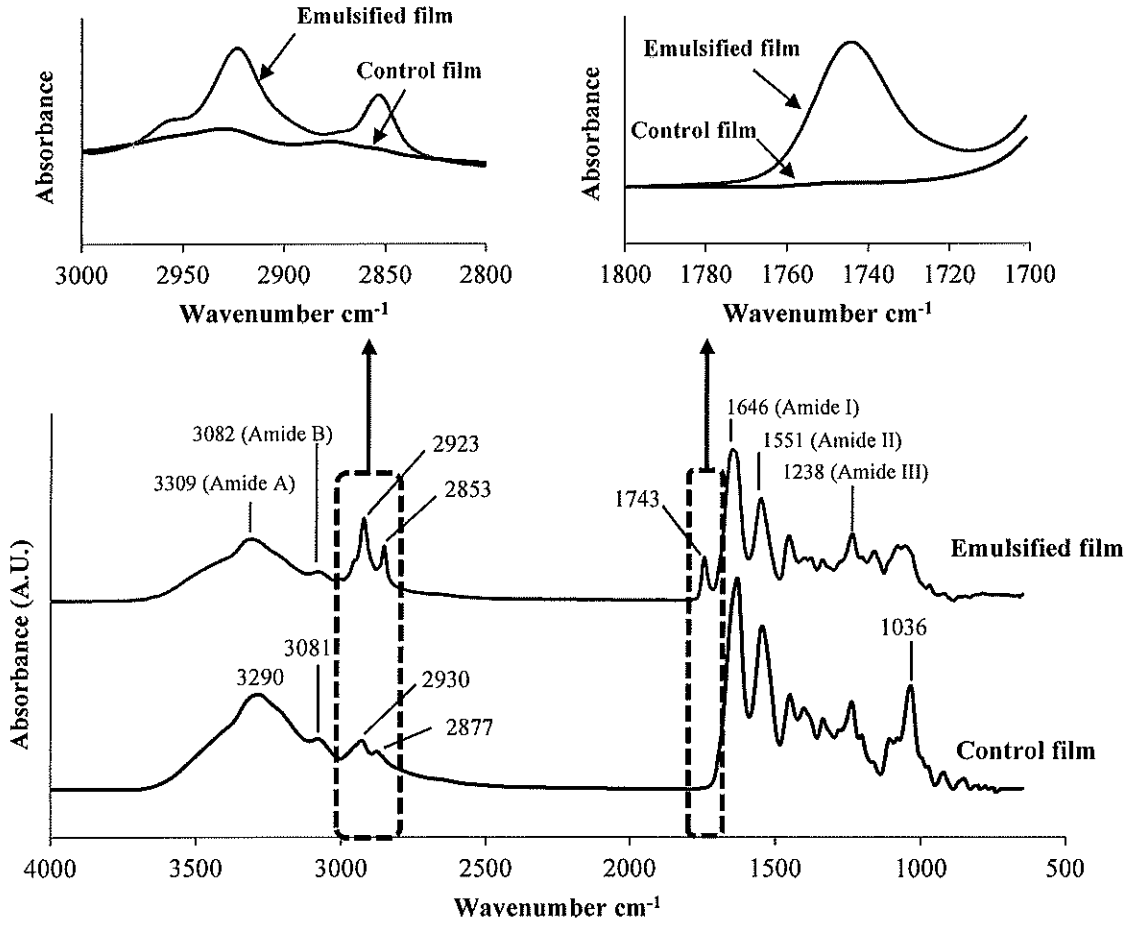

Figure 9. ATR-FTIR spectra of the control gelatin film and film incorporated with 75% palm oil and 10% glycerol.

This was because glycerol proportion in the emulsified film was lower than that of control film. Both films had the similar spectra in the range of 1700-1200 cm^{-1}, covering amide-I, II and III bands. Both films had the major bands at 1646 cm^{-1} (amide-I, illustrating C=O stretching/hydrogen bonding coupled with COO), 1551 cm^{-1} (amide-II, presenting the bending vibrations of N–H groups and stretching vibrations of C–N groups) and 1238 cm^{-1} (amide-III, illustrating the vibrations in-plane of C–N and N–H groups of bound amide or vibrations of CH_2 groups of glycine) (Aewsiri *et al.*, 2009; Muyonga *et al.*, 2004). It was noted that the amplitudes of amide-I, II and III of control film were slightly higher than film incorporated with palm oil. This was mainly due to the dilution effect caused by palm oil incorporated into gelatin film. Amide-A peak of control film found at wavenumber of 3290 cm^{-1}, which was lower than that of

emulsified film (3309 cm^{-1}). The amide-A band represents the NH-stretching coupled with hydrogen bonding. Glycerol at higher proportion in the control films might undergo interaction via hydrogen bonding with –NH in the gelatin chains to a higher extent as indicated by the shift to the lower wavenumbers. Emulsified film also showed the lower amplitude of amide-A band. The result suggested that the incorporation of palm oil into gelatin film showed the dilution effect on proteins in the film network. Amide-B band was found at 3081-3082 cm^{-1} for both film samples. Amide-B band illustrated NH-stretching vibration and asymmetric CH-stretching vibration (Kong and Yu, 2007). Additionally, peaks at wavenumbers of 2930 and 2877 cm^{-1} for the control film were shifted to lower wavenumbers of 2923 and 2853 cm^{-1} when palm oil was incorporated. The amplitude of both peaks was much higher in films added with palm oil, in comparison with the control film. Peaks at wavenumbers around 2853 cm^{-1} and 2924 cm^{-1} represented the methylene asymmetrical and symmetrical stretching vibration of the aliphatic C-H in CH_2 and CH_3 groups, respectively (Muik *et al.*, 2007). Those vibration stretching bands are obviously found in most lipids and hydrophobic substances (Guillén and Cabo, 2004). The result confirmed the presence of hydrophobic substance, palm oil, in the film. Furthermore, the peak at wavenumber of 1743 cm^{-1} was observed only in films containing palm oil. The carbonyl absorption of triglyceride ester linkage was observed at 1746 cm^{-1} (Setiowaty *et al.*, 2000). Thus, the stretching vibration peak assignable to the C=O group of triglycerides was noticeable when the palm oil was incorporated.

3.5 Conclusions

Incorporation of palm oil and glycerol affected mechanical, physical and thermal properties of gelatin films differently, depending upon the levels used. The lowest WVP of gelatin film was observed when 75% palm oil was incorporated and glycerol was excluded. The incorporation of palm oil directly affected colors, transparency and thermal properties of films. Thus, the use of 75% palm oil and 10% glycerol could improve water vapor barrier properties of gelatin-based film, whereas the satisfactory mechanical properties were still obtained.

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CHAPTER 4
EFFECTS OF SOY LECITHIN LEVELS AND MICROFLUIDIZATION CONDITIONS ON PROPERTIES OF FISH GELATIN-BASED FILM INCORPORATED WITH PALM OIL

4.1 Abstract

Properties of film-forming dispersion (FFD) and emulsion film incorporated with palm oil containing soy lecithin at various levels (50% and 75% w/w, based on palm oil) and emulsified with different microfluidization pressure (1, 2 and 3 kpsi) and pass number (2 and 4) were investigated. Microfluidized FFD containing 50% soy lecithin showed the smaller oil droplet size and the emulsion was more stable during storage. Films containing 50% soy lecithin had lower water vapor permeability (WVP) and elongation at break (EAB) with higher tensile strength (TS) than those containing 75% soy lecithin ($p < 0.05$). The lowest WVP and highest TS and EAB were found for films from FFD microfluidized at 3 kpsi. Decreases in L^* value and light transmittance with coincidental increases in b^*- and ΔE^* values were observed in films when the amount of soy lecithin incorporated increased ($p < 0.05$). The smooth surface and compact cross-section were observed in films from microfluidized FFD. Film from microfluidized FFD containing 50% soy lecithin showed higher thermal stability. Thus, the emulsion gelatin film with the improved properties could be prepared from FFD using 50% soy lecithin as a surfactant with the aid of microfluidization.

4.2 Introduction

Gelatin, a protein possessing an excellent film-forming property, is one of the first materials used for edible coating and film making (McHugh and Krochta, 1994). Nevertheless, the gelatin films still have drawback due to poor water vapor barrier property (Sobral *et al.*, 2001). The incorporation of hydrophobic substances such as lipid, fatty acid and wax has been implemented to improve water barrier property of gelatin film (Limpisophon *et al.*, 2010; Prodpran *et al.*, 2007; Soazo *et al.*, 2011). Recently, gelatin film incorporated with palm oil has been shown to have the improved water vapor barrier property (Nilsuwan *et al.*, 2015). Owning to hydrophobic nature,

palm oil is immiscible in film-forming dispersion (FFD) or emulsion before making the films. Nevertheless, the homogeneity of oil droplets and stability of emulsion in FFD play a significant role in properties of emulsion-based films. High emulsion stability during the film drying give rise to a homogeneous distribution of lipid particles in the film, which in turn contributes to the efficient control of water vapor migration (Debeaufort *et al.*, 1993). To disperse oil in film-forming aqueous phase, homogenization along with appropriate level of surfactant is required. Microfluidization is one of potential homogenization techniques to obtain fine and more uniform oil droplet dispersion as well as more emulsion stability, compared to typical homogenization. Surfactants or emulsifiers are the substances that reduce surface tension between oil and water, thereby enhancing emulsification and increasing emulsion stability (Dalgleish, 1997). Soy lecithin has been reported as an effective surfactant for stabilization of protein-based emulsion films (Prodpran *et al.*, 2007; Tongnuanchan *et al.*, 2014). Thus, this study aimed to investigate the influence of homogenization conditions and soy lecithin levels on oil droplet distribution and emulsion stability of FFD and properties of resulting fish skin gelatin-based emulsion films.

4.3 Materials and Methods

4.3.1 Chemicals and gelatin

Glycerol, soy lecithin and sodium dodecyl sulfate (SDS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade. Fish gelatin from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Palm oil was obtained from *OLEEN Company Limited* (Bangkok, Thailand).

4.3.2 Preparation of film-forming dispersion (FFD)

Gelatin (3.5 g) was dissolved in 90 ml of distilled water and then heated at 70 °C for 30 min. Glycerol was added to gelatin solution at the level of 10% (w/w, based on protein content). Palm oil was mixed with soy lecithin at levels of 50% and 75% (w/w, based on palm oil content) and then transferred into the prepared gelatin

solution to obtain the palm oil concentrations of 75% (w/w, based on protein content). The volume was adjusted to 100 mL using distilled water. The mixtures were homogenized at a speed of 22,000 rpm for 3 min using a rotor-stator homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The coarse emulsions were passed through a Microfluidizer (Model HC-5000, Microfluidizer, Newton, MA, USA) at 1, 2 and 3 kpsi for 2 and 4 passes. Fine emulsion termed 'film-forming dispersion: FFD' was subjected to analyses and also used for film preparation.

4.3.3 Effect of soy lecithin levels and homogenization condition on characteristics of FFD

FFD samples were stored at room temperature (28-30 °C) for 0 and 12 h. At the time designated, FFD were taken for analyses.

4.3.3.1 Oil droplet size

Size of oil droplets in FFD was determined using a ZetaPALs zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA). Prior to analysis, FFDs were diluted with 1% (w/v) sodium dodecyl sulfate (SDS) solution in order to dissociate flocculated droplets. The surface-weighted mean (d_{32}) and the volume-weighted mean (d_{43}) particle diameters of the oil droplets were calculated by equations (1) and (2), respectively (Fabra *et al.*, 2011).

$$d_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}$$

$$d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$$

where n_i and d_i are the number of droplets of a determined size range and the droplet diameter, respectively.

4.3.3.2 Flocculation factor and coalescence index

To determine flocculation factor (F_f) and coalescence index (C_i), FFDs were diluted with distilled water in the presence and absence of 1% (w/v) SDS. F_f and C_i were calculated using the following equations (Palazolo *et al.*, 2005):

$$F_f = \frac{d_{43\text{-SDS}}}{d_{43+\text{SDS}}}$$

$$C_i\ (\%) = \frac{(d_{43+\text{SDS, t}} - d_{43+\text{SDS, in}}) \times 100}{d_{43+\text{SDS, in}}}$$

where $d_{43+\text{SDS}}$ and $d_{43\text{-SDS}}$ are the volume-weighted mean particle diameters of the emulsion droplets in the presence and absence of 1% SDS, respectively. $d_{43+\text{SDS, in}}$ is initial value of the volume-weighted mean particle diameter of the emulsion droplets in the presence of 1% SDS; $d_{43+\text{SDS, t}}$ is the value of the volume-weighted mean particle diameter of the emulsion droplets in the presence of 1% SDS at the designated storage time.

4.3.4 Effect of soy lecithin levels and homogenization conditions on properties of film

4.3.4.1 Preparation of film

Prior to casting, FFD was subjected to air removal by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature. FFD (4 mL) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown for 12 h at room temperature (28-30 °C) prior to further drying at 25 °C and 50 ± 5% RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and subsequently analyzed.

4.3.4.2 Determination of film properties

4.3.4.2.1 Film thickness

The thickness of film was measured using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Five random locations around each film of ten film samples were used for average thickness determination.

4.3.4.2.2 Mechanical properties

Prior to testing, films were conditioned for 48 h at 25 °C and 50 ± 5% RH. Tensile strength (TS) and elongation at break (EAB) were determined as described

by Iwata *et al.* (2000) with a slight modification using the Universal Testing Machine (Lloyd Instrument, Hampshire, UK) equipped with tensile load cell of 100 N. Ten samples (2 × 5 cm^2) with initial grip length of 3 cm were used for testing. Cross-head speed was set at 30 mm/min.

4.3.4.2.3 Water vapor permeability (WVP)

WVP was measured using a modified ASTM method (ASTM, 1989) as modified by Shiku *et al.* (2004). The films were sealed on an aluminium permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and a rubber gasket to hold the films in place. The cups were placed in a desiccator containing the distilled water at 30 °C. The cups were weighed at 1-h intervals over a 10-h period. WVP of the film was calculated as follows:

$$WVP \text{ (g/m s Pa)} = \frac{wl}{At(P_2-P_1)}$$

where w is the weight gain of the cup (g); l is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); $P_2 - P_1$ is the vapor pressure difference across the film (4242.31 Pa at 30 °C)..

4.3.4.2.4 Color

Film samples were subjected to color measurement using a CIE colorimeter (Hunter associates laboratory, Inc., Reston, VA, USA). D_{65} (day light) and a measure cell with opening of 30 mm was used. The color of the films was expressed as L^*-value (lightness), a^*-value (redness/greenness) and b^*-value (yellowness/blueness). Total difference of color (ΔE^*) was calculated as follows (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences between the color parameter of the samples and those of the white standard (L^* = 93.61, a^* = -0.97, b^* = 0.44).

4.3.4.2.5 Light transmittance and transparency value

The light transmittance of films was measured at the ultraviolet and visible range (200-800 nm) using a UV-vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) according to the method of Shiku *et al.* (2004). The transparency value of film was calculated using the following equation (Han and Floros, 1997):

$$\text{Transparency value} = \frac{-logT_{600}}{x}$$

where T_{600} is the fractional transmittance at 600 nm and x is the film thickness (mm). The greater transparency value represents the lower transparency of film.

4.3.4.2.6 Scanning electron microscopy (SEM)

Morphology of surface and cross-section of film samples was visualized using a scanning electron microscope (SEM) (Quanta 400, FEI, Eindhoven, the Netherlands). For cross-section, samples were fractured under liquid nitrogen prior to visualization. Then, the samples were mounted on bronze stub and sputtered with gold (Sputter coater SPI-Module, West Chester, PA, USA) in order to make the sample conductive. The photographs were taken at an acceleration voltage of 15 kV.

4.3.4.2.7 Differential scanning calorimetry

Thermal properties of films were determined using a differential scanning calorimeter (DSC) (Perkin Elmer, Model DSC-7, Norwalk, CT, USA). Temperature calibration was performed using the indium thermogram. Film samples (2-5 mg) were accurately weighed into aluminium pans, hermetically sealed, and scanned over the temperature range of -20 to 150 °C, with a heating rate of 5 °C/min. Liquid nitrogen was used as cooling medium and the system was equilibrated at -20 °C for 5 min prior to the scan. An empty aluminium pan was used as the reference. A second scan was also performed in the same manner, followed the quench-cooling of the sample after completing the first scan.

4.3.4.2.8 Thermo-gravimetric analysis (TGA)

Films were scanned using a thermo-gravimetric analyser (TGA7, PerkinElmer, Norwalk, CT, USA) from 25 to 800 °C at a rate of 10 °C/min (Nuthong *et al.*, 2009). Nitrogen was used as the purge gas at a flow rate of 20 mL/min.

4.3.4.2.9 Fourier transform infrared (ATR-FTIR) spectroscopy

Films were scanned with a Bruker Model Equinox 55 FTIR spectrometer (Bruker Co., Ettlingen, Germany) equipped with a horisontal ATR Trough plate crystal cell (45° ZnSe; 80 mm long, 10 mm wide and 4 mm thick) (PIKE Technology Inc., Madison, WI, USA) at 25 °C as described by Nuthong *et al.* (2009). Films were placed onto the crystal cells and the cells were clamped into the mount of FTIR spectrometer. The spectra in the range of 650-4000 cm^{-1} with automatic signal gain were collected in 32 scans at a resolution of 4 cm^{-1} and ratioed against a background spectrum recorded from the clean empty cell at 25 °C.

4.3.5 Statistical analysis

Completely randomized design (CRD) was used throughout the study. All experiments were run in triplicate with different three lots of films. Data were subjected to analysis of variance (ANOVA), and mean comparisons were carried out by Duncan's multiple range test. For pair comparison, T-test was used (Steel *et al.*, 1980). Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL, USA).

4.4 Results and discussion

4.4.1 Effects of microfluidization pressures/pass numbers and soy lecithin levels on characteristics of film-forming dispersion containing palm oil

4.4.1.1 Droplet size distribution

Oil droplet sizes, expressed as d_{32} and d_{43}, of FFD containing soy lecithin at 50% and 75% (w/w, based on palm oil content) emulsified at pressure levels of 1, 2

and 3 kpsi for 2 and 4 passes are shown in Table 12. The d_{32} is related to specific surface area, while the d_{43} is index of coalescence and flocculation (Hebishy *et al.*, 2013). The smaller d_{32} contributes to the higher specific surface area (Hebishy *et al.*, 2013). The larger d_{43} reflects the association of individual droplets into larger flocs (Hebishy *et al.*, 2013). The d_{32} is more influenced by the small particles, whereas d_{43} is highly influenced by larger ones (Bengtsson and Tornberg, 2011). At the same soy lecithin level used, the smaller d_{32} and d_{43} were noticeable in all microfluidized FFD samples, compared with the control FFD prepared using typical homogenization ($p < 0.05$), regardless of storage time. The continuous decreases in d_{32} and d_{43} of FFD were generally observed when the pressure was increased from 1 to 3 kpsi ($p < 0.05$), irrespective of pass numbers. No differences in both d_{32} and d_{43} values between FFD with 2 and 4 passes were observed when the same pressure level was applied ($p > 0.05$), regardless of soy lecithin levels. The result suggested that microfluidization pressure showed an important role in size reduction of oil droplet in emulsion system. The greater turbulence and shear forces are associated with increased pressure applied (Hogan *et al.*, 2001). This result was in agreement with Takeungwongtrakul *et al.* (2014) who reported that d_{32} and d_{43} of shrimp oil droplet in emulsions containing whey protein concentrate and sodium caseinate were decreased when pressure of homogenization increased. At the same pressure, the oil droplet sizes of FFD passed through the microfluidizer at 2 and 4 passes were not different ($p > 0.05$). Thus, numbers of passes had no profound influence on the disruptive forces generated in the system. Moreover, the smaller oil droplet size was observed in the FFD containing 50% soy lecithin ($p < 0.05$), compared with that found in FFD with 75% lecithin, regardless of microfluidization pressures and pass numbers. The result suggested that the appropriated level of surfactant could enhance the size reduction and stabilization of oil droplet in the emulsion. Soy lecithin at high level (75%) might be self-assembled into micelles after emulsification. As a result, a lower amount of active surfactant could migrate and localize at oil-water interface. After 12 h of storage, the increases in both d_{32} and d_{43} were noticeable in all FFD samples ($p < 0.05$). With extended storage, the collapse of emulsion possibly took place via coalescence mechanism or assembly of individual droplets by flocculation as well as Oswald ripening mechanism (Djordjevic *et al.*, 2008; Intarasirisawat *et al.*, 2014). Oil droplets more likely aligned themselves

Table 12. Effects of pressure levels and number of pass of microfluidization on the oil droplet size and stability of film forming dispersion containing soy lecithin at different levels.

Soy lecithin (%)	Pressure (kpsi)	Number of passes	Storage time (h)	d_{32} (μm)	d_{43} (μm)	F_f	C_i (%)
50	0	0	0	0.205 ± 0.004^{*dA}	0.232 ± 0.008 ^{fA}	1.478 ± 0.007 ^{aA}	
			12	0.271 ± 0.012 ^{eA}	0.292 ± 0.005 ^{hA}	2.192 ± 0.030 ^{dA}	25.59 ± 2.29 ^{dA}
	1	2	0	0.175 ± 0.008 ^{cA}	0.209 ± 0.004 ^{dA}	1.497 ± 0.016 ^{aA}	
			12	0.208 ± 0.005 ^{dA}	0.251 ± 0.003 ^{gA}	2.382 ± 0.097 ^{eA}	20.24 ± 1.30 ^{cA}
		4	0	0.172 ± 0.007 ^{cA}	0.207 ± 0.003 ^{dA}	1.489 ± 0.031 ^{aA}	
			12	0.209 ± 0.006 ^{dA}	0.250 ± 0.003 ^{gA}	2.338 ± 0.024 ^{eA}	21.00 ± 1.68 ^{cA}
	2	2	0	0.145 ± 0.006 ^{bA}	0.189 ± 0.006 ^{cA}	1.621 ± 0.011 ^{bA}	
			12	0.170 ± 0.005 ^{cA}	0.219 ± 0.003 ^{cA}	2.738 ± 0.046 ^{fA}	16.01 ± 1.41 ^{bA}
		4	0	0.143 ± 0.005 ^{bA}	0.187 ± 0.006 ^{cA}	1.629 ± 0.020 ^{bA}	
			12	0.170 ± 0.003 ^{cA}	0.217 ± 0.003 ^{eA}	2.791 ± 0.123 ^{fA}	16.15 ± 1.59 ^{bA}
	3	2	0	0.117 ± 0.003 ^{aA}	0.157 ± 0.007 ^{aA}	1.765 ± 0.058 ^{cA}	
			12	0.134 ± 0.005 ^{bA}	0.174 ± 0.005 ^{bA}	3.911 ± 0.033 ^{gA}	10.90 ± 2.94 ^{aA}
		4	0	0.113 ± 0.005 ^{aA}	0.155 ± 0.007 ^{aA}	1.744 ± 0.018 ^{cA}	
			12	0.133 ± 0.004 ^{bA}	0.172 ± 0.006 ^{bA}	3.979 ± 0.134 ^{gA}	10.77 ± 3.58 ^{aA}

Different lowercase letters in the same column under the same soy lecithin level indicate significant differences ($p < 0.05$). Different uppercase letters under the same pressure level and number of pass indicate significant differences ($p < 0.05$). F_f : Flocculation factor; C_i : Coalescence index. * Mean ± SD (n = 3).

Table 12 (Cont.). Effects of pressure levels and number of pass of microfluidization on the oil droplet size and stability of film forming dispersion containing soy lecithin at different levels.

Soy lecithin (%)	Pressure (kpsi)	Number of passes	Storage time (h)	d_{32} (μm)	d_{43} (μm)	F_f	C_i (%)
75	0	0	0	0.255 ± 0.006^{dB}	0.282 ± 0.004^{fB}	1.288 ± 0.014^{aB}	
			12	0.301 ± 0.008^{eB}	0.330 ± 0.001^{hB}	1.994 ± 0.035^{dB}	17.22 ± 0.44^{dB}
	1	2	0	0.235 ± 0.007^{cB}	0.260 ± 0.002^{dB}	1.324 ± 0.012^{aB}	
			12	0.268 ± 0.004^{dB}	0.298 ± 0.002^{gB}	2.097 ± 0.031^{eB}	14.38 ± 0.62^{cB}
		4	0	0.232 ± 0.005^{cB}	0.258 ± 0.003^{dB}	1.335 ± 0.009^{aB}	
			12	0.269 ± 0.008^{dB}	0.296 ± 0.001^{gB}	2.110 ± 0.044^{cB}	14.46 ± 0.29^{cB}
	2	2	0	0.215 ± 0.007^{bB}	0.250 ± 0.002^{cB}	1.383 ± 0.018^{bB}	
			12	0.240 ± 0.006^{cB}	0.275 ± 0.005^{eB}	2.254 ± 0.046^{fB}	9.76 ± 1.85^{bB}
		4	0	0.213 ± 0.009^{bB}	0.248 ± 0.002^{cB}	1.387 ± 0.016^{bB}	
			12	0.240 ± 0.005^{cB}	0.274 ± 0.003^{eB}	2.254 ± 0.045^{fB}	10.30 ± 1.13^{bB}
	3	2	0	0.197 ± 0.005^{aB}	0.218 ± 0.003^{aB}	1.464 ± 0.015^{cB}	
			12	0.214 ± 0.006^{bB}	0.235 ± 0.001^{bB}	2.937 ± 0.025^{gB}	7.68 ± 0.28^{aB}
		4	0	0.193 ± 0.008^{aB}	0.217 ± 0.003^{aB}	1.468 ± 0.013^{cB}	
			12	0.213 ± 0.003^{bB}	0.234 ± 0.001^{bB}	2.946 ± 0.020^{gB}	7.84 ± 0.33^{aB}

Different lowercase letters in the same column under the same soy lecithin level indicate significant differences ($p < 0.05$). Different uppercase letters under the same pressure level and number of pass indicate significant differences ($p < 0.05$). F_f : Flocculation factor; C_i : Coalescence index. * Mean ± SD (n = 3).

closely, leading to flocculation and creaming as the storage time increased. Those phenomena could foster the coalescence of emulsion. At 12 h of storage, d_{32} and d_{43} of all microfluidizied FFD were lower than those of the control FFD ($p < 0.05$). The result indicated that the small oil droplets in FFD were more resistant to coalescence during the extended storage.

For flocculation factor (F_f) and coalescence index (C_i), FFD microfluidized at pressure above 2 kpsi showed the higher F_f than that of the control when the same soy lecithin level was used ($p < 0.05$). At the same pressure used, number of passes had no impact on F_f ($p > 0.05$). Generally, the continuous increases in F_f were observed when the pressure was increased from 1 to 3 kpsi ($p < 0.05$), irrespective of soy lecithin level and pass number. This was generally related with the decreases of d_{32} and d_{43} in these samples when higher homogenization pressure was implemented. Bonilla *et al.* (2012) reported that the great aggregation or flocculation was observed for small oil droplets. The FFD containing 75% soy lecithin showed the lower F_f than those containing 50% soy lecithin ($p < 0.05$), regardless of homogenization conditions. This might be related with the lower surface area of the large oil droplet (d_{32} and d_{43}) in FFD containing 75% soy lecithin, thereby lowering the bridging flocculation between small droplets. After 12 h of storage, the increases in F_f were observed for all FFD samples, compared to those found at 0 h (after emulsification). The results suggested that the assembly of individual droplets by flocculation occurred in the emulsion when the storage time increased.

The coalescence (C_i) was generally observed for all FFD samples after 12 h of storage ($p < 0.05$). Coalescence is the index for instability of the emulsion. The increase in C_i was attributed to the collapse of the oil droplets as evidenced by the higher d_{43} (Fredrick *et al.*, 2010). The highest C_i was noticeable in the control FFD as soy lecithin at both 50% and 75% was used as a surfactant. At the same level of soy lecithin, the continuous decrease of C_i was found when the homogenization pressures were increased ($p < 0.05$), regardless of pass numbers. Larger oil droplets in FFD were prone to coalescence. This was related with the marked increase in d_{43}, particularly for the control FFD. Overall, C_i of the FFD samples containing 50% soy lecithin was higher than those of FFDs containing 75% soy lecithin. This result suggested that the higher surfactant in FFD effectively prevented the rupture of the oil droplets through the

coalescence. However, the smaller oil droplet was noticeable in FFD when 50% soy lecithin was used. This was probably attributed to the micells of soy lecithin in the aqueous phase with the large size. Therefore, the FFD emulsification with high homogenization pressure (3 kpsi) along with addition of 50% of soy lecithin could provide oil droplets with smaller size in FFD.

4.4.2 Effects of homogenization pressures/pass number and surfactant level on properties of fish gelatin-based film incorporated with palm oil

4.4.2.1 Thickness

Thickness of fish skin gelatin films incorporated with palm oil containing soy lecithin at different levels (50% and 75% w/w, based on palm oil content) and emulsified with different microfluidization pressures (1, 2 and 3 kpsi) with various pass numbers (2 and 4 passes) is shown in Table 13. All films from microfluidized FFD had lower thickness of film than the control film (prepared with typical homogenization) at both soy lecithin levels used ($p < 0.05$). At the same soy lecithin level, the lowest thickness of film was obtained for film from FFD microfluidized at 3 kpsi ($p < 0.05$), regardless of pass number. This result suggested that the emulsification of FFD using the microfluidization provided the small oil droplets, which did not interrupt the alignment or interaction between peptide chains during film drying. This led to the compact structure of the film. However, no differences in thickness of film from FFD microfluidized with 2 and 4 passes ($p > 0.05$). Moreover, thickness of films containing 75% soy lecithin was higher than those containing 50% soy lecithin ($p < 0.05$) when the same pressure and pass number were used. The smaller oil droplets in FFD using 50% soy lecithin as a surfactant (Table 12) might relate well with the thinner corresponding films, compared with films having 75% soy lecithin. Additionally, the molecule of soy lecithin might disrupt the development of compact protein network, especially when the higher level was used. (Andreuccetti *et al.*, 2009) reported the variation of film thickness caused by the varying concentrations of soy lecithin in the formulations. Thus, homogenization pressure and soy lecithin levels directly affected free volume and internally structural organization of film, which in turn determined thickness of films.

Table 13. Effects of pressure levels and number of pass of microfluidization on the mechanical properties and water vapor permeability of emulsion based film containing soy lecithin at different levels.

Soy lecithin (%)	Pressure (kpsi)	Number of passes	Thickness (μm)	TS (MPa)	EAB (%)	WVP ($\times 10^{-12}$ g/m s Pa)
50	0	0	92.79 ± 1.20^{*cA}	29.72 ± 0.96 ^{aB}	38.32 ± 1.34 ^{aA}	5.70 ± 0.15 ^{dA}
	1	2	89.07 ± 0.71 ^{bA}	32.91 ± 0.77 ^{bB}	42.73 ± 1.12 ^{bA}	5.51 ± 0.09 ^{cA}
		4	88.43 ± 0.84 ^{bA}	33.25 ± 1.59 ^{bB}	44.36 ± 1.69 ^{bA}	5.50 ± 0.07 ^{cA}
	2	2	88.51 ± 0.98 ^{bA}	36.64 ± 1.69 ^{cB}	47.95 ± 2.97 ^{cA}	4.98 ± 0.08 ^{bA}
		4	87.95 ± 0.69 ^{bA}	36.44 ± 1.54 ^{cB}	48.40 ± 2.81 ^{cA}	4.89 ± 0.09 ^{bA}
	3	2	86.43 ± 0.90 ^{aA}	41.00 ± 0.47 ^{dB}	59.65 ± 1.11 ^{dA}	4.31 ± 0.07 ^{aA}
		4	86.36 ± 0.97 ^{aA}	41.54 ± 1.33 ^{dB}	61.39 ± 1.55 ^{dA}	4.23 ± 0.04 ^{aA}
75	0	0	99.52 ± 0.80 ^{cB}	23.04 ± 1.27 ^{aA}	49.76 ± 3.36 ^{aB}	6.17 ± 0.02 ^{dB}
	1	2	93.39 ± 0.90 ^{bB}	27.42 ± 1.77 ^{bA}	53.94 ± 1.68 ^{bB}	5.94 ± 0.06 ^{cB}
		4	93.35 ± 0.79 ^{bB}	29.22 ± 0.90 ^{bcA}	56.87 ± 2.00 ^{bcB}	5.91 ± 0.06 ^{cB}
	2	2	92.19 ± 0.82 ^{bB}	29.71 ± 1.70 ^{cA}	59.10 ± 2.42 ^{cB}	5.42 ± 0.05 ^{bB}
		4	92.29 ± 0.94 ^{bB}	30.95 ± 2.10 ^{cdA}	59.97 ± 3.26 ^{cB}	5.43 ± 0.05 ^{bB}
	3	2	91.00 ± 0.99 ^{aB}	31.97 ± 1.74 ^{deA}	65.20 ± 3.24 ^{dB}	5.10 ± 0.14 ^{aB}
		4	90.29 ± 0.75 ^{aB}	33.12 ± 1.09 ^{eA}	68.67 ± 2.74 ^{dB}	4.98 ± 0.10 ^{aB}

Different lowercase letters in the same column under the same soy lecithin level indicate significant differences ($p < 0.05$). Different uppercase letters in the same column under the same pressure level and number of pass indicate significant differences ($p < 0.05$). * Mean ± SD ($n = 3$).

4.4.2.2 Mechanical properties

Mechanical properties expressed as tensile strength (TS) and elongation at break (EAB) of films prepared from FFD containing soy lecithin at various levels (50% and 75% w/w, based on palm oil content) with different homogenization conditions are shown in Table 13. TS indicates the resistance to tension forces and EAB is related to film stretching capacity (Acevedo-Fani *et al.*, 2015). Films prepared from microfluidized FFD generally had the higher TS and EAB than the control film (prepared with typical homogenization), regardless of soy lecithin levels ($p < 0.05$). Increases in both TS and EAB were more pronounced with increasing microfluidization pressures. At the same soy lecithin level, the highest TS and EAB were found in the film microfluidized at 3 kpsi ($p < 0.05$). The smaller oil droplet might more homogenously distributed throughout the film matrix and less interfered the alignment of gelatin chains during film formation. This resulted in more homogenous and compact network of film, which in turn increase TS and EAB of film. In addition, the microfluidization pressure obviously reduced the size of oil droplet, which generally related to increase in hydrophobic surface. These surfaces of oil droplets might interact with hydrophobic domains of protein chains, thus facilitating the strength of film matrix. Simultaneously, the oil droplets could localize between protein chains and acted as plasticizer, rendering the more flexible films as indicated by the increased elongation. This result was in agreement with Pérez-Gago and Krochta (2001) who reported that the increase in strength and elasticity of the emulsified film could be due to the decrease in lipid particle size when homogenization became more intense. Moreover, the emulsion film containing 50% soy lecithin had the higher TS along with lower EAB than those containing 75% soy lecithin ($p < 0.05$). Excessive soy lecithin in FFD could lower the interaction between protein chains. This was evidenced by lower TS but higher EAB. Thus, microfluidization pressure and soy lecithin directly had the influence on mechanical properties of resulting films.

4.4.2.3 Water vapor permeability (WVP)

WVP of fish skin gelatin films from FFD containing soy lecithin at levels of 50% and 75% (w/w, based on palm oil) microfluidized with various pressures

and pass numbers is shown in Table 13. At the same soy lecithin level, film from microfluidized FFD had lower WVP than did the control film ($p < 0.05$). The continuous decreases in WVP of films from microfluidized FFD were found when the pressure applied was increased ($p < 0.05$). With increasing homogenization pressure, the oil droplet size became smaller (Table 12). Those oil droplets which were hydrophobic in nature could insert between protein chain and created diffusion path tortuosity, which limited the water vapor transfer through the films. This result was in agreement with Fabra *et al.* (2011) who reported that WVP was decreased as lipid particle size decreased in sodium caseinate based films containing 10% oleic acids. In general, WVP of films containing 75% soy lecithin was higher than those containing 50% soy lecithin ($p < 0.05$), irrespective of homogenization pressure and pass number. It was noted that the FFD containing 75% soy lecithin had the larger oil droplet than those containing 50% soy lecithin (Table 12). Smaller size oil droplets had the larger hydrophobic surface, which could prevent water vapor transfer through the film more effectively.

4.4.2.4 Color, light transmittance and film transparency

The color of various gelatin films from FFD containing soy lecithin at levels of 50% and 75% (w/w, based on palm oil content) microfluidized at different pressures and pass numbers is shown in Table 14. Overall, the level of surfactant exhibited the profound impact on the color of films. L^*-values decreased, while the b^*- and ΔE^*-values increased ($p < 0.05$) as soy lecithin levels increased from 50% to 75%. Films became more yellowish when soy lecithin at high level was incorporated. Brownish yellow color of soy lecithin mainly contributed to the lower L^*-value and higher b^*-value of resulting film. This result was in accordance with (Tongnuanchan *et al.*, 2013a) who reported that the color of fish skin gelatin film incorporated with leaf essential oil turned to be yellowish when soy lecithin was used as a surfactant.

Table 14. Effects of pressure levels and number of pass of microfluidization on the color of emulsion based film containing soy lecithin at different levels.

Soy lecithin (%)	Pressure (kpsi)	Number of passes	*L**	*a**	*b**	Δ*E**
50	0	0	87.15 ± 0.23 ^{*aB}	-1.78 ± 0.03 ^{aB}	13.09 ± 0.25 ^{aA}	14.22 ± 0.17 ^{aA}
	1	2	87.14 ± 0.22 ^{aB}	-1.79 ± 0.05 ^{aB}	13.11 ± 0.14 ^{aA}	14.25 ± 0.13 ^{aA}
		4	87.24 ± 0.15 ^{aB}	-1.79 ± 0.02 ^{aB}	13.22 ± 0.26 ^{aA}	14.30 ± 0.16 ^{aA}
	2	2	87.27 ± 0.18 ^{aB}	-1.79 ± 0.05 ^{aB}	13.13 ± 0.17 ^{aA}	14.20 ± 0.21 ^{aA}
		4	87.30 ± 0.20 ^{aB}	-1.81 ± 0.04 ^{aB}	13.16 ± 0.17 ^{aA}	14.22 ± 0.16 ^{aA}
	3	2	87.25 ± 0.21 ^{aB}	-1.82 ± 0.04 ^{aB}	13.13 ± 0.13 ^{aA}	14.21 ± 0.13 ^{aA}
		4	87.18 ± 0.28 ^{aB}	-1.79 ± 0.02 ^{aB}	13.24 ± 0.28 ^{aA}	14.34 ± 0.24 ^{aA}
75	0	0	85.08 ± 0.18 ^{aA}	-1.46 ± 0.03 ^{aA}	15.09 ± 0.09 ^{aB}	16.95 ± 0.11 ^{aB}
	1	2	85.01 ± 0.20 ^{aA}	-1.47 ± 0.02 ^{aA}	15.04 ± 0.10 ^{aB}	16.95 ± 0.15 ^{aB}
		4	85.12 ± 0.11 ^{aA}	-1.48 ± 0.04 ^{aA}	15.11 ± 0.17 ^{aB}	16.95 ± 0.16 ^{aB}
	2	2	85.18 ± 0.22 ^{aA}	-1.47 ± 0.02 ^{aA}	15.03 ± 0.14 ^{aB}	16.85 ± 0.21 ^{aB}
		4	85.02 ± 0.2 ^{aA}	-1.49 ± 0.03 ^{aA}	15.09 ± 0.16 ^{aB}	16.99 ± 0.15 ^{aB}
	3	2	85.18 ± 0.19 ^{aA}	-1.46 ± 0.03 ^{aA}	15.10 ± 0.18 ^{aB}	16.91 ± 0.12 ^{aB}
		4	85.07 ± 0.15 ^{aA}	-1.45 ± 0.05 ^{aA}	15.06 ± 0.16 ^{aB}	16.93 ± 0.18 ^{aB}

Different lowercase letters in the same column under the same soy lecithin level indicate significant differences ($p < 0.05$). Different uppercase letters in the same column under the same pressure level and number of pass indicate significant differences ($p < 0.05$). * Mean ± SD (n = 3).

Light transmission at selected wavelengths from 200 to 800 nm in UV and visible ranges and transparency value of varying films from fish skin gelatin are shown in Table 15. All films had the excellent barrier property against UV light at 200 and 280 nm (Table 15). Protein based films generally have high UV light barrier capacity owing to their high amount of aromatic amino acids that absorb UV light (Hamaguchi *et al.*, 2007). Similar results were reported for gelatin films from skins of tilapia (Tongnuanchan *et al.*, 2013a), bigeye snapper and brownstripe red snapper (Jongjareonrak *et al.*, 2006). Higher transmission of visible light in the range of 350-800 nm was generally observed in all films from microfluidized FFD, compared with control film. The lowest barrier property toward light transmission was obtained for films prepared from FFD with a pressure level of 3 kpsi. This was related well with the decrease in opaqueness of films containing the smallest oil droplets. When 50% soy lecithin was used, higher light transmittance at wavelengths of 350-800 nm was observed, compared with film containing 75% soy lecithin. The smaller oil droplets in FFD containing 50% of soy lecithin most likely rendered the film with less opaqueness. Those small oil droplets could allow the light to transmit through the film. For transparency values, the lower transparency value indicated that the film was more transparent. Microfluidized film had the lower transparency value than the control films ($p < 0.05$) (Table 15). This result suggested that the presence of smaller oil droplets yielded more transparent films. Basically, oil droplets localized in the film matrix lowered the transparency of gelatin film, more likely due to the light scattering effect (Tongnuanchan *et al.*, 2012). However, the distribution of small oil droplet prepared by microfluidization could enhance the light transmission through the film. In general, films with 50% soy lecithin showed the lower transparency value than those using 75% soy lecithin ($p < 0.05$), indicating that the formers were more transparent than the latter. Thus, the levels of soy lecithin and microfluidization pressure determined color, light transmittance and transparency of emulsified films.

4.4.2.5 Film morphology

SEM micrographs of the surface and freeze-fractured cross-section of films from fish skin gelatin incorporated with palm oil containing soy lecithin at various levels (50% and 75% w/w, based on palm oil) emulsified with different homogenization

Table 15. Effects of pressure levels and number of pass of microfluidization on the light transmittance and transparency value of emulsion based film containing soy lecithin at different levels.

Soy lecithin (%)	Pressure (kpsi)	Number of passes	Light transmittance (%) at different wavelength (nm)								Transparency value
			200	280	350	400	500	600	700	800	
50	0	0	0.00	0.12	4.67	9.80	18.81	26.50	33.04	38.68	$5.80 \pm 0.01^{*dA}$
	1	2	0.00	0.08	4.43	10.62	21.68	31.55	39.92	46.95	5.37 ± 0.01^{cA}
		4	0.00	0.08	4.56	10.91	22.98	31.64	40.49	47.71	5.35 ± 0.01^{cA}
	2	2	0.00	0.10	4.98	11.52	24.76	35.48	42.29	49.44	4.88 ± 0.02^{bA}
		4	0.00	0.09	4.80	11.84	24.10	35.48	42.56	51.72	4.88 ± 0.01^{bA}
	3	2	0.00	0.15	5.60	14.28	29.74	41.05	50.17	55.34	4.25 ± 0.02^{aA}
		4	0.00	0.14	6.31	14.51	29.57	41.25	50.04	56.77	4.26 ± 0.01^{aA}
75	0	0	0.00	0.10	3.38	6.64	12.68	18.20	23.19	27.72	7.97 ± 0.01^{dB}
	1	2	0.00	0.08	2.95	6.69	15.36	24.24	32.36	39.51	6.91 ± 0.02^{cB}
		4	0.00	0.07	3.05	7.28	15.72	24.64	31.80	39.30	6.88 ± 0.07^{cB}
	2	2	0.00	0.07	3.69	7.46	17.79	27.98	36.90	45.41	6.25 ± 0.02^{bB}
		4	0.00	0.08	3.80	8.96	17.55	28.35	37.43	46.84	6.22 ± 0.02^{bB}
	3	2	0.00	0.08	4.59	8.65	22.03	30.63	41.52	49.81	5.95 ± 0.01^{aB}
		4	0.00	0.08	4.06	9.97	22.71	30.77	42.82	49.95	5.93 ± 0.01^{aB}

Different lowercase letters in the same column under the same soy lecithin level indicate significant differences ($p < 0.05$). Different uppercase letters in the same column under the same pressure level and number of pass indicate significant differences ($p < 0.05$).
*Mean ± SD (n = 3).

conditions are illustrated in Figure 10. The control film (with typical homogenization) had the rough and irregular surface for both soy lecithin levels used. Smooth surface was obtained for film from microfluidized FFD. This result indicated that FFD subjected to microfluidization had the stable emulsion system and no collapse of emulsion occurred during casting and drying. For cross-section, the microfluidized films with both soy lecithin levels had the higher compact structure, compared with the controls. The cross-section of films became smoother when microfluidization technique was used for preparing FFD, in which more uniform network could be developed.

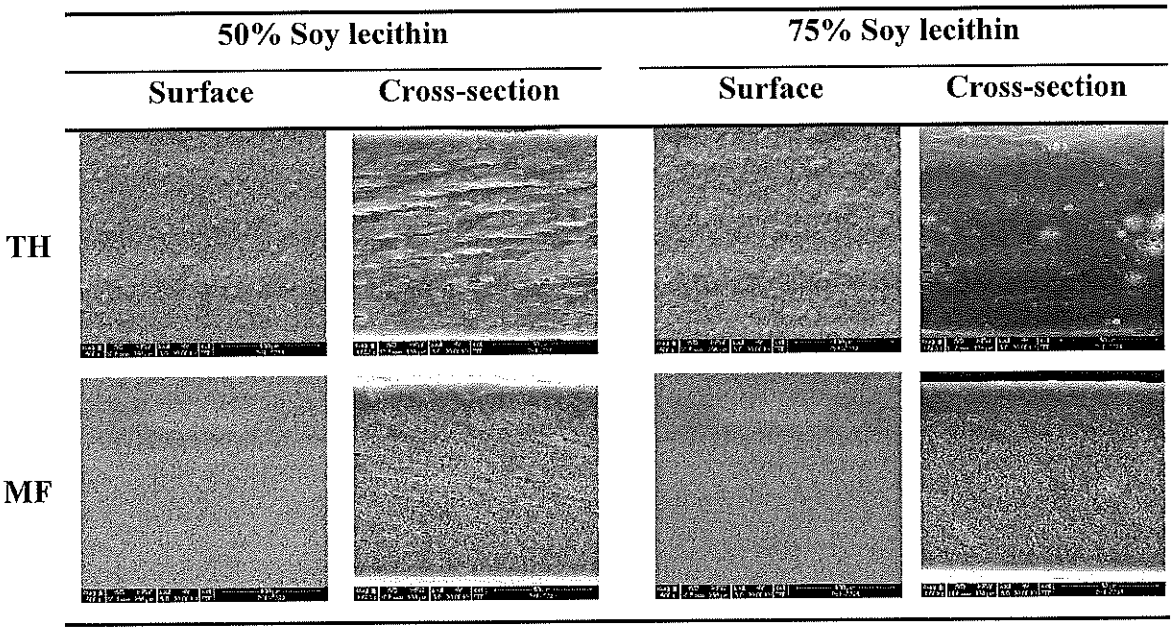

Figure 10. SEM micrographs of surface (magnification: 500×) and cross-section (magnification: 1800×) of films from fish skin gelatin incorporated with palm oil containing soy lecithin at various levels using different homogenization techniques. TH: Typical homogenization, MF: Microfluidization (3 kpsi, 2 passes)

Typically, the protein-protein disruption in the film matrix arose from droplets of palm oils was associated with the enhanced roughness of film cross-section (Tongnuanchan *et al.*, 2013b). Those oil droplets, particularly with the larger size, were localized inside the film network, thereby interrupting protein-protein chain interaction. This more likely brought about the discontinuous network as evidenced by the increase irregular network. However, films emulsified with microfluidization technique, which provided the small oil droplets, showed the uniformity. Thus, the oil droplet size and distribution

had the impact on the film microstructure, which was related to the mechanical properties and WVP of films incorporated with palm oil.

4.4.2.6 Differential scanning calorimetry (DSC)

DSC thermograms of the 1st and 2nd-heating scans of selected films from fish skin gelatin incorporated with palm oil containing soy lecithin at various levels (50% and 75% w/w, based on palm oil) emulsified with microfluidization pressure of 3 kpsi for 2 passes in comparison with typical homogenization are illustrated in Figure 11A and B, respectively. Glass transition temperature (T_g) of film is presented in Table 16. The glass transition is associated with molecular segmental motion of disordered (amorphous phase) structure which undergoes from a brittle glassy solid state to a rubbery or highly viscous state, whereas the melting transition indicated the temperature causing a disruption of ordered or aggregated structure (Tang *et al.*, 2009; Tongnuanchan *et al.*, 2015). The glass-transition temperature (T_g) of polymers is an important parameter in many applications, which affects the thermo-rheological behaviour (Tongnuanchan *et al.*, 2014). T_g of films from microfluidized FFD were higher than the control films (prepared with typical homogenization). It was suggested that the hydrophobic surfaces of small oil droplets could make the bridges via hydrophobic domains between protein chains, thus depressing the mobility of gelatin molecule in the films. In general, T_g of gelatin film from FFD containing 50% soy lecithin was higher than those containing 75% soy lecithin when the same homogenisation condition was used (Figure 11A). The result suggested that an excessive soy lecithin (75%) in FFD might lower the interaction between protein chains, thus increasing gelatin chain mobility. The DSC results were generally in agreement with the mechanical properties of films (Table 13). It was noted that the DSC thermogram of all films showed no endothermic transition after glass transition. This suggested that an ordered phase structure of gelatin was not existed in the obtained emulsified gelatin film, regardless of lecithin level and emulsification technique used. The addition of palm oil might disrupt the protein organization as well as protein-protein chain interaction in the resulting film. When oil droplets were dispersed in the gelatin film matrix, the weaker film structure was developed. The weaker film structure had the lower thermal stability, which required a lower enthalpy for destroying

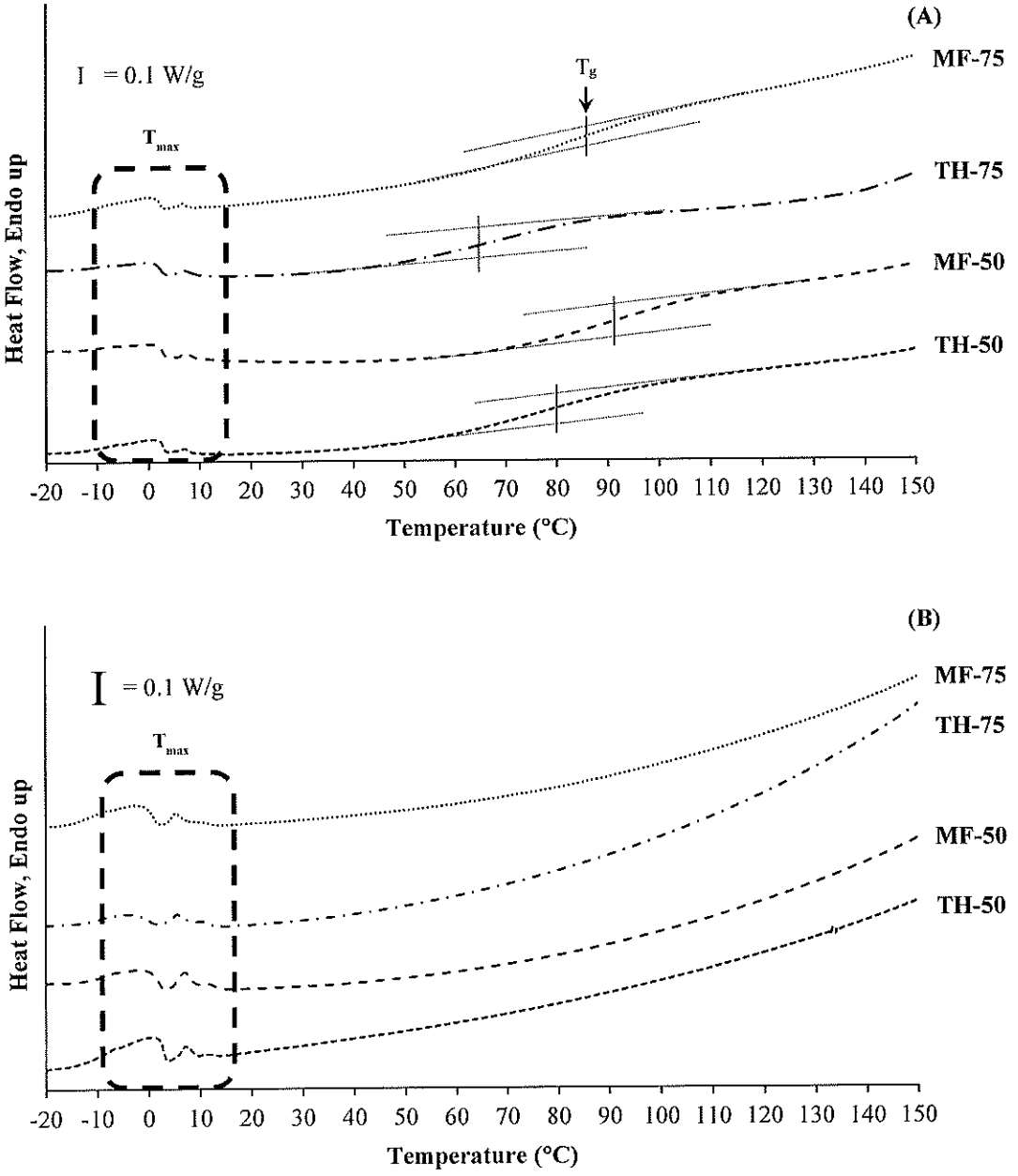

Figure 11. DSC thermograms of 1st-heating scan (A) and 2nd-heating scan (B) of the films from gelatin incorporated with palm oil containing soy lecithin at various levels using different homogenization conditions. TH-50 and TH-75: Films from FFD containing 50 and 75% soy lecithin prepared with typical homogenization. MF-50 and MF-75: Films from FFD containing 50 and 75% soy lecithin microfluidized at 3 kpsi for 2 passes.

Table 16. Effects of pressure levels and number of pass of microfluidization on glass transition temperature (°C), thermal degradation temperatures (T_d) and weight loss (Δw) of emulsion based film containing soy lecithin at different levels.

Soy lecithin (%)	Pressure (kpsi)	Number of passes	T_g (°C)	Δ_1		Δ_2		Δ_3		Δ_4		Residual (%)
				$T_{d1,onset}$ (°C)	Δw_1 (%)	$T_{d2,onset}$ (°C)	Δw_2 (%)	$T_{d3,onset}$ (°C)	Δw_3 (%)	$T_{d4,onset}$ (°C)	Δw_4 (%)	
50	0	0	80.02	63.09	2.69	186.01	4.28	272.41	36.86	384.87	40.52	15.65
	3	2	91.27	54.96	3.25	185.54	4.69	274.10	33.78	372.20	42.92	15.36
75	0	0	65.10	72.88	2.89	174.42	3.70	266.31	36.79	365.62	40.59	16.03
	3	2	86.52	58.50	2.73	175.36	4.94	267.46	36.79	355.58	39.13	16.41

Δ_1, Δ_2, Δ_3 and Δ_4 denote the first, second, third and fourth stage weight loss, respectively, of film during heating scan.

the inter-molecular interaction. Moreover, the thermograms of films incorporated with palm oil exhibited endothermic transition peak (T_{max}) observed at low temperature in the range of 0.52-0.93 °C. This endothermic transition was most likely attributed to the melting transition of palm oil droplets dispersed in the film network (Tongnuanchan *et al.*, 2015).

From the thermograms of the 2nd-heating scan, no glass transition of gelatin was noticed for all films (Figure 11B). After the first-heating scan, the adsorbed and bounded water acting as plasticizer could be removed out from the matrix of gelatin film. As a result, the gelatin chains could undergo more inter-molecular interaction. This led to decreased mobility of gelatin in the matrix and thus the glass transition could not be clearly detected. Nevertheless, the endothermic melting peak associated with incorporated oil phase was still observed for all films. Thus, the homogenization conditions and soy lecithin levels directly affected the thermal behaviour of gelatin film.

4.4.2.7 Thermo-gravimetric analysis (TGA)

TGA thermograms revealing thermal degradation behaviour of selected films are illustrated in Figure 12. The degradation temperatures (T_d), weight loss (Δw) and residue of all film samples are presented in Table 16. All film samples had four stages of weight loss. The first stage weight loss (Δw_1 = 2.69-3.25%) was observed over the temperature (T_{d1}) ranging from 54.96 to 72.88 °C. The weight loss at this temperature range was more likely associated with the loss of free water absorbed in the film. Film containing 50% soy lecithin generally had lower onset temperatures (T_{d1}) and weight loss than those containing 75% soy lecithin, except Δw_1 of film from microfluidized FFD was higher than the control counterpart. This might be due to the lower amount and interaction of water in film matrix, associated with higher hydrophobicity of film containing smaller oil droplet (Tongnuanchan *et al.*, 2015). Soybean lecithin at high level (75%) located in the aqueous phase might interact with water to a higher extent, as indicated by the lower Δw_1. The second stage of weight loss of films appeared approximately at the onset temperature of 174.42-186.01 °C (T_{d2}) with Δw_2 of 3.70-4.94%. Weight loss at this stage was mostly owing to the loss of low molecular weight protein fraction and glycerol as well as structural bound water (Hoque *et al.*, 2011). For the third stage of weight loss, T_{d3} of 266.31-274.10 °C and Δw_3 of

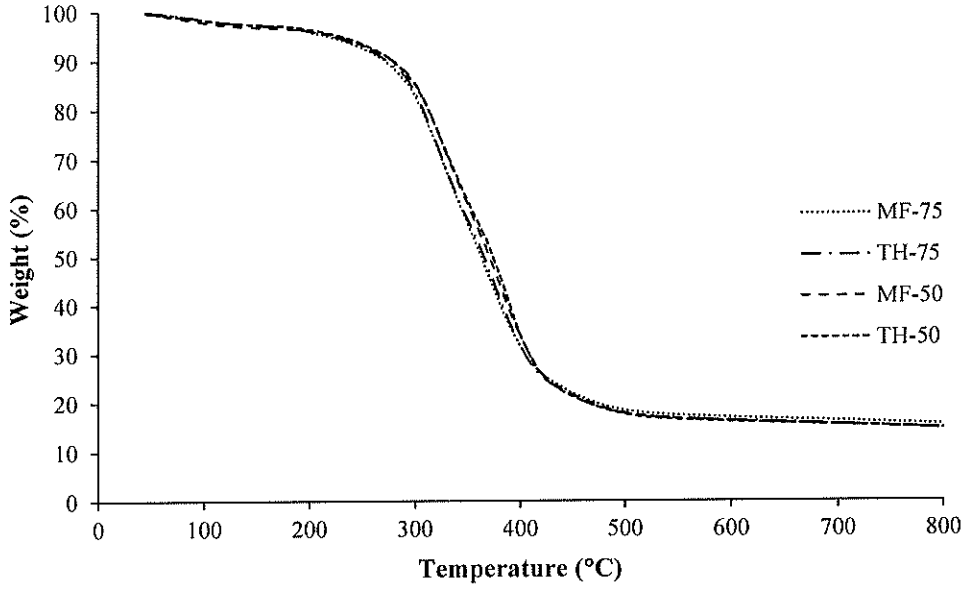

Figure 12. Thermo-gravimetric curves of the films from gelatin incorporated with palm oil containing soy lecithin at various levels using different homogenization conditions. TH-50 and TH-75: Films from FFD containing 50 and 75% soy lecithin prepared with typical homogenization. MF-50 and MF-75: Films from FFD containing 50 and 75% soy lecithin microfluidized at 3 kpsi for 2 passes.

33.78-36.86% were observed for all films. This stage of weight loss was most likely associated with the degradation or decomposition of larger size or highly interacted protein fractions. In general, T_{d2} and T_{d3} for films containing 50% soy lecithin was higher than those with 75% soy lecithin. The results suggested that film containing 50% soy lecithin showed the higher heat resistance than those containing 75% soy lecithin. Addition of 50% soy lecithin yielded a stronger film network due to the higher inter/intra-molecular protein interaction in film matrix. This led to the higher heat resistance of resulting films. It was noted that T_{d3} for films from microfluidized FFD were higher than the control films (prepared with typical homogenization). The result revealed that film from microfluidized FFD exhibited higher heat resistance than did the control film. Microfluidization yielded smaller oil droplet with increasing hydrophobic surface, thereby enhancing the interaction with protein in film matrix via hydrophobic-hydrophobic interaction. This confirmed that gelatin films from microfluidized FFD containing 50% soy lecithin had the higher interaction between

protein molecules in the film network as evidenced by higher TS (Table 13). For the fourth stage of weight loss, T_{d4} of 355.58-384.87 °C with Δw_4 of 39.13-42.92% were obtained for all emulsion films, suggesting the loss of high thermal stable components, possibly gelatin molecules closely associated with soy lecithin. The T_{d4} for films containing 50% soy lecithin was higher than those containing 75% soy lecithin. However, gelatin films incorporated with 75% soy lecithin had higher residue mass from thermal degradation, compared with film containing 50% soy lecithin. This might be related with the heat stable soy lecithin and their complex with proteins. The result indicated that homogenization conditions and soy lecithin levels had the pronounced impact on thermal stability of gelatin-based film.

4.4.2.8 Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra of selected films prepared from FFD containing soy lecithin at various levels (50% and 75% w/w, based on palm oil content) emulsified with different homogenization conditions are illustrated in Figure 13. Overall, films generally showed the similar major peaks, consisting of amide-I, II, III, A and B, commonly found in protein based films. The band situated at the wavenumber of 1055 cm^{-1} was found in all film samples, corresponding to the OH group, mainly from glycerol added as a plasticizer (Bergo and Sobral, 2007). All films had the similar spectra in the range of 1700-1200 cm^{-1}, covering amide-I, II and III bands. Amide-A peak of films from FFD containing 50% soy lecithin found at wavenumber of 3307 cm^{-1}, which was higher than that of those films containing 75% soy lecithin (3304 cm^{-1}). The amide-A band represents the NH-stretching coupled with hydrogen bonding. Addition of soy lecithin at level of 50% providing smaller oil droplet might interrupt interaction via hydrogen bonding with –NH in the gelatin chains to a higher extent as indicated by the higher wavenumbers. Film containing 50% soy lecithin also showed the higher amplitude of amide-A band. Amide-B band was found at 3082 cm^{-1} for all film samples. Amide-B band illustrated NH-stretching vibration and asymmetric CH-stretching vibration (Kong and Yu, 2007). Additionally, all films showed another two peaks at wavenumbers of 2924 and 2854 cm^{-1}. The amplitudes of these peaks were much higher in films from microfluidized FFD, especially when 50% soy lecithin was used as a surfactant. Peaks at wavenumbers around 2853 cm^{-1} and 2924 cm^{-1}

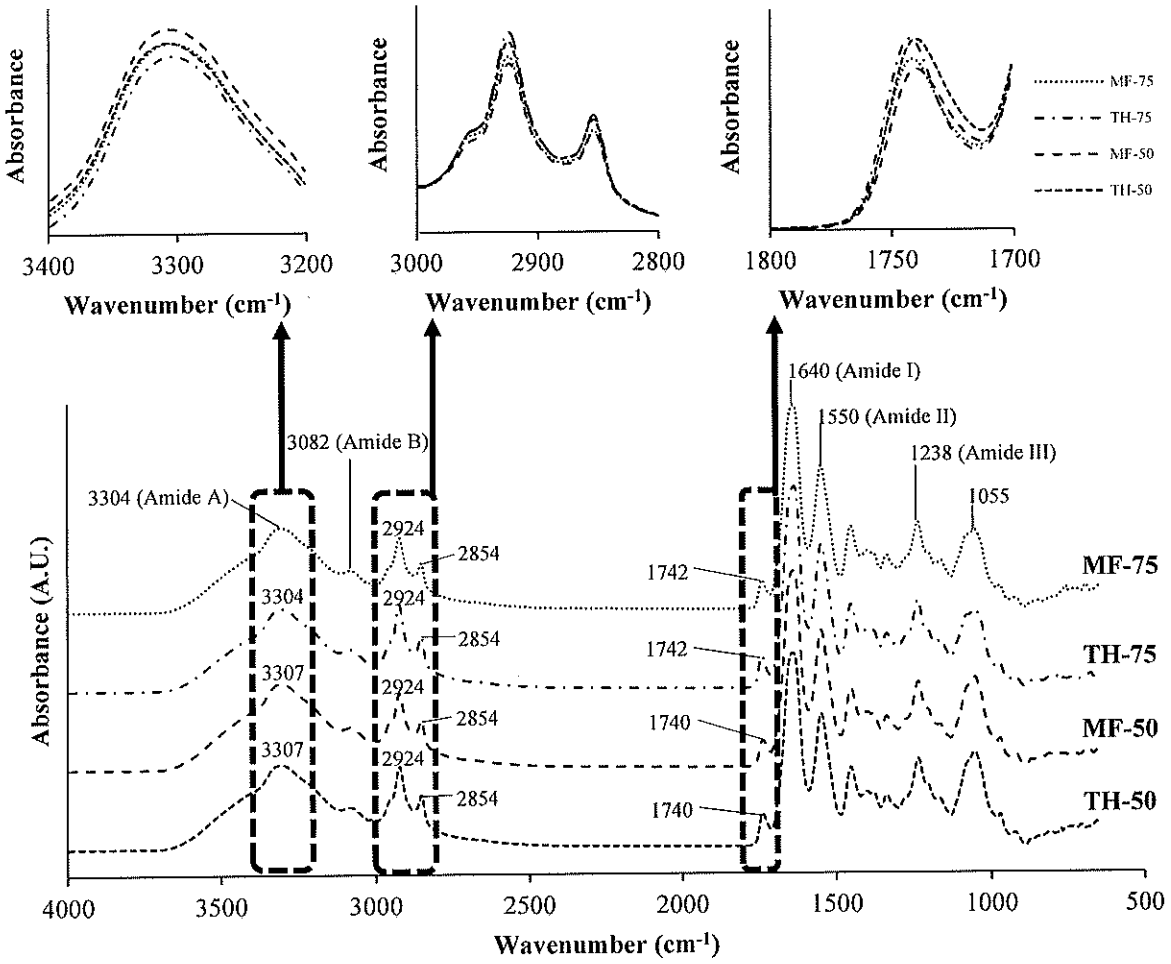

Figure 13. ATR-FTIR spectra of the films from gelatin incorporated with palm oil containing soy lecithin at various levels using different homogenization conditions. TH-50 and TH-75: Films from FFD containing 50 and 75% soy lecithin prepared with typical homogenization. MF-50 and MF-75: Films from FFD containing 50 and 75% soy lecithin microfluidized at 3 kpsi for 2 passes.

represented the methylene asymmetrical and symmetrical stretching vibration of the aliphatic C-H in CH_2 and CH_3 groups, respectively (Muik *et al.*, 2007). Those vibration stretching bands are obviously found in most lipids and hydrophobic substances (Guillén and Cabo, 2004). The result confirmed the presence of hydrophobic substance, palm oil, in the film. With higher lecithin used (75%), the proportion of palm oil in the and in the film oil became less as evidenced by the lower amplitude of those peaks. Furthermore, the peak at wavenumber of 1740-1742 cm^{-1} was observed in all films containing palm oil. The carbonyl absorption of triglyceride ester linkage was observed

at 1746 cm^{-1} (Setiowaty *et al.*, 2000). Thus, the stretching vibration peak assignable to the C=O group of triglycerides was noticeable when the palm oil was incorporated.

4.5 Conclusions

Microfluidization conditions and addition of soy lecithin affected the stability of FFD and properties of emulsion-based gelatin films. Microfluidized FFD containing 50% soy lecithin showed the small oil droplet size and the emulsion was more stable during storage. Films from microfluidized FFD showed the increased water vapor barrier property, strength and extensibility. Films from microfluidized FFD containing 50% soy lecithin had lower WVP with higher TS and EAB than those containing 75% soy lecithin. Decreases in L^* value and light transmittance with coincidental increases in b^*- and ΔE^* values were observed in films, especially when the amount of soy lecithin incorporated increased. The compact film with smoother surface was observed in films from microfluidized FFD with higher thermal stability than control emulsion film. Thus, the microfluidization with appropriated soy lecithin level could enhance stability of emulsion in FFD, which directly determined properties and morphology of film.

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CHAPTER 5
EFFECT OF FISH GELATIN FILM WITHOUT AND WITH PALM OIL INCORPORATED ON QUALITY CHANGES OF SHRIMP CRACKER DURING STORAGE

5.1 Abstract

Impact of fish gelatin film incorporated without (GF) and with palm oil (EF) on the quality changes of fried shrimp cracker during the storage of 15 days at room temperature was investigated, in comparison with nylon/linear low-density polyethylene (Nylon/LLDPE) film. The moisture content and water activity of shrimp cracker packaged with all films increased during storage ($p < 0.05$). The lowest moisture content and water activity were found in the sample packaged with Nylon/LLDPE film throughout the storage ($p < 0.05$). Sample packaged with fish gelatin films incorporated with palm oil generally had lower moisture content than those without oil added during the first 12 days of storage ($p < 0.05$). During 15 days of storage, shrimp cracker packaged with Nylon/LLDPE film generally had the lower PV and TBARS value as well as volatile compounds, except for n-nonanal, than those stored in fish gelatin films, regardless of oil incorporation. The decreases in crispiness and increases in toughness occurred for all samples during 15 days of storage. Nevertheless, the lower changes were observed in the sample packaged with Nylon/LLDPE film. Overall, gelatin film showed the excellent oxygen barrier property, which was associated with the retardation of lipid oxidation. The incorporation of oil into gelatin film could lower WVP but negatively increased oxygen permeability of resulting film. Thus, the improvement of gelatin film is still required.

5.2 Introduction

During the last decade, there has been an increasing interest in edible or biodegradable films based on biopolymers. The main advantage of biopolymers over synthetic polymers is their biodegradability. Biopolymers such as polysaccharides and proteins have been used as biodegradable and/or edible food packaging materials (Artharn *et al.*, 2009; Gennadios *et al.*, 1997). Proteins are important biopolymers possessing good film forming ability. Among proteins, fish gelatin has been used as

film-forming material (Gómez-Guillén *et al.*, 2009). Properties of fish gelatin-based films depend on various factors such as the source of gelatin, concentration of gelatin, plasticizers and substances incorporated into the film forming solutions (Jongjareonrak *et al.*, 2006; Limpisophon *et al.*, 2010; Tongnuanchan *et al.*, 2012). Gelatin film generally has good mechanical and oxygen barrier properties. However, it has high water absorptivity due to hydrophilicity of gelatin molecules and the presence of hydrophilic plasticizers such as glycerol (Gennadios *et al.*, 1994; Krochta and De Mulder-Johnston, 1997; McHugh and Krochta, 1994). To improve water vapor barrier properties of the gelatin films, hydrophobic substances such as fats and oils have been added (Bertan *et al.*, 2005; Tongnuanchan *et al.*, 2012). Recently, gelatin film incorporated with palm oil has been prepared by Tongnuanchan *et al.* (2015) and it had the lowered water vapor permeability. With the improved water vapor barrier property, the gelatin films incorporated with fats and oils could become an environmental friendly edible packaging, which could be used to maintain the quality of foods, especially those prone to the textural changes induced by moisture adsorption. Thus, the aim of this work was to study the use of the gelatin films incorporated without and with palm oil to extend the shelf-life of shrimp cracker.

5.3 Materials and methods

5.3.1 Materials and chemicals

Glycerol, soy lecithin (HLB = 4.0) and trichloroacetic acid were obtained from Merck (Darmstadt, Germany). Ammonium thiocyanate and 1,1,3,3-tetramethoxypropane (MDA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade. Fish gelatin from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Palm oil was obtained from *OLEEN Company Limited* (Bangkok, Thailand).

5.3.2 Films from fish gelatin incorporated without and with palm oil

5.3.2.1 Preparation of film-forming solution and dispersion

Gelatin (3.5 g) was dissolved in 90 mL of distilled water and then heated at 70 °C for 30 min. Glycerol was added into solution to obtain the concentration of

30% (w/w) based on protein content. The mixtures was stirred using a magnetic stirrer for 5 min. The resulting solution was termed as 'film-forming solution, FFS'. For another portion of gelatin solution, glycerol was added to gelatin solution at the level of 10% (w/w, based on protein content). Palm oil was mixed with soy lecithin at a level of 50% (w/w, based on palm oil) and then transferred into the prepared gelatin solution to obtain the palm oil concentration of 75% (w/w, based on protein content). The volume was adjusted to 100 mL using distilled water. The mixture was homogenized at a speed of 22,000 rpm for 3 min using a rotor-stator homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The coarse emulsion was passed through a Microfluidizer (Model HC-5000, Microfluidizer, Newton, MA, USA) at 3 kpsi for 2 passes. Fine emulsion was referred to as 'film-forming dispersion, FFD'. Both FFS and FFD were subjected to film preparation.

5.3.2.2 Film casting and drying

Prior to casting, FFS and FFD were subjected to air removal by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature. FFS or FFD (4 mL) were cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown for 12 h at room temperature (28-30 °C) prior to further drying at 25 °C and 50 ± 5% RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and subsequently analyzed.

5.3.2.3 Determination of film properties

5.3.2.3.1 Film thickness

The thickness of film was measured using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Five random locations around each film of ten film samples were used for determination of average thickness.

5.3.2.3.2 Mechanical properties

Prior to testing, films were conditioned for 48 h at 25 °C and 50 ± 5% RH. Tensile strength (TS) and elongation at break (EAB) were determined as described by Iwata *et al.* (2000) using the Universal Testing Machine (Lloyd Instrument,

Hampshire, UK) equipped with tensile load cell of 100 N. Ten samples (2×5 cm^2) with an initial grip length of 3 cm were used for testing. Cross-head speed was set at 30 mm/min.

5.3.2.3.3 Water vapor permeability (WVP)

WVP was measured using a modified ASTM method (ASTM, 1989) as modified by Shiku *et al.* (2004). The films were sealed on an aluminium permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and a rubber gasket to hold the films in place. The cups were placed in a desiccator containing the distilled water at 30 °C. The cups were weighed at 1-h intervals over a 10-h period. WVP of the film was calculated as follows:

$$WVP \text{ (g/m s Pa)} = \frac{wl}{At(P_2-P_1)}$$

where w is the weight gain of the cup (g); l is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); $P_2 - P_1$ is the vapor pressure difference across the film (4242.31 Pa at 30 °C).

5.3.2.3.4 Oxygen transmission

Oxygen transmission rate (OTR) of films was measured according to the ASTM D3985-05 method. An Oxygen Permeation Analyzer (Illinois model 8000, Illinois Instruments Inc., USA) was used to measure oxygen transmission rates through the film. Each film was placed on a stainless steel mask. The mask was then placed in a test cell and exposed an oxygen atmosphere flow on one side and a nitrogen atmosphere flow on the other. Oxygen transmission rates was measured at 25 ± 1 °C and 50 ± 2% relative humidity. The film was allowed to equilibrate for 10 h before measurements.

5.3.3 Preparation of shrimp cracker

Dried shrimp crackers were obtained from a local market in Songkhla, Thailand. They had an average diameter of 5 mm with thickness of 1.5 mm. Crackers were fried using an electric deep fryer containing 4 L frying oil. Palm oil used as a frying medium was preheated to 180 °C before frying. Samples were placed in the

frying basket and immersed into the oil for 30 sec until samples are completely puffed. Thereafter, fried samples were placed on the screen to drain the oil for 5 min. The fried shrimp cracker was blotted with paper towels to remove an excess oil on the surface and allowed to cool at room temperature (26-28 °C), approximately 15 min before being packaged.

5.3.4 Study on quality changes of fried shrimp cracker during storage

Fried shrimp cracker (1.4 g) was placed into an aluminum cup and sealed with fish gelatin films incorporated without and with palm oil or Nylon/LLDPE film. Fried shrimp cracker stored in aluminum cup without packaging was prepared as the control. The samples were stored at 28 ± 0.5 °C and 65 ± 5% RH. Shrimp cracker samples were taken every 3 days for totally 15 days for analyses.

5.3.4.1 Moisture content and water activity

Moisture content was determined by the method of AOAC (2000). Water activity (a_w) was measured using a water activity meter (4TEV, Aqualab, Pullman, WA, USA).

5.3.4.2 Peroxide value

Peroxide value (PV) was determined as per the method of Richards and Hultin (2000) with a slight modification. Ground shrimp cracker (1 g) was homogenized at a speed of 13,500 rpm for 2 min in 11 mL of chloroform/methanol (2:1, w/v). Homogenate was filtered using Whatman No.1 filter paper. Two milliliters of 0.5% NaCl were added to 7 mL of the filtrate. The mixture was vortexed at a moderate speed for 30 s and then centrifuged at 3000xg for 3 min to separate the sample into two phases. Two milliliters of cold chloroform/methanol mixture (2:1) were added to 3 mL of the lower phase. Twenty-five microliters of ammonium thiocyanate and 25 µl of iron (II) chloride were added to the mixture (Shanta and Decker, 1994). Reaction mixture was allowed to stand for 20 min at room temperature prior to reading the absorbance at 500 nm. A standard curve was prepared using cumene hydroperoxide at a concentration range of 0.5-50 ppm. PV was expressed as mg cumene/kg sample.

5.3.4.3 Thiobarbituric acid-reactive substances (TBARS)

TBARS values were determined as described by Buege and Aust (1978). Ground shrimp cracker (0.2 g) was mixed with 2.5 mL of a TBA solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl. The mixture was heated in a boiling water bath (95-100 °C) for 10 min to develop a pink color, cooled with running tap water and then sonicated for 30 min, followed by centrifugation at 5000xg at 25 °C for 10 min. The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane (MDA) at the concentration ranging from 0 to 10 ppm and TBARS value was expressed as mg MDA equivalent/kg sample.

5.3.4.4 Textural properties

Textural property of shrimp cracker was measured at room temperature by a compressive test using a texture analyzer (Stable Micro Systems, Guildford, UK). Ottawa cell with flat square probe was used to determine the crispiness, in which the maximum force required to break the shrimp cracker samples was recorded. Toughness was determined by measuring the area under the curve during compressing. The studies were conducted using a pre-test speed of 1.0 mm/s, test speed of 2.0 mm/s, distance of 5.0 mm, and load cell of 50 kg.

5.3.4.5 Volatile compounds

Volatile compounds in fried shrimp cracker samples were determined using a solid-phase micro-extraction gas chromatography mass spectrometry (SPME-GC-MS) following the method of Iglesias and Medina (2008) with a slight modification.

5.3.4.5.1 Extraction of volatile compounds by SPME fiber

To extract volatile compounds, 1 g of sample was mixed with 4 mL of deionized water and stirred continuously to disperse the sample. The mixture was heated at 60 °C in 20 mL headspace vial with equilibrium time of 10 h. The SPME fiber (50/30 μm DVB/Carboxen™/ PDMS StableFlex™) (Supelco, Bellefonte, PA, USA) was conditioned at 270 °C for 15 min before use and then exposed to the headspace.

The 20 mL-vial (Agilent Technologies, Palo Alto, CA, USA) containing the sample extract and the volatile compounds were allowed to absorb into the SPME fiber at 60 °C for 1 h. The volatile compounds were then desorbed in the GC injector port for 15 min at 270 °C.

5.3.4.5.2 GC–MS analysis

GC–MS analysis was performed in a HP 5890 series II gas chromatography (GC) coupled with HP 5972 mass-selective detector equipped with a splitless injector and coupled with a quadrupole mass detector (Hewlett Packard, Atlanta, GA, USA). Compounds were separated on a HP-Innowax capillary column (Hewlett Packard, Atlanta, GA, USA) (30 m × 0.25 mm ID, with film thickness of 0.25 μm). The GC oven temperature program was: 35 °C for 3 min, followed by an increase of 3 °C/min to 70 °C, then an increase of 10 °C/min to 200 °C, and finally an increase of 15 °C/min to a final temperature of 250 °C and holding for 10 min. Helium was employed as a carrier gas, with a constant flow of 1 mL/min. The injector was operated in the splitless mode and its temperature was set at 270 °C. Transfer line temperature was maintained at 260 °C. The quadrupole mass spectrometer was operated in the electron ionisation (EI) mode and source temperature was set at 250 °C. Initially, full scan mode data was acquired to determine appropriate masses for the later acquisition in scan mode under the following conditions: mass range: 25-500 amu and scan rate: 0.220 s/scan. All the analyses were performed with ionization energy of 70 eV, filament emission current at 150 μA, and the electron multiplier voltage at 500 V.

5.3.4.5.3 Analyses of volatile compounds

Identification of volatile compounds in the samples was done by consulting ChemStation Library Search (Wiley 275.L). Identification of compounds was performed, based on the retention time and mass spectra in comparison with those of standards from ChemStation Library Search (Wiley 275.L). Quantification limits were calculated to a signal-to-noise (S/N) ratio of 10. The identified volatile compounds were expressed in the terms of relative abundance.

5.3.5 Statistical analysis

All experiments were run in triplicate with different three lots of films. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test. Analysis was performed using the SPSS package (SPSS for windows, Inc., Chicago, IL, USA).

5.4 Results and discussions

5.4.1 Properties of different films

Films from fish gelatin incorporated without and with palm oil and nylon/linear low-density polyethylene (Nylon/LLDPE) had varying properties as shown in Table 17. Different films showed various thickness. Film from fish gelatin incorporated with palm oil (EF) showed the higher thickness, while gelatin film (GF) had the lowest thickness ($p < 0.05$). For tensile strength and elongation at break (EAB), Nylon/LLDPE film had the highest values ($p < 0.05$). When comparing between GF and EF samples, the latter showed the higher TS and EAB than the former ($p < 0.05$). Oil droplet incorporated in matrix might strengthen the resulting films. Oil droplets might reduce the interaction between gelatin chain as indicated by higher EAB. This reduction in protein-protein interaction might take place at appropriate degree, which brought about the increased extension of film before breaking. Upon film stretching without breaking, gelatin molecule in the film matrix could undergo more orientation, which could withstand more tensile load. This led to the increased strength (i.e. TS) of palm oil incorporated gelatin film, compared to control film. For water vapor permeability (WVP), Nylon/LLDPE film, which is the synthetic film, had the lowest value ($p < 0.05$). Gelatin film showed the decrease in WVP when oil was incorporated in the film (EF). Oil droplets distributed in film acted as barrier for water vapor adsorption and migration through the film. Overall, film from Nylon/LLDPE had the highest oxygen transmission rate (OTR). In general, protein based film showed higher oxygen barrier properties than the plastic films such as Nylon/mLLDPE, LDPE and HDPE films (Cho *et al.*, 2010; Rhim *et al.*, 2006). Among all films, GF had the lowest OTR ($p < 0.05$). Gelatin based film has been reported to possess the excellent oxygen barrier property (Jongjareonrak *et al.*, 2006). It was noted that the incorporation of oil

increased OTR of gelatin film. When oil was added, the matrix was disconnected and the hydrophobic of gelatin film generally increased, which could thus increase adsorption or solubility of oxygen, a non-polar molecule, in the film. This brought about the lowering oxygen barrier property of film. The increasing oxygen transmission rate of films incorporated with oil was related with the microstructural changes, in which the film became more porous with the addition of oil (Altiok *et al.*, 2010).

Table 17. Thickness, tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP) and oxygen gas transmission rate (OTR) of films from fish gelatin incorporated without (GF) and with (EF) palm oil and nylon/linear low-density polyethylene (Nylon/LLDPE) film.

Films	Thickness (mm)	TS (MPa)	EAB (%)	WVP ($\times 10^{-12}$ g/m s Pa)	OTR (cc/m^2 day)
Nylon/LLDPE	0.080±0.001 ^{b*}	48.75±1.06 ^{a}	132.5±3.70 ^{a}	0.81±0.16 ^{c}	92.5
GF	0.055±0.002 ^{c}	26.90±1.19 ^{c}	40.17±1.37 ^{c}	23.90±0.69 ^{a}	3.53
EF	0.086±0.001 ^{a}	41.00±0.47 ^{b}	59.65±1.11 ^{b}	4.50±0.04 ^{b}	63.2

Different lowercase letters in the same column indicate significant differences ($p < 0.05$). *Mean ± SD ($n = 3$).

5.4.2 Quality changes of shrimp cracker during storage as affected by various films

5.4.2.1 Moisture content and water activity

Moisture contents of fried shrimp cracker kept in aluminum cup covered with fish gelatin films incorporated without (GF) and with palm oil (EF) in comparison with nylon/linear low-density polyethylene (Nylon/LLDPE) film during 15 days of storage are shown in Figure 14 (A and B). The initial moisture content of cracker was 1.13%. In general, the moisture content of fried shrimp cracker in aluminum cup covered with all films drastically increased within the first 3 days of storage ($p < 0.05$). The moisture content was increased to a higher extent for the sample packaged in both gelatin films (GF and EF) than that found in sample packaged in Nylon/LLDPE film throughout 15 days ($p < 0.05$). At 15 days of storage, samples packaged with GF, EF

and Nylon/LLDPE film had the increase in moisture content by 8.40, 8.46, and 4.54-fold, respectively. The result was in agreement with the lowest WVP of Nylon/LLDPE film (Table 17). When comparing moisture content of shrimp cracker covered with GF and EF, the former showed the higher moisture content ($p < 0.05$). The result confirmed that the incorporation of oil could lower the migration of water vapor to the sample. This was in accordance with the lower WVP of EF in comparison with GF (Table 17).

For water activity, fried shrimp cracker covered with EF showed the lower water activity than those covered with GF during 3-9 days of storage ($p < 0.05$). Initial water activity of fried crackers was 0.22. Much lower water activity was also found in sample packaged with Nylon/LLDPE film throughout 15 days of storage ($p < 0.05$). The result indicated that water vapor barrier property of gelatin film was lower than that of Nylon/LLDPE film. Generally, water vapor barrier property of gelatin film was poorer than that of other biopolymer films, especially for non-hydrophilic material or synthetic polymer, due to the hydrophilic nature of gelatin and hydrophilic plasticizer required for film preparation (Limpisophon *et al.*, 2009). The increase in moisture content and water activity of fried shrimp cracker was due to the diffusion of water vapor from the surrounding atmosphere through the packaging material. Generally, the incorporation of hydrophobic substances such as oil improved the water vapor barrier property of gelatin film (Tongnuanchan *et al.*, 2015). Therefore, the incorporation of palm oil could improve the water vapor barrier property of gelatin film, as evidenced by the lower moisture content and water activity of fried shrimp cracker during the storage. Among all samples, that stored in air (without film covering) had the higher moisture content and water activity. Therefore, films, either synthetic or gelatin films, were able to serve as the barrier for water vapor migration, in which the quality changes of cracker could be retarded.

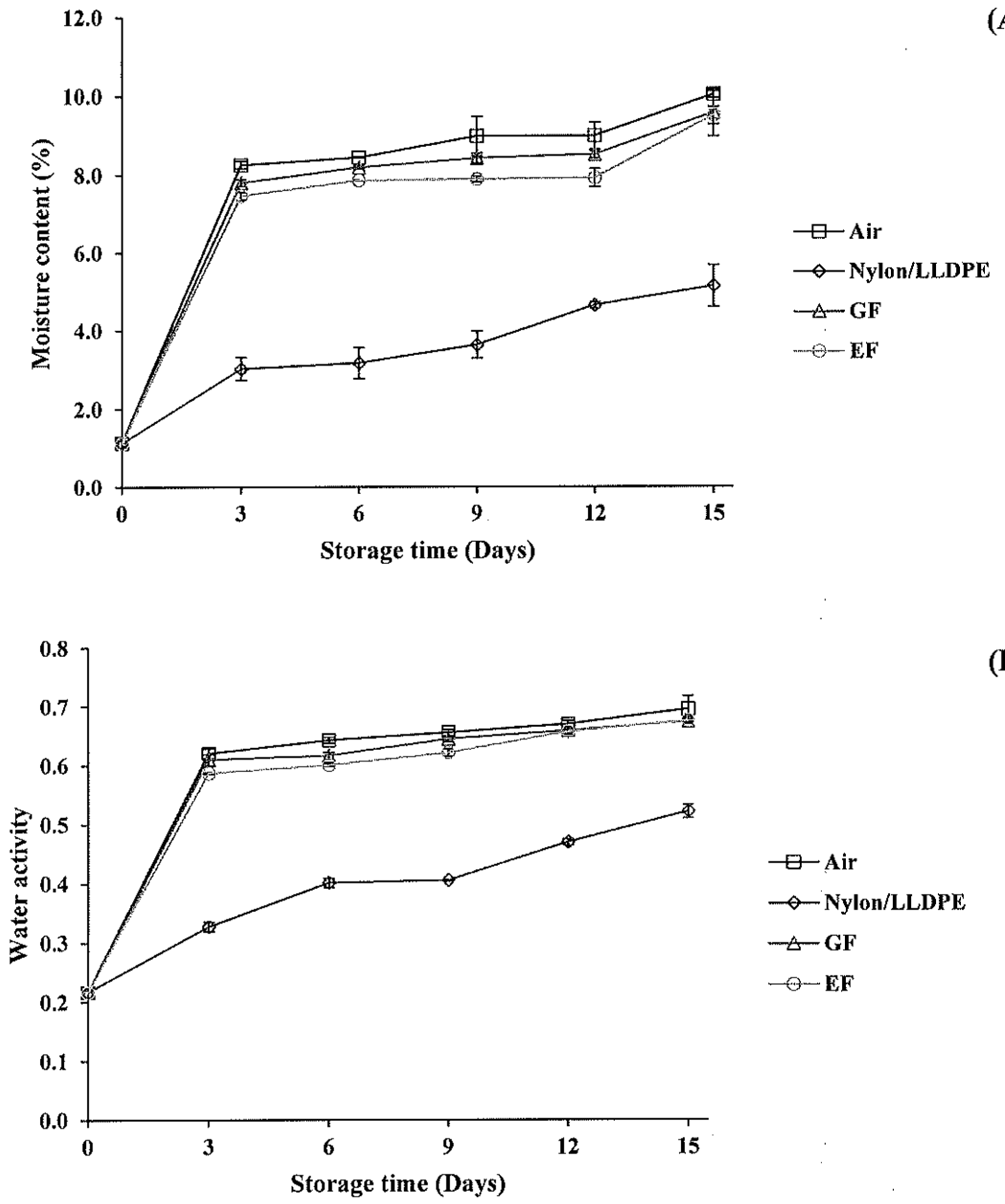

Figure 14. Changes in moisture content (A) and water activity (B) of fried shrimp cracker packaged with fish gelatin film incorporated without (GF) and with palm oil (EF) and nylon/linear low-density polyethylene (Nylon/LLDPE) film during storage of 15 days at 28 ± 0.5 °C and 65 ± 5% RH. Air: Fried shrimp cracker without packaging. Bars represent the standard deviation (n=3).

5.4.2.2 Peroxide value and TBARS

Lipid oxidation of fried shrimp cracker stored in aluminum cup covered with GF and EF in comparison with Nylon/LLDPE film during 15 days of storage was monitored as shown in Figure 15 (A and B). PV of samples covered with either GF or EF increased during 15 days of storage ($p < 0.05$). During storage, the samples packaged with Nylon/LLDPE film had no difference in PV, compared with those packaged with GF ($p > 0.05$). It was found that sample covered with EF had similar PV to the control throughout the storage ($p > 0.05$). Oxidative changes in crackers were initiated by the formation of free radicals, the precursors for the hydroperoxides, which were the primary oxidation products (Maisuthisakul *et al.*, 2007). Crackers are susceptible to lipid oxidation due to the large surface area in contact with the air, in which carbonyl compounds and hydroperoxides are generated (Noomhorm *et al.*, 1997). Due to the excellent oxygen barrier property of GF, the PV was expected to be lower in the sample covered with GF. Nevertheless, GF had the poorer WVP than Nylon/LLDPE film. As a consequence, the water in the cracker was increased. The higher amount of water might facilitate the movement of reactants, especially prooxidant. Thus, the oxidative reaction was increased although the amount of oxygen used for oxidation was limited.

For TBARS value, the continuous increases were observed in all samples throughout the storage of 15 days. The lower TBARS values were obtained in sample stored with Nylon/LLDPE film during 9-12 days of storage ($p < 0.05$). Lipid oxidation can be accelerated at high RH since more water molecules can act as reaction media (Partanen *et al.*, 2008). As the moisture content and water activity of all samples increased during 15 days of storage, lipid oxidation could be promoted, especially for the samples covered with EF. However, there was no difference in TBARS value between sample covered with GF and Nylon/LLDPE films at day 15 ($p > 0.05$). The result was in agreement with PV. Thus, packaging materials used was a prime factor affecting water vapor and oxygen permeability, thereby influencing oxidative stability. The result suggested that the incorporation of palm oil into gelatin could lower water vapor migration, but it decreased the barrier property toward oxygen molecule (Table 17).

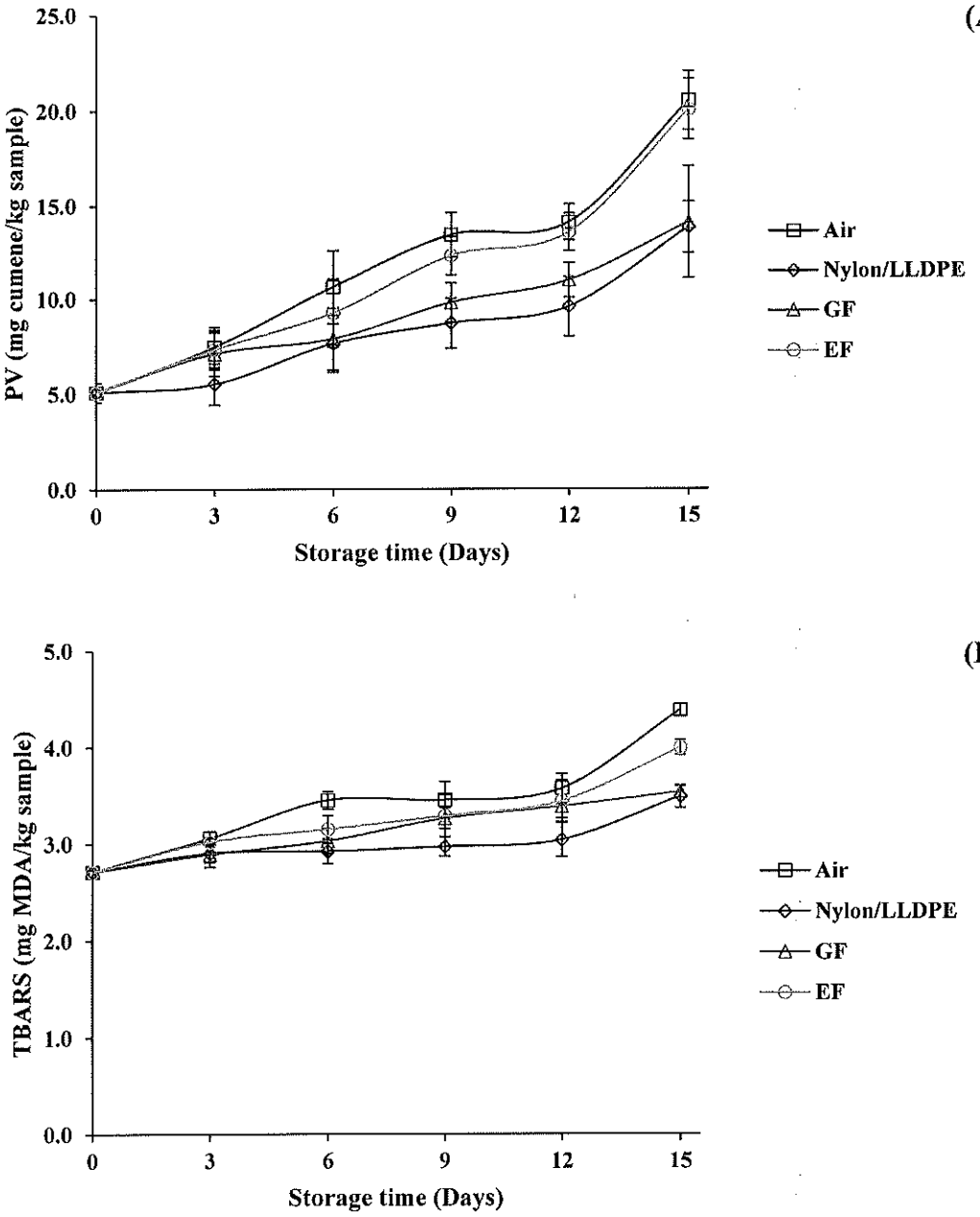

Figure 15. Changes in peroxide value (A) and TBARS (B) of fried shrimp cracker packaged with fish gelatin film incorporated without (GF) and with palm oil (EF) and nylon/linear low-density polyethylene (Nylon/LLDPE) film during storage of 15 days at 28 ± 0.5 °C and 65 ± 5% RH. Air: Fried shrimp cracker without packaging. Bars represent the standard deviation (n=3).

5.4.2.3 Textural properties

Crispiness (force necessary to break a product) and toughness (area under the curve during compressing) of fried shrimp cracker stored in aluminum cup, covered with GF and EF in comparison with Nylon/LLDPE film during 15 days of storage are shown in Figure 16 (A and B). Physical properties, such as density, shrinkage and porosity, are the main factors affecting the texture and transport phenomena of fried foods (Ziaiifar *et al.*, 2010). Crispiness is related to the deformation and fracturing of subsequent layers in a cell structure of food products (Luyten *et al.*, 2004). Crispiness of samples packaged with all films sharply decreased with the first 3 days of storage ($p < 0.05$), except that covered with Nylon/LLDPE film. Similar rate of decrease in crispiness was observed during 3-15 days of storage. During storage, shrimp cracker covered with Nylon/LLDPE film showed the higher crispiness than others ($p < 0.05$). No difference in crispiness between samples packaged with GF and EF was observed ($p > 0.05$). Crispiness of sample covered with gelatin films was similar to that of the control. On the other hand, the toughness of shrimp cracker samples covered with GF and EF and that kept in air markedly increased within the first 3 day of storage ($p < 0.05$). No change in toughness of sample packaged with Nylon/LLDPE film was obtained during the first 6 day of storage ($p > 0.05$). When storage time was increased, the water vapor might migrate through the films and contact with the sample surface to a higher extent. Water more likely reduced the strength of air cell structure of cracker (Luyten *et al.*, 2004). Water might fluidize the cracker network, where it became tougher in texture. The change was coincidental with the increases in toughness of cracker. Thus, packaging with the ability in lowering water vapor migration was required for crispy cracker, in which textural property could be maintained. Although the incorporation of palm oil into gelatin film in this study could lower WVP of resulting film to some degree, the film still showed the lower barrier property toward water, in comparison with the commercial Nylon/LLDPE film.

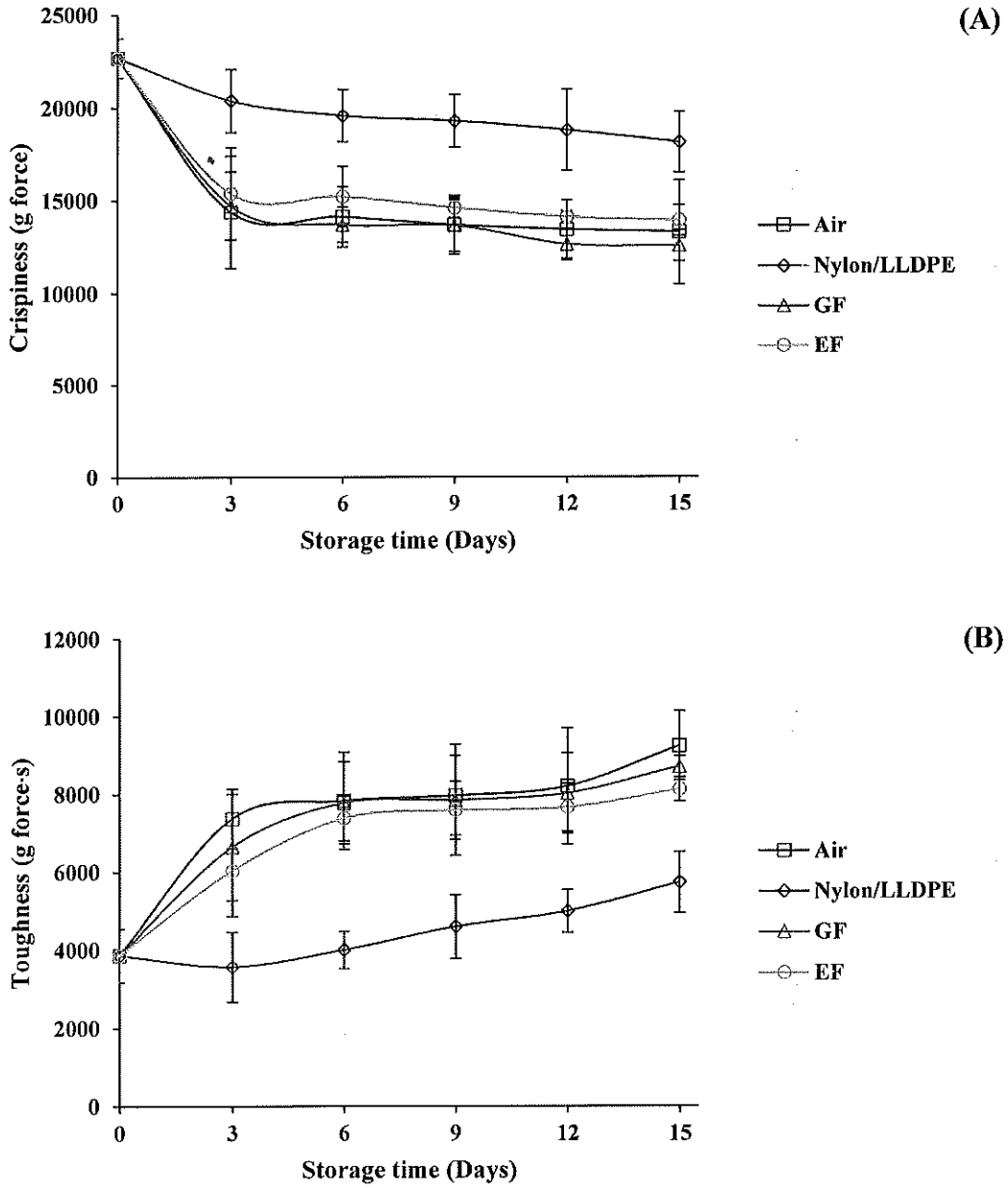

Figure 16. Changes in crispiness (A) and toughness (B) of fried shrimp cracker packaged with fish gelatin film incorporated without (GF) and with palm oil (EF) and nylon/linear low-density polyethylene (Nylon/LLDPE) film during storage of 15 days at 28 ± 0.5 °C and 65 ± 5% RH. Air: Fried shrimp cracker without packaging. Bars represent the standard deviation (n=5).

5.4.2.4 Volatile compounds

Selected volatile compounds in freshly fried shrimp cracker and those stored in aluminum cup covered with GF and EF in comparison with Nylon/LLDPE film after 15 days of storage are shown in Table 18. At day 0, fried shrimp cracker contained some volatile compounds including aldehyde (hexanal, n-nonanal and 2-decenal), alcohol (1-pentanol and 2-furanmethanol), noncyclic hydrocarbons (n-tridecane and n-undecane) and nitrogen-containing compounds (2,6-dimethylpyrazine). The result suggested that shrimp cracker underwent lipid oxidation to some degree during frying. After 15 days of storage, volatile compounds were also found in all fried shrimp cracker, but the abundance and types of compounds varied. Aldehydes have been used as the index of lipid oxidation in a number of foods because they possess low threshold values and are the major contributors to off-odor and off-flavor (Ross and Smith, 2006). Numerous aldehydes produced during oxidation included octanal, nonanal, pentanal, hexanal, etc. (Ross and Smith, 2006). Amongst all aldehydic compounds, hexanal and n-nonanal were found as the major aldehydes in all stored shrimp cracker samples, followed by 2-Decenal and 2,4-Decadienal (Table 18). Sample stored in air had the highest abundance of all aldehydes. It was noted that sample packaged with GF showed similar profile to that stored with Nylon/LLDPE film. Nevertheless, some differences in hexanal and n-nonanal were noticeable. This reconfirmed the excellent oxygen barrier property of GF film (Table 17). Alcohols are the secondary products produced by the decomposition of hydroperoxide (Eymard *et al.*, 2009). The 1-pentanol and 2-furanmethanol were found in shrimp cracker samples (Table 18). All shrimp cracker samples after 15 days of storage showed the higher abundance of those alcoholic compounds than freshly prepared sample. It was postulated that higher lipid oxidation and greater decomposition of hydroperoxide occurred. Higher abundance of volatile lipid oxidation products found in fried shrimp cracker without packaging correlated well with high TBARS value as shown in Figure 15B. 1-Methyoxy-2-propamal was found only in sample stored in air after 15 days of storage. Four nitrogen-containing compounds mainly pyrazine derivatives, were found in fried shrimp cracker. The 2,5-dimethylpyrazine was detected in all samples after 15 days of storage. Conversely, 2,6-dimethylpyrazine was formed only in freshly prepared

Table 18. Selected volatile compounds in freshly prepared shrimp cracker and those stored with different films after 15 days.

Volatile compounds	Day 0	Day 15			
		Air	Nylon/LLDPE	GF	EF
Aldehydes					
Hexanal	0.7	25.7	2.6	3.9	16.1
n-Nonanal	0.5	13.2	3.9	1.8	12.3
2-Decenal	2.8	8.1	2	1.9	7.5
2,4-Decadienal	ND	3.2	0.8	0.4	3
Alcohols					
1-Methoxy-2-propanol	ND	7.1	ND	ND	ND
1-Pentanol	1.9	5.8	2.5	2.6	2.8
2-Furanmethanol	0.3	1.5	0.4	0.5	1.0
Noncyclic hydrocarbons					
Heptane	ND	5.1	0.8	3.8	5.0
n-Tridecane	0.4	9.5	8.8	9.3	9.2
n-Dodecane	ND	19.6	18.4	19.0	20.2
Nitrogen-containing compounds					
Methylpyrazine	ND	0.8	ND	ND	ND
2,5-Dimethylpyrazine	ND	7	4.4	5	4.9
2,6-Dimethylpyrazine	2.5	1.3	0.5	0.5	0.6
Ethylpyrazine	ND	1.1	0.5	0.8	0.8

* Values are expressed as abundance ($\times 10^9$). ND: non-detectable. GF and EF: Fish gelatin film incorporated without and with palm oil, respectively. Nylon/LLDPE: Nylon/linear low-density polyethylene film. Air: Fried shrimp cracker without packaging.

sample (day 0). Pyrazines were reported to be formed by Maillard reaction from degradations of various nitrogen sources such as amino acids (Jaffrès *et al.*, 2011). Methylpyrazine was found only in fried shrimp cracker kept without packaging (air), while ethylpyrazine was detected only in the stored shrimp cracker. The presence of pyrazine indicated that browning reaction mediated by Maillard reaction occurred in shrimp cracker samples during 15 days of storage. Furthermore, some hydrocarbon compounds including heptane, n-tridecane, n-dodecane and n-undecane were found in fried shrimp cracker after 15 days of storage. The abundance of those compounds increased after 15 days of storage. Alkanes is mainly formed from lipid auto-oxidation of fatty acids released from triglycerides (Latorre-Moratalla *et al.*, 2011). Based on

volatile compounds, fried shrimp cracker stored with different packaging showed varying volatile compounds.

5.5 Conclusions

Fish gelatin films incorporated with palm oil could lower the increase in moisture content and water activity of fried shrimp cracker to some extent, but its capability was still lower than Nylon/LLDPE film. The preventive effect toward lipid oxidation of films from fish gelatin was equivalent to Nylon/LLDPE film. However, fish gelatin film could not maintain the textural property of cracker during storage. Therefore, the improvement of barrier property against water and oxygen of fish gelatin film is still needed.

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CHAPTER 6
SUMMARY AND FUTURE WORKS

6.1 Summary

1. The incorporation of 50 or 75% palm oil using soy lecithin as a surfactant in the presence of 30% glycerol could enhance stability of emulsion in FFD, which directly determined properties and morphology of film.

2. The use of 75% palm oil and 10% glycerol could improve water vapor barrier properties of gelatin-based film, whereas the satisfactory mechanical properties were still obtained.

3. The microfluidization of FFD at the pressure of 3 kpsi for 2 passes with 50% soy lecithin could enhance stability of emulsion, which directly governed properties and morphology of film.

4. Fish gelatin films incorporated with palm oil could lower the increase in moisture content and water activity of fried shrimp cracker to some extent, but its capability was still lower than Nylon/LLDPE film. Therefore, the improvement of barrier property against water vapor of fish gelatin film is still needed.

6.2 Future works

1. The preparation techniques of emulsion film based on gelatin incorporated or modified with hydrophobic substances should be further studied.

2. The effect of film compositions on thermal properties as well as heat-sealing properties of emulsion film should be investigated.

3. The application of emulsion film as edible pouch for shelf-life extension of food products should be investigated.

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List of Publication

1. Nilsuwan, K., Benjakul, S. and Prodpran, T. 2015. Emulsion stability and properties of fish gelatin-based films as affected by palm oil and surfactants. J. Sci. Food Agric. DOI: 10.1002/jsfa.7371.
2. Tongnuanchan, P., Benjakul, S., Prodpran, T. and Nilsuwan, K. 2015. Emulsion film based on fish skin gelatin and palm oil: Physical, structural and thermal properties. Food Hydrocolloid. 48: 248-259.

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1. Nilsuwan, K., Benjakul, S. and Prodpran, T. 2015. Effect of palm oil and surfactants on properties of fish gelatin-based film. The 17th Food Innovation Asia Conference. Bangkok, Thailand. 18-19 June, 2015. Poster presentation.