Gelatin Film Incorporated with Essential Oil and Palm Oil: Properties, Characteristics and Application as Edible Pouch

Phakawat Tongnuanchan

A Thesis Submitted in Fulfillment of the Requirements for the Degree of
Doctor of Philosophy in Food Science and Technology
Prince of Songkla University
2015

Copyright of Prince of Songkla University

Thesis Title	Gelatin Film Incorporated with Essential Oil and Palm Oil: Properties, Characteristics and Application as Edible Pouch
Author	Mr. Phakawat Tongnuanchan
Major Program	Food Science and Technology

Major Advisor:

…….....
(Prof. Dr. Soottawat Benjakul)

Co-advisor:

…….....
(Asst. Prof. Dr. Thummanoon Prodpran)

Examining Committee:

…….Chairperson
(Asst. Prof. Dr. Manee Vittayanont)

…….....……
(Prof. Dr. Soottawat Benjakul)

…….....……
(Asst. Prof. Dr. Thummanoon Prodpran)

…….....……
(Dr. Supachai Pisuchpen)

…….....……
(Assoc. Prof. Dr. Rungsinee Sothornvit)

The Graduate School, Prince of Songkla University, has approved this thesis as fulfillment of the requirements for the Doctor of Philosophy Degree in Food Science and Technology.

…….
(Assoc. Prof. Dr. Teerapol Srichana)
Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

……..……..….Signature
(Prof. Dr. Soottawat Benjakul)
Major Advisor

……..……..….Signature
(Mr. Phakawat Tongnuanchan)
Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

……...Signature
(Mr. Phakawat Tongnuanchan)
Candidate

ชื่อวิทยานิพนธ์ ฟิล์มเจลาตินที่เติมน้ำมันหอมระเหยและน้ำมันปาล์ม: สมบัติ คุณลักษณะ และการประยุกต์ใช้เป็นซองบรรจุภัณฑ์ชนิดบริโภคได้

ผู้เขียน นายภควรรษ ทองนวลจันทร์

สาขาวิชา วิทยาศาสตร์และเทคโนโลยีอาหาร

ปีการศึกษา 2557

บทคัดย่อ

จากการศึกษาผลของปัจจัยต่างๆ ได้แก่ ปริมาณกลีเซอรอล (ร้อยละ 20 และ 30 ของปริมาณโปรตีน) ปริมาณของน้ำมันหอมระเหย (ร้อยละ 25, 50 และ 100 ต่อปริมาณโปรตีน) และชนิดของสารลดแรงตึงผิว (Tween-20, Tween-80 และ soy lecithin) ต่อสมบัติของฟิล์มเจลาตินจากหนังปลาที่เติมน้ำมันหอมระเหยชนิดต่างๆ 11 ชนิด พบว่าฟิล์มที่เติมน้ำมันหอมระเหยทุกชนิด โดยเฉพาะอย่างยิ่งที่ปริมาณสูงขึ้น มีค่าการต้านทานแรงดึง (TS) และค่าการซึมผ่านไอน้ำ (WVP) ต่ำกว่า แต่มีค่าระยะยืดเมื่อขาด (EAB) และความหนาสูงกว่าฟิล์มที่ไม่เติมน้ำมันหอมระเหย ($p < 0.05$) ปริมาณของกลีเซอรอลที่สูงขึ้นทำให้ค่าระยะยืดเมื่อขาด ค่าการซึมผ่านไอน้ำ และความหนาของฟิล์มเพิ่มขึ้น แต่ทำให้ค่าการต้านทานแรงดึงลดลง ฟิล์มที่เตรียมโดยใช้ Tween-20 เป็นสารลดแรงตึงผิว มีค่าการต้านทานแรงดึงสูงกว่าฟิล์มที่เตรียมด้วยสารลดแรงตึงผิวชนิดอื่น ($p < 0.05$) สำหรับฟิล์มที่เติมน้ำมันหอมระเหยชนิดเดียวกันพบว่าฟิล์มที่เตรียมโดยใช้ soy lecithin เป็นสารลดแรงตึงผิวมีค่าการซึมผ่านไอน้ำต่ำสุด เมื่อเปรียบเทียบกับฟิล์มที่เตรียมด้วย Tween-20 และ Tween-80 เป็นสารลดแรงตึงผิว ($p < 0.05$) ฟิล์มที่เติมน้ำมันหอมระเหยชนิดต่างๆ มีค่า L^* ลดลง แต่มีค่า b^* เพิ่มขึ้น แสดงถึงการเพิ่มขึ้นของสีเหลือง เมื่อใช้ soy lecithin เป็นสารลดแรงตึงผิว พบว่าฟิล์มมีสีเหลืองเพิ่มขึ้น ฟิล์มที่เติมน้ำมันหอมระเหยมีค่าสีและลักษณะโปร่งแสงที่หลากหลายขึ้นกับชนิดและปริมาณของน้ำมันหอมระเหยที่เติม นอกจากนี้พบว่าฟิล์มที่เติมน้ำมันหอมระเหยมีฤทธิ์ต้านอนุมูลอิสระ ซึ่งขึ้นกับชนิดของน้ำมันหอมระเหยที่เติม โดยฟิล์มที่เติมน้ำมันหอมระเหยจากใบโหระพามีกิจกรรมการจับอนุมูล DPPH และ ABTS สูงสุด ($p < 0.05$) ขณะที่ฟิล์มที่เติมน้ำมันหอมระเหยผิวมะนาวมีกิจกรรมการจับโลหะสูงที่สุด ($p < 0.05$)

เมื่อศึกษาคุณลักษณะและสมบัติทางเคมีกายภาพของฟิล์มจากเจลาตินที่เติมน้ำมันหอมระเหย จากสเปกตรา FTIR แสดงให้เห็นว่าฟิล์มที่เติมน้ำมันหอมระเหย โดยเฉพาะอย่างยิ่งเมื่อปริมาณของน้ำมันหอมระเหยสูงขึ้น มีไฮโดรโฟบิกซิตีสูงกว่าฟิล์มชุดควบคุม ซึ่งสามารถสังเกตได้จากแอมพลิจูดของพีคที่เลขคลื่น 2874–2926 cm^{-1} และ 1731-1742 cm^{-1} โดยไม่ขึ้นกับชนิดของน้ำมันหอมระเหยและสารลดแรงตึงผิว อันตรกิริยาระหว่างโปรตีนในโครงสร้างของฟิล์มลดลงเมื่อ

มีการเติมน้ำมันหอมระเหย ฟิล์มที่เติมน้ำมันหอมระเหยมีอุณหภูมิการเสื่อมสภาพโดยความร้อน (T_d) อุณหภูมิการเปลี่ยนสถานะคล้ายแก้ว (T_g) และเอนทาลปี (ΔH) ต่ำ และการสูญเสียน้ำหนัก (ΔW) สูงกว่าฟิล์มชุดควบคุม โดยเฉพาะอย่างยิ่งเมื่อปริมาณของกลีเซอรอลและน้ำมันหอมระเหยสูงขึ้น ฟิล์มที่เติมน้ำมันหอมระเหยทุกชนิดมีโครงสร้างภาคตัดขวางที่ขรุขระ เมื่อเปรียบเทียบกับฟิล์มชุดควบคุม โดยไม่ขึ้นกับปริมาณกลีเซอรอล แต่อย่างไรก็ตามฟิล์มที่เติมน้ำมันหอมระเหยที่ระดับตั้งแต่ร้อยละ 50 ต่อปริมาณโปรตีน ขึ้นไปมีโครงสร้างแบบสองชั้น (bilayer) นอกจากนี้ฟิล์มที่เติมน้ำมันหอมระเหยมีผิวหน้าของฟิล์มและการกระจายตัวของน้ำมันที่สม่ำเสมอ เมื่อใช้ soy lecithin เป็นสารลดแรงตึงผิว และพบว่าฟิล์มที่เติมน้ำมันหอมระเหยมีโครงสร้างแบบสองชั้นเมื่อใช้ Tween-80 เป็นสารลดแรงตึงผิว

จากการศึกษาสมบัติของฟิล์มอิมัลชันจากเจลาตินจากหนังปลาที่เติมน้ำมันปาล์มที่ปริมาณต่างๆ พบว่าขนาดหยดน้ำมันมวลรวม (d_{32} และ d_{43}) ของสารละลายฟิล์มอิมัลชันที่มีปริมาณน้ำมันทุกระดับ (ร้อยละ 25, 50, 75 และ 100 ต่อปริมาณโปรตีน) มีค่าใกล้เคียงกันหลังจากการเตรียม (0 ชม.) ($p > 0.05$) และมีค่าเพิ่มเล็กน้อยเมื่อระยะเวลาการเก็บรักษานานขึ้น โดยเฉพาะอย่างยิ่งที่ 12 ชม. ($p < 0.05$) ฟิล์มที่เติมน้ำมันปาล์มมีค่าการทนต่อแรงดึงและค่ามอดูลัสของความยืดหยุ่นต่ำลง แต่มีค่าระยะยืดเมื่อขาดสูงขึ้นเมื่อปริมาณของน้ำมันเพิ่มขึ้น ($p < 0.05$) ค่าการซึมผ่านไอน้ำและความชื้นลดลงในฟิล์มที่มีปริมาณน้ำมันปาล์มเพิ่มขึ้น ($p < 0.05$) การเติมน้ำมันปาล์มโดยทั่วไปมีผลให้ไฮโดรโฟบิกซิตีเพิ่มขึ้น และอันตรกิริยาระหว่างโปรตีนในโครงสร้างของฟิล์มลดลง ซึ่งส่งผลให้ความเสถียรต่อความร้อนของฟิล์มลดลง

เมื่อศึกษาเปรียบเทียบสมบัติของฟิล์มชนิดต่างๆ ที่ใช้กลีเซอรอลเป็นพลาสติกไซเซอร์และเติมน้ำมันหอมระเหยจากใบโหระพา น้ำมันปาล์ม หรือน้ำมันผสมระหว่างน้ำมันหอมระเหยจากใบโหระพากับน้ำมันปาล์ม (1:1 โดยน้ำหนัก) และเตรียมด้วยสารลดแรงตึงผิวชนิดต่างๆ (Tween-20 และ soy lecithin) พบว่าการเติมน้ำมันทำให้ทั้งค่า T_g และสัดส่วนของโครงสร้างโมเลกุลที่เป็นระเบียบลดลง ฟิล์มที่เติมน้ำมันและสารลดแรงตึงผิวทุกชนิดมีค่าความแข็งแรงของการปิดผนึกด้วยความร้อนต่ำกว่าฟิล์มชุดควบคุม ($p < 0.05$) ฟิล์มที่เติมน้ำมันปาล์มมีค่าความแข็งแรงของการปิดผนึกด้วยความร้อนและประสิทธิภาพของการปิดผนึกด้วยความร้อนสูงกว่าฟิล์มที่เติมน้ำน้ำมันหอมระเหยจากใบโหระพาและน้ำมันผสม ($p < 0.05$) โดยไม่ขึ้นกับชนิดของสารลดแรงตึงผิว การใช้ Tween-20 มีผลให้ฟิล์มสามารถปิดผนึกด้วยความร้อนสูงกว่า soy lecithin ($p < 0.05$) ฟิล์มชุดควบคุมและฟิล์มที่เติมน้ำมันชนิดต่างๆ และเตรียมฟิล์มโดยใช้ Tween-20 เป็นสารลดแรงตึงผิว ให้คุณภาพของการปิดผนึกด้วยความร้อนที่ดี (การเชื่อมประสานของฟิล์มที่

สมบูรณ์) ดังนั้นน้ำมันและสารลดแรงตึงผิวมีผลต่อสมบัติเชิงกล สมบัติทางความร้อน รวมทั้งความสามารถในการปิดผนึกด้วยความร้อนของฟิล์ม

จากการศึกษาการใช้ซองบรรจุภัณฑ์บริโภคได้ที่ทำจากฟิล์มเจลาตินจากหนังปลาที่ไม่เติมและเติมน้ำมันหอมระเหยจากใบโหระพา น้ำมันปาล์ม หรือน้ำมันผสมระหว่างน้ำมันหอมระเหยจากใบโหระพากับน้ำมันปาล์ม สำหรับการเก็บรักษาผงเนื้อไก่และน้ำมันจากหนังไก่เปรียบเทียบกับซองจากไนลอน/พอลิเอธิลีนความหนาแน่นต่ำ (Nylon/LDPE) เป็นเวลา 15 วัน ที่อุณหภูมิ 28 ± 0.5 องศาเซลเซียส และความชื้นสัมพัทธ์ 65 ± 5 % พบว่าความชื้นของผงเนื้อไก่ที่เก็บรักษาในซองที่ทำจากฟิล์มเจลาตินจากหนังปลาที่ไม่เติมและเติมน้ำมันชนิดต่างๆ เพิ่มขึ้นระหว่างการเก็บรักษาเป็นเวลา 15 วัน ($p < 0.05$) ตัวอย่างที่เก็บรักษาในซองที่ทำจากฟิล์มอิมัลชันมีความชื้นต่ำกว่าตัวอย่างที่เก็บรักษาในซองที่ทำจากฟิล์มเจลาตินที่ไม่เติมน้ำมัน ตัวอย่างทั้งหมดที่เก็บรักษาในซองที่ทำจากฟิล์มทุกชนิดเกิดปฏิกิริยาออกซิเดชันของไขมันเพิ่มขึ้นเล็กน้อย ซึ่งบ่งชี้โดยการเพิ่มขึ้นของค่า PV และ TBARS ระหว่างการเก็บรักษาเป็นเวลา 15 วัน ตัวอย่างทั้งหมดมีสีเข้มขึ้นและสีเหลืองเพิ่มขึ้นระหว่างการเก็บรักษา น้ำมันจากหนังไก่ที่เก็บรักษาในซองจาก Nylon/LDPE มีค่า TBARS และ *p*-anisidine สูงกว่าน้ำมันที่เก็บในซองจากฟิล์ม เจลาตินจากหนังปลาที่ไม่เติมและเติมน้ำมันชนิดต่างๆ ระหว่างการเก็บรักษาเป็นเวลา 15 วัน ($p < 0.05$) ดังนั้นซองบรรจุภัณฑ์ทำจากฟิล์มเจลาตินจากหนังปลาที่เติมน้ำมันสามารถใช้เป็นอีกหนึ่งบรรจุภัณฑ์ทางเลือกที่ย่อยสลายได้สำหรับการยืดอายุการเก็บรักษาอาหารที่มีไขมัน

Thesis Title Gelatin Film Incorporated with Essential Oil and Palm Oil: Properties, Characteristics and Application as Edible Pouch

Author Mr. Phakawat Tongnuanchan

Major Program Food Science and Technology

Academic Year 2014

ABSTRACT

Properties of fish skin gelatin films incorporated with different 11 essential oils as influenced by different levels of glycerol (20% and 30% based on protein), levels of essential oils (25%, 50% and 100%, based on protein) and surfactants (Tween-20, Tween-80 and soy lecithin) were investigated. Films incorporated with all essential oils, especially at higher amount, showed the lower tensile strength (TS) and water vapor permeability (WVP) but higher elongation at break (EAB) and thickness, compared with the film without essential oil incorporated ($p < 0.05$). Higher glycerol content increased EAB, WVP and thickness but decreased TS of films. Films added with Tween-20 showed higher TS, compared with those having other surfactants ($p < 0.05$). For the film added with the same essential oil, those containing soy lecithin as surfactant had the lowest WVP, in comparison with those having Tween-20 or Tween-80 ($p < 0.05$). Films incorporated with various essential oils had the decreased L^*-value and increased b^*-value, suggesting the increased yellowness. When soy lecithin was used as surfactant, films became more yellowish. Films containing essential oils had varying color and transparency, depending on types and levels of essential oils incorporated. Films incorporated with essential oils showed varying antioxidative activities, depending on types of essential oils. Among films containing essential oils, that with basil essential oil had the highest DPPH and ABTS radical scavenging activities ($p < 0.05$), while those incorporated with lemon essential oil had the highest chelating activity ($p < 0.05$).

Characteristics and physicochemical properties of films added with essential oils were determined. Based on Fourier transforms infrared (FTIR) analysis, films added with essential oils, especially with increasing essential oil concentration, exhibited higher hydrophobicity with higher amplitude at wavenumber of 2874–2926 cm^{-1} and 1731–1742 cm^{-1} than the control film, regardless of types of essential oils

and surfactants used. The decreased protein-protein interaction took place in the matrix of gelatin film when essential oils were incorporated. Films containing essential oils had lower thermal degradation temperature (T_d), glass-transition (T_g) and enthalpy (ΔH) with higher weight loss (Δw), particularly with higher levels of glycerol and essential oils, compared with the control film. Films containing all types of essential oils had rough cross-section, compared with control films, irrespective of glycerol levels. However, bilayer films could be formed as essential oils at level above 50% were incorporated. Moreover, smoother surface and more homogeneous oil distribution was observed in films containing essential oils when soy lecithin was used as surfactant. Films incorporated with essential oils also exhibited bi-layer morphology when Tween-80 was used as surfactant.

Properties of emulsion film based on fish skin gelatin containing palm oil at different levels were tested. Particle sizes (d_{32} and d_{43}) of oil of film-forming emulsion at all oil levels (25%, 50%, 75% and 100%, based on protein) was similar after preparation (0 h) ($p > 0.05$) and slightly increased with increasing storage time, especially at 12 h ($p < 0.05$). Films incorporated with palm oil had lower TS and elastic modulus (EM) but higher EAB as the amount of palm oil increased ($p < 0.05$). Decreased WVP and moisture content were observed for films having the increasing amount of palm oil ($p < 0.05$). The addition of palm oil generally resulted in the increased hydrophobicity and the weaker protein-protein interaction in film network, leading to the lower thermal stability.

Different films plasticized with glycerol and incorporated with basil essential oil, palm oil or a mixture of basil essential oil/palm oil (1:1 by weight), in the presence of different surfactants (Tween-20 and soy lecithin) were comparatively characterized. The incorporation of oils yielded films with the decreases in both T_g and extent of ordered-phase. All films added with different oils and surfactants had lower seal strength than the control film ($p < 0.05$). Film containing palm oil exhibited the higher seal strength and seal efficiency than those with basil essential oil or the mixture ($p < 0.05$), regardless of surfactants. Tween-20 yielded the film with the higher heat sealability than soy lecithin ($p < 0.05$). Good quality seal (complete fusion) was observed in the control film and those films containing various oils using

Tween-20 as surfactant. Both oil and surfactants could therefore affect the mechanical properties, thermal properties as well as heat sealability of resulting films.

Edible pouches made from fish gelatin films incorporated without and with palm oil, basil essential oil and oil mixture were prepared and used to store chicken powder and chicken skin oil in comparison with nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days at 28 ± 0.5 ºC and 65 ± 5 % RH. The moisture content of chicken powder packaged in pouches from fish gelatin films incorporated without and with various oils increased during 15 days of storage ($p < 0.05$). Samples packaged in pouches made from emulsion films had lower moisture content than those packaged in pouch from gelatin film without oil added. All samples packaged in pouches made from all films had the slight increase in lipid oxidation as indicated by increased PV and TBARS during 15 days of storage. The color of all samples became darker and more yellowish during storage. Chicken skin oil packaged in Nylon/LDPE pouch had higher TBARS and *p*-anisidine value than those stored in pouches made from fish gelatin films incorporated without and with various oils during 15 days of storage ($p < 0.05$). Therefore, pouches from gelatin films incorporated with oils could be used as alternative biodegradable packaging to prolong the shelf-life of fat containing foods.

ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest 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 overall guidance during my study since the first day of being student. I would not have achieved this far and this thesis would not have been completed without all the support that I have always received from him. I feel very honor to work under his supervision.

I gratefully acknowledge my co-advisor, Assist. 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 inspired me to be a responsible scientist. In addition, he has provided recommendation and encouragement throughout my graduate program.

I would also like to express my profound gratitude to Assoc. Prof. Dr. Kazufumi Osako of the Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Japan for his guidance in my oversea research. His warmly welcome, generous contribution and unconditional help, particularly during my stay in Japan is also acknowledged.

I am also very grateful to my examining committees, Asst. Prof. Dr. Manee Vittayanont of the Department of Food Technology and Dr. Supachai Pisuchpen of the Department of Material Product Technology, Faculty of Agro-Industry, Prince of Songkla University Faculty. 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, helpful suggestion and advices on my thesis.

I would like to express my deepest appreciation to my beloved family for the great suggestion, understanding and teaching me with their good attitude. My deep acknowledgement is due to all of my friends of Fish Chemistry and Biochemistry lab (2205), and staffs who gave me their helps during study. Thanks are also given to all Food Processing Lab members; for making my visit at Tokyo very

enjoyable and memorable experience which I will never forget. Finally, I would like to thank the Department of Food Technology and Department of Material Product Technology, Faculty of Agro-Industry, Prince of Songkla University for providing laboratory equipment and experimental space.

This study could not be succeeded without the scholarship support from National Research University Project of Thailand's Office of the Higher Education Commission, the TRF Senior Research Scholar Program and Graduate School, Prince of Songkla University for an oversea thesis research study and funding this project.

Phakawat Tongnuanchan

CONTENTS

Page

CONTENTS (Continued)

Chapter **Page**

CONTENTS (Continued)

Chapter **Page**

CONTENTS (Continued)

Chapter **Page**

CONTENTS (Continued)

Chapter **Page**

CONTENTS (Continued)

Chapter **Page**

CONTENTS (Continued)

Chapter **Page**

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Potential processing and management are very important for fish industry, where great economic, nutritional and environmental values can be obtained by increasing the yield and better uses of byproducts. Fish processing generates solid wastes that can be as high as 50–80% of the original raw material (Shahidi *et al.*, 1994). These wastes are an excellent raw material for preparation of high protein foods. About 30% of the wastes consists of skin and bone with high collagen content (Wasswa *et al.*, 2007). Gelatin can be obtained by partial hydrolysis of collagen in skin and bone. Due to bovine spongiform encephalopathy crisis, there has been a growing interest for finding an alternative source for gelatin production, especially from aquatic sources. Gelatin has numerous applications, particularly as an ingredient to improve the elasticity, consistency and stability of foods. It can be used for encapsulation as well as pharmaceutical and photographic industries (Wasswa *et al.*, 2007). Moreover, gelatins have been widely used as film forming material, particularly for preparation of biodegradable or edible films (Arvanitoyannis, 2002; Cao *et al.*, 2007; Hoque *et al.*, 2011b; Jongjareonrak *et al.*, 2008; Rattaya *et al.*, 2009; Staroszczyk *et al.*, 2012).

Biodegradability of biopolymer films has led to a growing 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 to protect it against external factors e.g. water, oxygen, carbon dioxide and lipids (Krochta, 1997). Proteins from diverse sources have been used as material for biodegradable film because of their relative abundance and good film-forming ability (Hamaguchi *et al.*, 2007; Krochta, 2002). Among proteins, gelatin from either mammalian or fish has been used as the material for biodegradable and active film (Seydim and Sarikus, 2006). The 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.*, 2009a).

Although gelatin yields transparent, colorless and highly extensible films, those films have poor water resistance and are almost completely soluble in water. This could be a drawback when they are applied to 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 use of essential oils from plants can be another approach to improve water vapor barrier property of gelatin films. Due to antioxidant and antimicrobial activities of essential oils (Burt, 2004; Kordali *et al.*, 2005), they may make the film become active. As a consequence, smart films with varying property can be produced, especially for shelf-life extension of foods. Development of convenient food products with longer shelf-life, and the changes in retailing practices and/or in way of life have promoted the development of new and/or improved packaging materials. Consumer demands for more natural foods, and also for environmental protection lead to the development of new packaging materials. Active, intelligent, biodegradable and edible packaging is emerging for food industry.

Since gelatin film is solubilized in water, it can be used for making pouch or bag, which is ready to dissolve in the presence of water, especially at high temperature. Therefore, it can be the convenient packaging, which carries the ingredients or condiments for some types of food, e.g. instant noodle, etc. However, heat sealability is a prime factor determinating the potential use of film or sheet as food packaging, especially bag or pouch. Heat sealing capacity of films is critical and also determines the integrity of the package. Heat sealing quality depend on the processing conditions such as temperature, time and pressure as well as previous film treatments (López *et al.*, 2011).

Pouch prepared from active packaging films incorporated with active compounds, e.g. essential oils, via heat sealing can be used as convenient packaging, which is able to retard deterioration caused by chemical reaction, especially lipid oxidation. Pouch be also able to protect the moisture absorption of dried foods. Therefore, foods can be stored for an extended time. Also, gelatin from fish processing byproduct can be better used and novel packaging can be produced,

thereby increasing the value of byproducts from fish processing industry. The information gained will be of benefit for fish processing and packaging industries. Novel marketable packaging with safety can be developed and serve for growing convenient food market.

1.2 Review of Literature

1.2.1 Biodegradable film

Biodegradable film and coating have been received the increasing attention owing to their biocompatibility and alternative packaging to synthetic polymers or plastics. Almost food packagings are generally made from plastics, which are non-biodegradable synthetic polymers and have the negative impact on environment (Kester and Fennema, 1986; Krochta and De Mulder-Johnston, 1997).

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 (Cuq *et al.*, 1995b; Guilbert and Gontard, 1995; Guilbert, 2002). Over the last decade, there has been a widespread interest in films made from renewable and natural polymers which can degrade naturally and more rapidly than petroleum-based plastics. Among all biopolymers, proteins have been paid increasing attention as a potential material for biodegradable films and coating (Cuq *et al.*, 1997; Gennadios *et al.*, 1994; Park and Chinnan, 1995).

Biopolymeric materials used for biodegradable films can be divided into 4 categories: biopolymer hydrocoilloids (proteins and polysaccarides), lipids, resins and composites (Krochta *et al.*, 1994). Physical and chemical characteristics of the biopolymers greatly influence the properties of resulting films and coatings (Sothornvit and Krochta, 2000). In general, plasticizers are required to increase the flexibility of film by lowering the interaction between polymers. Films can be incorporated with other additives for different purposes and applications (Table 1).

1.2.1.1 Proteins as film forming material

The ability of different proteins to form films and coatings is highly dependent on their molecular characteristics: molecular weight, conformations, electrical properties (charge vs pH), flexibilities, and thermal stabilities (Vargas *et al.*, 2008). Proteins are thermoplastic heteropolymers containing 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, 1999). 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 *et al.*, 2005; Krochta, 2002). 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. In addition, properties of protein based-films depend on various factors such as the source of protein, pH of protein solution, plasticizers, film thickness, preparation conditions, formation process and additives incorporated into the film forming solutions (Benjakul *et al.*, 2008; Cuq *et al.*, 1996; Park and Chinnan, 1995; Sobral *et al.*, 2005).

Protein used as film-forming materials are derived from both animal and plant sources, such as animal tissues, milks, eggs, grains, and oilseeds, etc. (Table 1). In general, film formation involves protein solubilization, followed by solvent evaporation (casting or molding) (Krochta, 1997). Protein-based films show impressive gas barrier properties and mechanical properties, compared with those prepared from polysaccharides and fat-based films, since proteins have a unique structure which confers a wider range of functional properties, especially a high intermolecular binding potential. However, the poor water vapor resistance limits their application. Improvement of protein film properties could be attained by

Table 1. Materials used for edible films and coatings.

Functional compositions	Materials	References
Film-forming materials	*Proteins*: myofibrillar protein, whey protein, casein, wheat gluten, soy protein, collagen, gelatin, corn zein, egg protein, pea protein, rice bran, sunflower, cottonseed protein, peanut protein, serum albumin, porcine plasma protein, mung bean protein	Artharn *et al.* (2007); Fang *et al.* (2002); Abu Diak *et al.* (2007); Rhim *et al.* (2006); Deiber *et al.* (2011); Jongjareonrak *et al.* (2006a); Ghanbarzadeh *et al.* (2006); Gennadios *et al.* (1996); Kowalczyk and Baraniak, (2011); Adebiyi *et al.* (2008); Salgado *et al.* (2010); Yue *et al.* (2012); Jangchud and Chinnan (1999); Białopiotrowicz and Jańczuk (2001); Nuthong *et al.* (2009a); Hoque *et al.* (2011c)
	Polysaccharides: starch, modified starch, modified cellulose (CMC, MC, HPC), alginate, carrageenan, pectin, pullulan, chitosan, gellan gum, xanthan gum	López *et al.* (2011); Kim *et al.* (2003); Su *et al.* (2012); Nishio and Takahashi (1984); Pranoto *et al.* (2005); Debeaufort and Voilley (1995); Rhim (2004) ; Alvesa *et al.*(2011b); Galus and Lenart (2013); Diab *et al.* (2001); Martínez-Camachoa *et al.* (2010); Xu *et al.* (2007); da Matta *et al.* (2011)
	Lipids: waxes (beeswax, carnauba wax, candelilla wax, rice bran wax), resins (shellac, terpene), acetoglycerides	Fabra *et al.* (2009); Talens and Krochta (2005); Bosquez-Molina *et al.* (2003); Shih *et al.* (2011); Soradech *et al.* (2013); Benavides *et al.* (2012); Rhim *et al.* (2005)

Table 1. Materials used for edible films and coatings (cont.).

Functional compositions	Materials	References
Plasticizers	Glycerin, propylene glycol, sorbitol, mannitol, sucrose, polyethylene glycol (PG200, PG400), polyols, water	McHugh and Krochta (1994); Fadini *et al.* (2013); Weng *et al.* (2006); Sothornvit and Krochta (2000); Arvanitoyannis *et al.* (1998)
Antioxidant agents	BHT, BHA, PG, α-tocopherol, green tea extract, borage, lysozyme, TPCN, seaweed extract, polyphenol-rich plant extracts, gelatin hydrolysate, lignin	Chen *et al.* (1992); Jongjareonrak *et al.* (2008); Siripatrawan and Harte (2010); Gómez-Estaca *et al.* (2009b); Bao *et al.* (2009); Rattaya *et al.* (2009); Gómez-Estaca *et al.* (2009a); Gómez-Guilléna *et al.* (2007); Giménez *et al.* (2009a); Núñez-Flores *et al.* (2013)
Antimicrobial agents	Essential oils, lysozyme, potassium sorbate, sorbic acid, nano-clay, chitosan, nisin, EDTA, Ovotransferrin	Seydim and Sarikus (2006); Hosseini *et al.* (2009); Atarés *et al.* (2010b); Buonocore *et al.* (2005); Sayanjali *et al.* (2011); Hauser and Wunderlich (2011); Sothornvit *et al.* (2009); Shen *et al.* (2010); Sivarooban *et al.* (2008); Seol *et al.* (2009)
Cross-linking agents	Enzymatic cross-linking (EDC, transglutaminase), genipin, oxidized phenolic compounds, aldehyde,	Kolodziejska and Piotrowska (2007); Bigi *et al.* (2002); Hoque *et al.* (2011b); Nuthong *et al.* (2009b), Hernández-Muñoz *et al.* (2004)

*CMC = carboxy methylcellulose; MC = methylcellulose; HPC = hydroxypropyl cellulose; EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; EDTA = ethylenediaminetetraacetic acid; BHT = butylated hydroxytoluene; BHA = butylated hydroxyanisole; PG = Propyl gallate; TCPN = tea polyphenol-loaded chitosan nanoparticles; EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

modifying the properties of protein by chemical and enzymatic methods (Kester and Fennema, 1986; Krochta, 1997). Additionally, several approaches such as enzymatic modifications (De Carvalho and Grosso, 2004; Staroszczyk *et al.*, 2012) incorporation of appropriate plasticisers (Vanin *et al*., 2005) and hydrophobic materials (Limpan *et al*., 2010; Prodpran *et al*., 2007), etc. have been implemented.

1.2.1.2 Polysaccharides as film forming material

Polysaccharides including starch, non-starch carbohydrates, gums, and fibers can be used as film forming material (Guilbert, 1986; Guilbert, 2002). The sequence of polysaccharides is simple, compared to proteins. However, the conformation of polysaccharide structures is more complicated and unpredictable. Most carbohydrates are neutral, while some gums are mostly negatively charged (Chandrasekaran and Radha, 1995). Although this electrostatic neutrality of carbohydrates may not affect significantly the properties of formed films and coatings, the occurrence of relatively large numbers of hydroxyl groups or other hydrophilic moieties in the structure indicate that hydrogen bonds may play significant roles in film formation and characteristics of resulting films (Han *et al.*, 2005). Polysaccharides render transparent and homogeneous edible films with moderate mechanical properties. However, the application of these films is limited by their water solubility and poor WVP. To solve this shortcoming, blending with different biopolymers (Chen *et al.*, 2008), the addition of hydrophobic materials such as oils or waxes (Anker *et al.*, 2002; Ayranci and Tunc, 2003), or chemical modification of polymer structure (Garg and Jana, 2007; Qiu *et al.*, 2013) have been proposed.

1.2.1.3 Lipids as film forming material

Lipids and resins are also used as film-forming materials, but they are not polymers and, evidently, "biopolymers" is a misnomer for them. Nevertheless, they are edible, biodegradable, and cohesive biomaterials. Most lipids and edible resins are soft-solids at room temperature and possess characteristic phase transition temperatures (Rhim *et al.*, 2005). They can be fabricated to any shape by casting and

molding systems after heat treatment, causing reversible phase transitions between fluid, soft-solid, and crystalline solid (Nussinovitch, 2009). Because of their hydrophobic nature, films or coatings made from lipid film-forming materials have very high water resistance and low surface energy (Han *et al.*, 2005). Lipids can be combined with other film-forming materials, such as proteins or polysaccharides, as emulsion particles or multi-layer coatings in order to increase the resistance to water penetration (Gennadios *et al.*, 1997; Perez-Gago and Krochta, 2002; Prodpran *et al.*, 2007; Yang and Paulson, 2000).

1.2.1.4 Composite film forming materials

Biopolymer composites can modify film properties and create desirable film structures for specific applications. Similar to multi-layered composite plastic films, biopolymer films can be produced as multiple composite layers, such as protein coatings (or film layers) on polysaccharide films, or lipid layers on protein/polysaccharide films. This multi-layered film structure optimizes the characteristics of the final film. Composite films can also be created by mixing two or more biopolymers, yielding one homogeneous film layer (Debeaufort *et al.*, 1998; Were *et al.*, 1999; Yildirim and Hettiarachchy, 1997). Various biopolymers can be mixed together to form a film with unique properties that combine the most desirable attributes of each component (Wu *et al.*, 2002). The main objective of producing composite films is to improve the permeability or mechanical properties as dictated by the need of a specific application. These heterogeneous films are applied either in the form of an emulsion, suspension, or dispersion of the non-miscible constituents, or in successive layers (multilayer coating or films), or in the form of a solution in a common solvent (Guilbert, 1986). Moreover, the association among the polymers can be achieved through blending, extruding, laminating, or coating with other polymers with desirable properties. Blending is an easier and effective way to prepare associated polymeric materials (Alves *et al.*, 2011a; Hoque *et al.*, 2011c; Pranoto *et al.*, 2007; Zhong and Xia, 2008). According to Liu *et al.* (2007), composite films were prepared from pectin incorporated with fish skin gelatin or soybean flour protein. The addition of protein promoted molecular interactions, resulting in a well-organized homogeneous structure. The composite films showed an increase in stiffness and

strength and a decrease in water solubility and water vapor transmission rate, in comparison with films cast from pectin alone (Liu *et al.*, 2007). Denavi *et al.* (2009) reported that a reduction of thickness, water vapor permeability, film solubility and soluble protein was observed with increasing soybean-protein isolate concentration of the composite films obtained from soybean-protein isolate and cod gelatin. The formulation containing 25% SPI: 75% cod-skin gelatin had the maximum force at the breaking point, which was 1.8-fold and 2.8-fold greater than those of 100% gelatin and 100% SPI films, respectively. Moreover, Gounga *et al.* (2007) reported that blend film from whey protein isolate-pullulan with the ratio 1:1 had a good appearance with the greatest values of oxygen permeability, water vapor permeability, moisture content, film solubility, and light transmittance. However, addition of pullulan at low concentration was good enough to significantly modify these properties, hence improving the characteristics of whey protein isolate based films for food applications. Benbettaïeb *et al.* (2014) studied the development of composite-blended edible films using polysaccharide (chitosan) and protein (bovine gelatin) mixtures. The higher proportions of gelatin in film matrixes could be an advantageous component that corresponds to enhancement of the oxygen and water vapor barrier properties as well as mechanical properties of blended films. This was possibly due to the strong intra and intermolecular bondings between both materials during film formation via hydrogen interaction, which evidenced by a shift in peak position of the amide-I and amide-III groups, thereby yielding the obtained films with compact structure. It has been reported that the molecular interactions between gelatin and chitosan were mainly by hydrogen bondings, which providing the good compatibility of both polymers coexisted in the blend film matrix (Uriarte-Montoya *et al.*, 2010).

1.2.1.5 Plasticizers

Plasticizers are required for edible films and coatings, especially for polysaccharides and proteins. Those films are often brittle and stiff due to extensive interactions between polymer molecules (Krochta, 2002). 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 processability (Guilbert and Gontard, 1995; Krochta, 2002). Plasticizers increase the free volume of polymer structures or the molecular mobility of polymer molecules (Sothornvit and Krochta, 2000). These properties imply that the 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 and Gontard, 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.

There are two main types of plasticizers (Sothornvit and Krochta, 2000; Sothornvit and Krochta, 2001):

1. Agents capable of forming many hydrogen bonds, thus interacting with polymers by interrupting polymer-polymer bonding and maintaining the farther distance between polymer chains.

2. Agents capable of interacting with large amounts of water to retain more water molecules, thus resulting in higher moisture content and larger hydrodynamic radius.

Owing to the hydrophilic nature of water, biopolymers, and plasticizers, and due to the abundantly existing hydrogen bonds in their structures, it is very difficult to separate these two mechanisms. 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, the repulsive forces between molecules of the same charge or between polar and non-polar polymers can increase the distance between polymers, thus achieving the plasticization in the case of charged polymeric film structures. 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. Four theories have been proposed to explain the mechanism of the plasticizing effect (Di Gioia and Guilbert, 1999; Sears and Darby, 1982; Sothornvit and Krochta, 2005) as follows:

1. Lubricity theory – a plasticizer is considered as a lubricant to facilitate the movements of the macromolecules over each other.

2. Gel theory – a plasticizer disrupts the polymer–polymer interactions including hydrogen-bonds and van der Waals and ionic forces.

3. Free volume theory – a plasticizer may depress the glass transition temperature by increasing polymer free volume and mobility of polymeric chains. The fundamental concept underlying these theories is that a plasticizer can interpose itself between the polymer chains and decrease the forces holding the chains together.

4. Coiled spring theory – plasticizing effects from the point of review of tangled macromolecules.

Several plasticizers with different properties have been used in protein based films. Types and levels of plasticizers directly determine the properties of films. Sothornvit and Krochta (2001) and Jongjareonrak *et al.* (2006b) reported that polyethylene glycol of a smaller size was more efficient in interacting with β-lactoglobulin and fish gelatin molecules, respectively. Tensile strength, elastic modulus of these films decreased and the elongation at break increased with the addition of polyethylene glycol. Both sorbitol and mannitol are hexabasic alcohols, and they are isomeric compounds. Sorbitol has been extensively used as plasticizer for gelatin-based films (Jongjareonrak *et al.*, 2006a). Plasticizer causes no apparent tendency to re-crystallization in the film structure, but alters 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 those containing ethylene glycol, diethylene glycol and polypropylene glycol. This is attributed to the higher hydrophilic nature of the glycerol (Bergo and Sobral, 2007; 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.* (2013) 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 80g glycerol/100 g gelatin, respectively. The addition of higher quantities of glycerol, would not be recommendable since the properties are not modified and moreover it is not profitable (Rivero *et al.*, 2013). Sucrose is a disaccharide, which has eight hydroxyls. The bulky ring structure of α-D-glucose and β-D-fructose hindered the interaction of the hydroxyl groups in sucrose with the reactive groups of protein. However, the water binding abilities of ring structures are poorer, which resulted in greater reduction of the plasticizing efficiency (Cao *et al.*, 2009; Wan *et al.*, 2005).

1.2.1.6 Additives

Edible films and coatings can carry various active agents, such as emulsifiers, antioxidants, antimicrobials, nutraceuticals, flavors, and colorants, thus enhancing food quality and safety, up to the level where the additives do not interfere with physical and mechanical properties of films (Baldwin *et al.*, 1995; Baldwin *et al.*, 1997; Guilbert *et al.*, 1996; Han, 2002; Han, 2003; Howard and Gonzales, 2001; Kester and Fennema, 1986). Because of the various chemical characteristics of these active additives, film composition should be modified to keep a homogeneous film structure when heterogeneous additives are incorporated into the film-forming materials (Debeaufort *et al.*, 1998).

Emulsifiers are surface active agents of amphiphilic nature and are able to reduce the surface tension of the water-lipid interface or the water-air surface. Emulsifiers are essential for the formation of protein or polysaccharide films containing lipid emulsion particles. They also modify surface energy to control the

adhesion and wettability of the film surface (Krochta, 2002). Although many biopolymers possess the certain levels of emulsifying capacity, it is necessary to incorporate emulsifiers into film-forming solutions to produce lipid-emulsion films. In the case of protein films, some film-forming proteins have sufficient emulsifying capacity due to their amphiphilic structure (Krochta, 1997). Andreuccetti *et al.* (2011) reported films containing yucca extract showed higher tensile strength values and moisture contents and less elongation and water vapor permeability values, 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 films added with yucca extract, when compared to gelatin-based film without surfactant. 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). Similar behavior, with lower tensile strength values, 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, glycerol and surfactants (Tween 20, Span 80 and lecithin), the addition of surfactants without the addition of a plasticizer, led to slightly weaker and less flexible films with lower tensile strength as compared to those containing glycerol and surfactant (Rodríguez *et al.*, 2006). Moreover, water vapor permeability was significantly reduced in films with structural changes induced by hydrophobic substances (Chen *et al.*, 2009; Chen *et al.*, 2010).

Antioxidants and antimicrobial agents can be incorporated into film-forming solutions to achieve active packaging or coating functions (Han, 2002; Han, 2003). They provide additional active functions to the edible film and coating system to protect food products from oxidation and microbial spoilage, resulting in quality improvement and safety enhancement. When nutraceutical and pharmaceutical substances are incorporated into edible films and coatings, the system can be used for drug delivery purposes (Han, 2003). Incorporated flavors and colorants can improve the taste and the visual perception, respectively. Direct surface application of active substances by spraying or dipping is not highly effective because the active substances can react with food components, evaporate or diffuse into the food, thereby showing reduced antimicrobial activity. As a result, large antimicrobial

concentrations are required (Han and Floros, 1998; Ouattara *et al.*, 2000; Quintavalla and Vicini, 2002). Instead, the incorporation of antimicrobial agents to packaging materials slows down their release and helps keeping high concentrations of the active compounds on the product surface for extended periods. The incorporation of partially purified lysozyme from hen egg white into zein film showed antimicrobial effect on *Bacillus subtilis* and *Lactobacillus plantarm* (Mecitoglu *et al.*, 2006). The grape seed extract, nisin and EDTA incorporated soy protein edible film was effective to variable degrees in inhibiting the growth of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella typhimurium* (Sivarooban *et al.*, 2008).

To improve the film properties, several chemicals such glyoxal, caffeic acid, feruric acid or tannin acid (Cao *et al.*, 2007; Hernández-Muñoz *et al.*, 2004; Nuthong *et al.*, 2009c) and enzymes such as transglutaminase have been incorporated for improvement of mechanical property of films (Chambi and Grosso, 2006; De Carvalho and Grosso, 2004; Di Pierro *et al.*, 2007). Moreover, Hernández-Muñoz *et al.* (2004) reported that the incorporation of cross-linking agents (formaldehyde, glyoxal and glutaraldehyde) in glutenin-rich films could enhance the mechanical properties of films as well as improve the water barrier properties. Amongs those agents, formaldehyde was the most appropriate cross-linking agents, which potentially enhanced properties of glutenin-rich films. De Carvalho and Grosso (2004) also reported that the chemical modification (formaldehyde) of gelatin based film was better than enzymatic modification (transglutaminase) to improve the mechanical properties of films, whereas the greatest reduction in water vapor permeability was found for the enzymatic modified films. However, synthetic protein cross-linking agents can be associated with possible toxicity. Thus, the use of cross-linking agents from natural source has been investigated to improve the mechanical properties of protein films (Nuthong *et al.*, 2009b). The improved properties of these films confirm that chemical and enzymatic approach could be a useful tool for preparing edible film for food coating and pharmaceutical applications.

1.2.2 Functions and advantages of edible films

1.2.2.1 Edibility and biodegradability

The most beneficial characteristics of edible films and coatings are their edibility and inherent biodegradability (Guilbert *et al.*, 1996; Krochta, 2002). To maintain edibility, all film components (i.e. biopolymers, plasticizers, and other additives) should be food-grade ingredients and all process facilities should be acceptable for food processing (Guilbert *et al.*, 1996). With regard to biodegradability, all components should be biodegradable and environmentally safe. Human toxicity and environmental safety should be evaluated by standard analytical protocols by authorized agencies (Han *et al.*, 2005).

1.2.2.2 Physical and mechanical protection

Edible films and coatings protect packaged or coated food products from physical damage caused by mechanical impact, pressure, vibrations, and other mechanical factors. Edible films have lower tensile strength than common plastic films, while their elongation-at-break varies widely (Han *et al.*, 2005). Some edible films generally have elongation values, comparable to those of common plastic films (Guilbert, 1986; Guilbert, 2002). Many edible film and coating materials are very sensitive to moisture (Guilbert and Gontard, 1995; Guilbert *et al.*, 1996; Krochta, 2002). At higher relative humidity conditions, their physical strength is lower than that at lower relative humidity since absorbed moisture actions as a plasticizer. Temperature is also an important variable affecting the physical and mechanical properties of edible films and coatings (Guilbert *et al.*, 1997; Miller *et al.*, 1998; Wu *et al.*, 2002). The physical strength of materials dramatically decreases when temperature increases above the glass transition temperature. High relative humidity and large amounts of plasticizers lower the glass transition temperature of film-forming materials (Irissin-Mangata *et al.*, 2001).

1.2.2.3 Migration, permeation, and barrier function

The quality of most food products deteriorates via mass transfer phenomena, including moisture absorption, oxygen invasion, flavor loss, undesirable odor absorption, and the migration of packaging components into the food (Debeaufort *et al.*, 1998; Kester and Fennema, 1986; Krochta, 2002; Miller *et al.*, 1998). These phenomena can occur between food and the atmospheric environment, food and packaging materials, or among heterogeneous ingredients in the food product itself (Krochta, 1997). Atmospheric oxygen penetration into foods causes oxidation of food ingredients; inks, solvents and monomeric additives in packaging materials can migrate into foods; essential volatile flavors of beverages and confections may be absorbed into plastic packaging materials; and some foods absorb moisture from fillings/toppings, leading to the loss of crispiness. Edible films and coatings may wrap these food products or be located between heterogeneous parts of food products to prevent these migration phenomena and preserve quality (Guilbert *et al.*, 1997; Krochta, 2002). Certain edible films are excellent oxygen barriers. Except for lipid-based materials, the water vapor permeability of most edible films is generally higher than that of common plastic films (Rhim *et al.*, 2005). All barrier properties of edible films and coatings are affected greatly by film composition and environmental conditions (relative humidity and temperature) (Gontard *et al.*, 1996; Grondahl *et al.*, 2004). Plasticizers in edible film-forming materials reduce glass transition temperatures and increase the permeability of most migrants. Oxygen permeability is very sensitive to relative humidity (Guilbert *et al.*, 1997; Mate and Krochta, 1998). At higher relative humidity conditions, oxygen permeability increases substantially. Therefore, it is very important to maintain low relative humidity environments to maximize the effectiveness of edible films as gas barriers (Han *et al.*, 2005). Temperature is also an important factor of migration (Amarante and Banks, 2001; Guilbert *et al.*, 1997; Wu *et al.*, 2002). A temperature increase provides more energy to the migrating substances and increases the permeability. At temperatures far from the phase transition, changes of migration coefficients such as permeability and diffusivity follow the Arrhenius equation (Guilbert *et al.*, 1997; Miller *et al.*, 1998).

1.2.3 Film forming mechanisms and processes

1.2.3.1 Film forming mechanism

An edible 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 (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 films are produced from whey-protein gels by dehydration after heat-set or cold-set gel formation (Han *et al.*, 2005). 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 *et al.*, 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.*, 1995a).

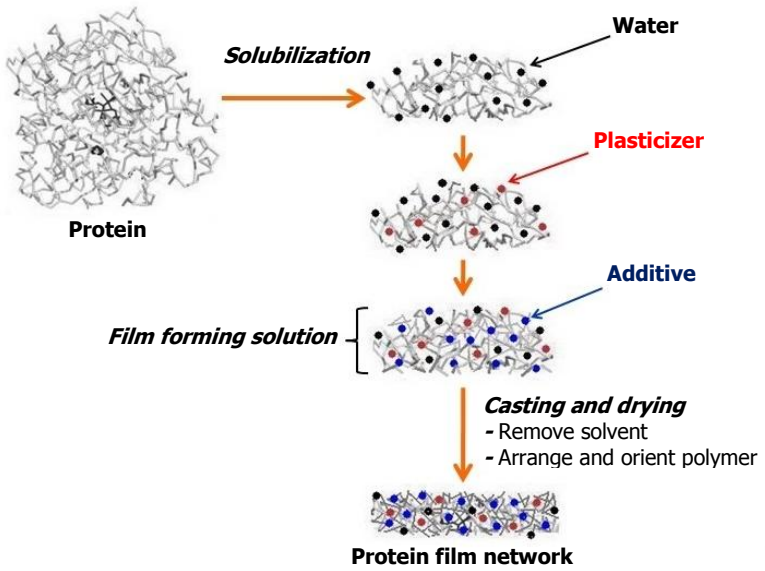


Figure 1. Mechanism of protein film formation.
Source: Adapted from Marquie and Guilbert (2002).

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 (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; Mecitoglu *et al.*, 2006).

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; Micard *et al.*, 2000b; Miller *et al.*, 1997), orientation, radiation (Gennadios *et al.*, 1998; Micard *et al.*, 2000b), and ultrasound treatment (Banejee *et al.*, 1996).

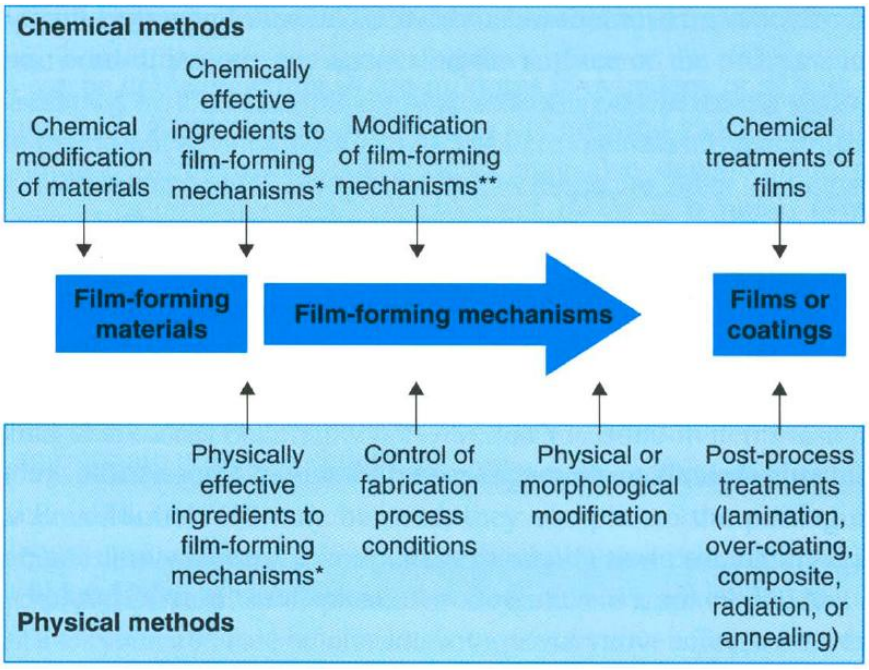


Figure 2. Chemical and physical modifying methods for preparation of edible film and coatings.

* 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 *et al.* (2005).

Physico-chemical 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). The determination of most physical and mechanical characteristics of film structures is related to physical chemistry parameters, which include mechanical strength, elasticity, moisture and gas permeation, cohesion of polymers, film adhesion onto food surfaces, surface energy, surface roughness/smoothness, light transmittance, color (opaque/gloss), viscosity, thermoplastic characteristics and others (Sothornvit and Krochta, 2000). Cohesion of film-forming materials is a very important parameter that influences the mechanical

strength of films, especially homogeneously continuous film structures (Guilbert *et al.*, 1996). Cohesion is the attractive force between molecules of the same substance (Anonymous, 1992). If the film-forming materials contain heterogeneous ingredients that are not compatible with the main biopolymers, the cohesion of the film forming materials decreases and the film strength weakens (Han *et al.*, 2005). When the use of new biopolymers or additives is investigated, the compatibility of all film-forming ingredients should be maintained to obtain strong cohesion (Han *et al.*, 2005). Plasticizers are the agents reducing the cohesion of film-forming polymers (Guilbert *et al.*, 1996). Adhesion of film-forming materials is an important parameter, practically, for film casting and coating processes (Guilbert *et al.*, 1996). Adhesion is the attractive force between the surface molecules of different substances, such as between coating materials and food surfaces (Anonymous, 1992). A low adhesion force results in incomplete coatings on the surface, or easy peel-off of the coating layers from the surface. Surface active agents, such as emulsifiers and other amphiphilic chemicals in the film forming solution reduce the surface tension of the coating solution, thus decreasing the difference between the solid surface energy and the surface tension of the coating solution and ultimately increasing the work of adhesion (Guilbert, 2002; Han *et al.*, 2005).

1.2.3.2 Film formation processes

There are two categories of film formation processes; dry and wet (Guilbert *et al.*, 1997) (Figure 3). The dry process of edible film production does not use liquid solvents, such as water or alcohol. Molten casting, extrusion, and heat pressing are good examples of dry processes. For the dry process, 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. 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 thermoplasticity of the film-forming materials (Guilbert *et al.*, 1997; Krochta, 2002). The wet process uses solvents for the dispersion of film-forming materials, followed by drying to remove the solvent and form a film structure. For the wet process, 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 (Cuq *et al.*, 1995b; Guilbert and Gontard, 1995; Han and Floros, 1997; Han *et al.*, 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 (Jongjareonrak *et al.*, 2006a), fish muscle protein (Artharn *et al.*, 2008; Benjakul *et al.*, 2008), porcine plasma protein (Nuthong *et al.*, 2009a) were prepared by casting methods, in which film forming solutions were firstly prepared prior to casting.

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 *et al.*, 2005). To produce a homogeneous film structure without phase separation, various emulsifiers can be added to the film-forming solution (Andreuccetti *et al.*, 2009; Krochta, 2002; Prodpran *et al.*, 2007). 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 *et al.*, 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).

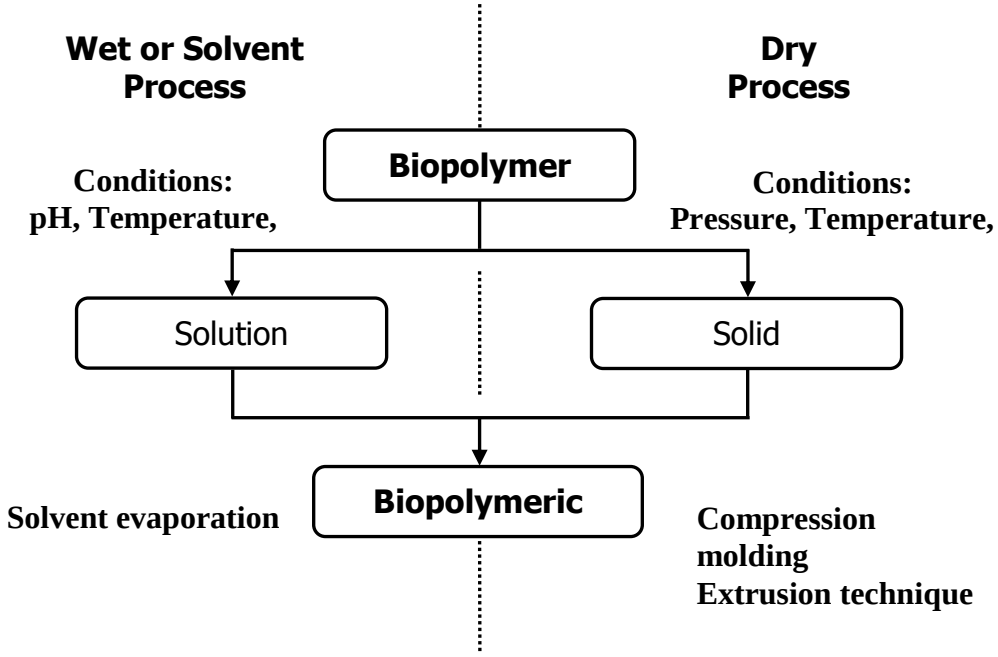


Figure 3. Processing methods: wet (or solvent) and dry process.
Source: Adapted from Guerrero *et al.* (2010).

1.2.4 Gelatin films

Gelatin is derived from the fibrous insoluble protein or collagen, which is the principal constituent of animal skin, bone, and connective tissue. Gelatin is produced via the partial hydrolysis of native collagen (Schrieber and Gareis, 2007). The extraction of gelatin from collagen involves several steps such as alkali and/or acid pretreatments for collagen hydrolysis followed by the main extraction in water at temperature above 45 °C (Montero and Gómez-Guillén, 2000; Wainewright, 1977). The pretreatment and extraction methods greatly affect the physicochemical properties of the extracted gelatin (Montero and Gómez-Guillén, 2000). Two types of gelatins are obtained from selected treatments: acid pretreatment yields Type A gelatin, whereas alkaline pretreatment yields Type B gelatin (Gennadios *et al.*, 1994). Gelatin is one of the important biopolymers widely used in the manufacture of gel desserts, edible films in food industries, and hard/soft capsules in pharmaceutical industries (Baziwane and He, 2003; Choi and Regenstein, 2000). Gelatin is used to encapsulate low moisture or oil phase food ingredients and pharmaceuticals. Such encapsulation provides protection for the core against oxygen and light (Gennadios *et*

al., 1994). In food industry, it has been used as a sausage casing component or coating materials (Donhowe and Fenema, 1994).

Gelatin possesses the excellent film-forming properties and is one of the first materials applied to edible coatings and films (Gennadios *et al.*, 1994). Edible films made from gelatin of different fish, including bigeye snapper and brown stripe red snapper (Jongjareonrak *et al.*, 2006a; Jongjareonrak *et al.*, 2008), baltic cod (Kołodziejska *et al.*, 2006; Kołodziejska and Piotrowska, 2007), tilapia (Pranoto *et al.*, 2007), tuna (Gómez-Guillén *et al.*, 2007), blue shark (Limpisophon *et al.*, 2009), Alaska pollock and salmon (Avena-Bustillos *et al.*, 2006), cuttlefish (Hoque *et al.*, 2010) and squid (Nagarajan *et al.*, 2013), have been prepared.

Since the amino acid compositions of gelatin from varying fish species are significantly different, the functionality of selected fish gelatins such as film-forming properties are also different (Table 2). Protein content, plasticizers type and concentration have been reported to affect the properties of gelatin based films (Vanin *et al.*, 2005). TS of gelatin film from shark (*Prionace glauca*) skin were affected by the protein concentration (1, 2 and 3%) of film-forming solution (FFS) (Limpisophon *et al.*, 2009). TS of the film from a 2% protein FFS was the highest. EAB and water vapor permeability (WVP) increased with increasing FFS protein concentration. The addition of glycerol improved flexibility and enhanced the UV barrier property at 280 nm. Transparency at the visible range and WVP also increased with increasing glycerol content.

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 (Cuq *et al.*, 1997). 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 acid and polyethylene glycol (PEG)

with different molecular weight (300, 400, 600, 800, 1500, 4000, 10000, 20000) where PEG of lower molecular weights exhibited better plasticizing effects for gelatin film and such films had better visual properties.

Heat treatment at as 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). In general, the physical properties of gelatin are mainly governed by the source and the extracting conditions (Bigi *et al.*, 2004). Hoque *et al.* (2011a) 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 their resulting films. Increased amount of -NH_2 and -COOH groups from hydrolysis process and -OH group of glycerol formed H-bond with water molecules resulted in the increased WVP. Therefore, the chain length of gelatin molecules and plasticizer concentration were crucial factors governing the properties of gelatin-basedfilms from cuttlefish skin (Hoque *et al.*, 2011a). 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).

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 (Gomez-Guillen *et al.*, 2009). Krishna *et al.* (2012) reported that water vapor permeability values of extruded gelatin films (the highest value being 2.9 ± 0.2 g mm h^{-1} cm^{-2} Pa^{-1}) were higher than those of solution-cast films, while the glass transition temperatures (Tg) of the extruded films were generally lower than those of solution-cast films.

1.2.5 Heat sealing

Generally, heat sealability of material is important for packing industry, particularly for manufacturing of bags, pouches or sachets, which can be used for packaging foods or ingredients or drinks. Heat sealing is the process of sealing one flexible film to another similar film via heat and pressure. Biodegradable or edible films with heat sealability have been reported for several biomaterials such as fish gelatin (Rouhi *et al.*, 2013), bovine skin gelatin (Nur Hanani *et al.*, 2014), whey protein (Hernandez-Izquierdo and Krochta, 2009; Kim and Ustunol, 2001), wheat gluten (Cho *et al.*, 2007), sago starch (Abdorreza *et al.*, 2011) and corn starch (López *et al.*, 2011). The quality of heat sealing generally depends on the processing conditions such as sealing techiques, temperature, pressure and time (heating time and cooling time), film materials and treatments. Various sealing techniques are available and the appropriate technique depends on film material, the packaging and the product (López *et al.*, 2011). Regardless of various parameters for heat sealing quality, the seal must be strong enough to maintain the packaging integrity and has mechanical resistance for holding the product in the package.

In general, plasticizers, added to reduce hydrogen bonding between polymer chains via competition to polymer-polymer interactions, could serve as heat sealing promoter. Due to their high surface tension and viscosity, the adhesive force via hydrogen bonding between plasticizer and polymer as well as the plasticizer molecules during interdiffusion of surfaces of polymer is dominant. It has been reported that the application of glycerol as seal adhesive yielded the higher peel strength of wheat gluten films prepared by compression-mold, however it could not improve the seal strength for lap-shear test. For polysaccharide-based film, films without plasticizer were brittle and the seal was suddenly separated after application of force during testing, whereas films plasticized with sorbitol had the increase in seal strength with increasing concentration. However, the decrease in seal strength was observed in film plasticized with glycerol as the amount increased (Abdorreza *et al.*, 2011). Thus, sorbitol was the appropriate plasticizer, which potentially enhanced heat sealing ability of sago starch film, in comparison with glycerol. However, the highest seal strength of sago starch films was observed when film plasticized with the sorbital/glycerol mixture (3:1) at 40% (w/w, starch basis). The type and the mixtures of plasticizers, as well as the appropriate concentration, directly affected seal strength of film. López *et al*. (2011) also reported that the addition of glycerol did not improve the seal strength of films based on acetylated, native corn starch and their blend.

Normally, the heat sealing was conducted near or higher than the melting transition temperature of polymer (Hernandez, 1997). Transition temperature (endothermic melting temperature) of polymer is generally determined by differential scanning calorimetry (DSC) and is affected by plasticizer. It has been reported that the film was nearly completely molten during heat sealing when T_{onset} and $T_{peak,\ max}$ of film were very close (Tanner *et al.*, 2001). Abdorreza *et al.* (2011) reported that sago starch films plasticized with sorbitol had lower T_{onset}. This temperature was closer to the heat sealing temperature, compared with those plasticized with glycerol. The temperature decreased with increasing concentrations of plasticizers, which potentialy enhanced the heat sealing ability, especially for sorbitol. However, glycerol yielded the whey protein isolate/lipid emulsion films with lower T_{onset} (108-122 °C) than those plasticized with sorbitol (126-127 °C) due to the differences in plasticizing effects of plasticizers. The highest seal strength of glycerol- and sorbitol-plasticized

film was found when sealed at 130°C and 110°C, respectively. Therefore, the difference in thermal transition temperature, which directly corresponds to the heat sealing ability of films, depends on types of polymers and plasticizers. The crystalline polymer of contacted surface of two films melts by applied heat between heated plates. During heating, the molten polymers from those films are merged themselves together with the concomitant interfacial interactions between the contacted surfaces, which enhanced by pressure and time. Thereafter, the recrystallization of polymers at seal occurs during cooling (Meka and Stehling, 1994; Mueller *et al.*, 1998). For protein/lipid laminated films or protein emulsion films, the lipid-oriented side did not heat sealable or formed seals that are easily delaminated (Kim and Ustunol, 2001). Kim and Ustunol (2001) reported that seal strength of whey protein isolate/lipid emulsion film plasticized with sorbitol ranged from 105 to 301 N/m and those plasticized with glycerol ranged from 141 to 323 N/m.

Heat sealing temperature had a significant influence on seal strength, whereas pressure variation did not affect seal strength significantly. Increase in heating time increased seal strength of film (Kim and Ustunol, 2001). Seal strength of glycerol plasticized whey protein isolate films with heating time of 2.00 and 2.50 s was higher than that of film sealed for 1.50 s for solution-cast film, whereas the heating time of 2.00, 2.25 and 2.50 s did not show the differentces in seal strength of extruded films (Hernandez-Izquierdo and Krochta, 2009). Moreover, seal strengths of films prepared with solution-cast method were significantly higher than those prepared with extrusion method for the heating time of 2.00 and 2.50 s. Cho *et al*. (2007) studied lap-shear strength of wheat gluten film plasticized with glycerol. Glycerol was added as a thin adhesive layer over the surfaces prior to sealing and different compression-molding temperatures (100, 110, 120 and 130°C) were used. Heat sealing temperatures (120, 150 and 175°C) had no effect on seal strength of resulting films. However, molding temperature below 130°C had no influence on the peel strength of films, whereas the increased sealing temperature yielded the higher peel strength. Therefore, heat sealing time as well as preparation method had an important effect on heat sealing ability of film.

Furthermore, the incorporation of nanoparticles to composite materials also has the influence on polymer properties including thermal, mechanical and gas

barrier properties (Kurian *et al.*, 2006). Abdorreza *et al.* (2013) reported that the addition of nanorod-rich ZnO (ZnO-nr) at low level into bovine gelatin and sago starch films could improve the seal strength of film due to the improvement of hydrogen and other bonds on the surface by ZnO-nr. However, the seal strength decreased with increasing level of ZnO-nr, which was probably due to the reduction in moisture content and flexibility of the films. The similar observation was also reported by Rouhi *et al.* (2013). Furthermore, Tabasi *et al.* (2014) reported that the blending of polylactic acid (PLA) and polycaprolactone (PCL) could decrease the crystallinity and yield more amorphous phase and chain mobility of film, thereby lowering the seal and hot-tack initiation temperatures. Leuangsukrerk *et al.* (2014) reported that konjac glucomannan (KGM) films with low concentration of whey protein isolate (WPI) were not heat-sealable. The endothermic melting transition was observed for WPI portion. Thus, the blend films with higher portion of WPI could form the heat sealable matrix. Seal strength of biopolymer based films from various sources are presented in Table 3.

Table 2. Properties of gelatin based film from different fish species.

Fish Species	Protein Conc.	Plasticizer conc.	Thickness (mm)	Mechanical property		WVP ($x10^{-10}$g m^{-1} s^{-1} Pa^{-1})	Transparency (%)	References
				TS (MPa)	EAB (%)			
Alaska pollock (*Theragra chalcogramma*) [1]	5% (w/w) of FFS	-	-	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/w) of FFS	-	-	49.7 – 60.0	3.36 – 3.8	0.85 – 1.08 [a]	-	Chiou *et al.* (2008)
Bigeye snapper (*Priacanthus tayenus*) [3]	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)
Bigeye snapper (*Priacanthus marcracanthus*)	2 and 3 % (w/v) of FFS	Glycerol, 25% (w/w) of protein	0.023-0.035	28.28 and 44.28	2.67 and 7.0	1.22 and 1.37	-	Jongjareonrak *et al.* (2006a)
	3% (w/v) of FFS	Glycerol, 0, 25, 50 and 75% (w/w) of protein	-	7.97 - 57.34	3.04 – 50.30	1.31- 2.73	-	
Brownstripe red snapper (*Lutjanus vitta*)	2 and 3 % (w/v) of FFS	Glycerol, 25% (w/w) of protein	0.024-0.037	41.09 and 58.09	7.02 and 8.20	1.33 and 1.35	-	Jongjareonrak *et al.* (2006a)
	3% (w/v) of FFS	Glycerol, 0, 25, 50 and 75% (w/w) of protein	-	18.80 – 67.78	5.24 – 95.40 [b]	1.32 – 2.28	-	
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)

Table 2. Properties of gelatin based film from different fish species (cont.).

Fish Species	Protein Conc.	Plasticizer conc.	Thickness (mm)	Mechanical property		WVP ($x10^{-10}$g m^{-1} s^{-1} Pa^{-1})	Transparency (%)	References
				TS (MPa)	EAB (%)			
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	3.19 – 7.59	Limpisophon *et al.* (2009)
Cuttlefish (*Sepia pharaonis*) [4]	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	1.32 – 1.35	Hoque *et al.* (2010)
Giant squid (*Dosidicus gigas*) [5]	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.* (2009b)
Giant squid (*Dosidicus gigas*) [6]	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.* (2009c)
Giant squid (*Dosidicus gigas*) [7]	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.* (2009c)
Grouper (*Epinephelus chlorostigma*)	3% (w/v) of FFS	Sorbitol, 30% (w/w) of protein	0.076	9.25	37.48	975 [c]		Jeya Shakila *et al.* (2012)
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)
Tuna (*Thunnus tynnus*) [9]	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]	0.48 – 0.80	Gómez-Guillén *et al.* (2007)

Table 2. Properties of gelatin based film from different fish species (cont.).

Fish Species	Protein Conc.	Plasticizer conc.	Thickness (mm)	Mechanical property		WVP ($x10^{-10}$g m^{-1} s^{-1} Pa^{-1})	Transparency (%)	References
				TS (MPa)	EAB (%)			
Tilapia (*Oreochromis niloticus*)[8]	5% (w/v) of FFS	Gellan and *k*-carrageenan, 0.01 and 0.02 g/g gelatin	-	101.23 – 109.76	5.08 - 6.81	1.75 - 2.4[a]	-	Pranoto *et al.* (2007)
Sole (*Solea spp*.) [aa]	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.* (2009b)
Atlantic halibut (*Hippoglossus hippoglossus*) [ab]	2% (w/v) of FFS	Sorbitol, 30% (w/w) of protein	0.080 ± 0.006	3.8 ± 0.8	294.5 ± 47.8	12.0 ± 2.2 [d]	-	Carvalho *et al.* (2008)
Atlantic halibut (*Hippoglossus hippoglossus*) [ac]	2% (w/v) of FFS	Sorbitol, 30% (w/w) of protein	0.079 ± 0.006	11.1 ± 2.6	170.3 ± 36.4	13.0 ± 2.2 [d]	-	Carvalho *et al.* (2008)
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]	0.95 - 1.80	Denavi *et al.* (2009)

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

Table 3. Seal strength of biopolymer based films from various sources.

Film forming materials, Conc.	Plasticizer conc.	Additives, Conc.	Thickness (mm)	Seal strength (N/m)		References
				Peel test	Lab-shear test	
Native corn starch, 5% (w/w) of FFS	Without and with glycerol, 30 % (w/w) of starch	-	0.084 and 0.106	0.07 and 0.15	-	López *et al*. (2011)
Acetylated corn starches, 5% (w/w) of FFS	Without and with glycerol, 30 % (w/w) of starch	-	0.108 and 0.131	0.11 and 0.27	-	López *et al*. (2011)
Native and acetylated corn starches mixture (1:1), 5% (w/w) of FFS	Without and with glycerol, 30 % (w/w) of starch	-	0.097 and 0.125	0.08 and 0.22	-	López *et al*. (2011)
Sago starch, 4% (w/v) of FFS	Sorbitol, 30, 40 and 50% (w/w) of starch basis	-	0.116 - 0.126	73.66 - 301.34	-	Abdorreza *et al.* (2011)
	Glycerol, 30, 40 and 50% (w/w) of starch basis	-	0.123 - 0.155	15.63 - 69.19	-	
	Mixture (1:1), 30 and 40% (w/w) of starch basis	-	0.127 - 0.141	53.57 - 187.50	-	
	Mixtures (1:3 and 3:1), 30 and 40% (w/w) of starch basis	-	0.119 - 0.138	13.39 - 375.00	-	

Table 3. Seal strength of biopolymer based films from various sources (cont.).

Film forming materials, Conc.	Plasticizer conc.	Additives, Conc.	Thickness (mm)	Seal strength (N/m)		References
				Peel test	Lab-shear test	
Sago starch, 4% (w/w) of FFS	Mixture (3:1), 40% (w/w) of starch basis	ZnO-nr, 1-5% (w/w) of total solid	-	365.04 - 504.43	-	Abdorreza *et al*. (2013)
Bovine gelatin, 8% (w/w) of FFS	Mixture (3:1), 40% (w/w) of starch basis	ZnO-nr, 1-5% (w/w) of total solid	-	823.01 - 1340.71	-	
Whey protein isolate, 5% (w/v) of FFS	Glycerol, 5.0% (w/v) of FFS	-	0.120 ± 0.015	171 - 323	-	Kim and Ustunol (2001)
	Glycerol, 4.8% (w/v) of FFS	Butterfat, 0.2% (w/v) of FFS	0.120 ± 0.015	147 - 291	-	
	Glycerol, 4.2% (w/v) of FFS	Candella wax, 0.8% (w/v) of FFS	0.120 ± 0.015	141 - 297	-	
	Sorbital, 3.5% (w/v) of FFS	-	0.140 ± 0.019	110 - 301	-	
	Sorbital, 3.3% (w/v) of FFS	Butterfat, 0.2% (w/v) of FFS	0.140 ± 0.019	112 - 268	-	
	Sorbital, 2.7% (w/v) of FFS	Candella wax, 0.8% (w/v) of FFS	0.140 ± 0.019	105 - 296	-	
Fish gelatin, 5% (w/v) of FFS	Sorbitol and glycerol, 0.15 and 0.15 g/g gelatin	ZnO-nr, 1-5% (w/w) of gelatin	-	12.02 - 41.56	-	Rouhi *et al*. (2013)

Table 3. Seal strength of biopolymer based films from various sources (cont.).

Film forming materials, Conc.	Plasticizer conc.	Additives, Conc.	Thickness (mm)	Seal strength (N/m)		References
				Peel test	Lab-shear test	
Wheat gluten	Glycerol, 30% (w/w) of wheat gluten dought	Glycerol as an seal adhesive	-	0.053 - 0.243 [d]	0.070 - 0.229 [e]	Cho *et al*.(2007)
		Without glycerol as an seal adhesive	-	0.093 - 0.263	0.076 - 0.020	
Beef skin gelatin	Glycerol, 0.2 – 1.1 % (w/w) of gelatin	Corn oil	0.020 - 0.023	-	445.40 - 604.59	Nur Hanani *et al.* (2013)
Beef skin gelatin	-	Corn oil	0.023 - 0.028	-	231.78 - 329.16	Nur Hanani *et al.* (2014)
Whey protein isolate, 5% (w/w) of FFS	Glycerol, 40 % (w/w) of whey protein isolate [a]	-	0.13 ± 0.01	259.18 - 419.95	-	Hernandez-Izquierdo and Krochta (2009)
	Glycerol, 48.8 and 49.5% (w/w) of protein [b]	-	0.18 ± 0.02	196.85 - 262.47	-	
Soy protein isolate laminated with corn zein layer, 5% (w/v) of FFS	Sorbitol and glycerol, 1.25% and 1.25% (w/v) of FFS	-	114.60 ± 2.70	25.62 – 344.91	-	Choa *et al.* (2010)

* FFS = Film forming solution; ZnO-nr = zinc oxide nanorods; [a] Films were prepared by solution casting; [b] Films were prepared by extrusion followed by compression molding; [c] Seal strength unit (g/25.4mm); [d] Seal strength unit (N/mm); [e] Seal strength unit (MPa).

1.2.6 Improvement of the property of protein based film

Several methods have been used to improve gelatin film properties. Chemical and enzyme treatment can be applied to modify the polymer network through the cross-linking of the polymer chains to improve the properties of protein film (De Carvalho and Grosso, 2004; Mahmoud and Savello, 1993; Yildirim and Hettiarachchy, 1997). Cross-linking agents are natural and synthetic molecules containing at least two reactive groups that are able to form a covalent inter- and /or intra molecular links between protein chains. These agents, when used to prepare protein based-films, strengthen the materials through the formation of new covalent bonds, while reducing film elasticity and solubility in water (Gennadios *et al.*, 1994). The chemical agents used for cross-linking agents including glutaraldehydes (Bigi *et al.*, 2001), calcium chloride (Galietta *et al.*, 1998), carbodiimide (Kołodziejska *et al.*, 2006), genipin (Bigi *et al.*, 2002), ferrulic acid, tannin acid (Cao *et al.*, 2007) and others.

1.2.6.1 Use of aldehydes

Chemical cross-linking of natural proteins has been used for development of protein-based film. The increase in degree of cross-linking may cause a reduction in water vapor permeability through the reduction in diffusivity and an improvement of mechanical characteristics (Cuq, 1996). Chemical modifications using formaldehyde, glutaraldehyde and gossypol in the production of biofilms based on proteins extracted from cottonseed indicated a significant decrease in solubility and an increase in puncture force (Marquie *et al.*, 1995). Reactions with formaldehyde led to the formation of more resistant films, indicating that these films may react with other groups besides ε-lysine, producing a more cohesive protein chain (Marquie *et al.*, 1997). With formaldehyde modification, a slight reduction in water vapor permeability (WVP) in gluten protein based films (Micard *et al.*, 2000a) and film based on soy protein (Ghorpade *et al.*, 1995) was found. De Carvalho and Grosso (2006) evaluated the cross-linking effect of formaldehyde (3.8 to 8.8 mmoles/100 mL of filmogenic solution) and glyoxal (6.3 to 26.3 mmoles/100 mL of filmogenic solution) on properties of bovine hide type B gelatin-based films. The treatments

caused a reduction in permeability to water vapor and in solubility. Greater thermal stability, with an increase in the melting point, was observed for the chemically (glyoxal and formaldehyde) treated films from bovine hide gelatin, indicating a greater degree of crosslinking, as confirmed by the decreasing number of free α-amine groups remaining after the modification reactions (De Carvalho and Grosso, 2004).

Treatment with formaldehyde resulted in the increase in TS and puncture strength by two-fold, but reduced WVP (by about 6%) and water solubility (by about 42%) of soy protein isolate films (Rhim *et al.*, 2000). The WVP and oxygen permeability of the films decreased after aldehyde treatment (Liu *et al.*, 2004). The ability of formaldehyde and glutaraldehyde to promote covalent intermolecular cross-linking of peanut protein film was therefore effective to increase the mechanical and barrier properties of the films.

Glutaraldehyde is more specific than formaldehyde. It can react with lysine, cysteine, histidine and tyrosine (Marquié and Guilbert, 2002). The incorporation of glutaraldehyde resulted in lower water vapor permeability for salmon gelatin films and higher oxygen permeability for pollock gelatin films (Chiou *et al.*, 2008). The cross-linking reduced free volume in these films, resulting in the better barrier properties. Alves *et al.* (2011a) studied the properties of films based on blends of gelatin and polyvinyl alcohol (PVA) cross-linked with glutaraldehyde. Both gelatin and PVA can be cross linked with glutaraldehyde. In the case of gelatin, the glutaraldehyde reacts with each ε-NH_2 functional group of adjacent lysine residues, while for PVA, the glutaraldehyde reacts with two adjacent hydroxyl groups, forming acetal bridges.

1.2.6.2 Uses of transglutaminase

The enzymatic crosslinking included by transglutaminase has been used for improvement of protein based films. Transglutaminase (TGase, protein-glutamine: amine γ-glutamyl transferase) catalyzes the introduction of ε-(γ-glutamyl)-lysine cross-links into proteins via an acyl transfer reaction. The γ-carboxyamide group of glutamine serves as the acyl donor and the ε-amino group of lysine serves as the acyl acceptor (Folk, 1980).

The solubility at pH 3 and 6 of TGase-modified gelatin films was decreased from 100% to 30% and that of films modified with TGase in the presence of dithiothreitol or cysteine was decreased to 5% and 15%, respectively. Under the same conditions, the solubility of chemically modified films was about 10% (Piotrowska *et al.*, 2008). The gelatin based films modified with TGase, glyxoal and formaldehyde had 20% reduction in solubility for all modified films (De Carvalho and Grosso, 2004). A substantial increase in the high molecular weight protein components was observed in the film forming solution of gelatin and casein cross-linked with TGase (Chambi and Grosso, 2006). A nanoclay composite film was produced using warm water fish gelatin as a base material with microbial transglutaminase treatment (Bae *et al.*, 2009). Molecular weight of fish gelatin solutions increased after treatment with microbial TGase (MTGase). Tensile strength decreased from 61.60 (0 min) to 56.42 MPa (30 min), while EAB increased from 13.94 (0 min) to 15.78% (30 min) when 2% (w/w) MTGase was added. The oxygen permeability and water vapor permeability were not changed after treatment with 2% (w/w) MTGase (Bae *et al.*, 2009).

1.2.6.3 Uses of phenols

Polyphenols can interact non-covalently or covalently with proteins. Under non-oxidizing conditions, non-covalent interactions including hydrogen bond and hydrophobic forces stabilize tannin-protein complexes (Chen and Hagerman, 2004). The non-covalent interaction may occur between polyphenols and proteins by hydrogen bonding and by hydrophobic interaction. In some cases, ionic bonding may be possible. Covalent attachment can occur depending on the polarity of the polyphenol (Hagerman *et al.*, 1998; Rawel *et al.*, 2003; Siebert *et al.*, 1996; Suryaprakash *et al.*, 2000). The interaction may occur via multisite interaction (several phenolic compounds bound to one protein molecule) or multidentate interaction (one phenolic compound bound to several protein molecules). The type of interaction depends on the type and the molar ratio of both phenolic compound and protein (Prigent *et al.*, 2003). Tannin can interact non-specifically with proteins such as bovine serum albumin (BSA) or specifically with protein such as gelatin (Frazier *et al.*, 2003; Nuthong *et al.*, 2009b).

Fish skin gelatin films incorporated with seaweed extract at pH 9 and 10 exhibited the higher EAB than the control film. However, no differences in TS and transparency between films without and with seaweed extract were observed ($p>0.05$). Water vapor permeability, and film solubility decreased as seaweed extract was incorporated, regardless of pH. This was associated with the formation of non-disulfide covalent bond in the film matrix, most likely induced by the interaction between oxidized phenols in seaweed extract and gelatin molecules (Rattaya *et al.*, 2009). The polyphenol–protein interaction was found to be more extensive, when tuna-skin gelatin film was added with oregano and rosemary extracts (Gómez-Estaca *et al.*, 2009a). The tuna-skin gelatin underwent the interactions with the polyphenols in both extracts, thereby altering the attributes of the corresponding films, namely, a higher glass transition temperature, decreased deformability, and water solubility (Gómez-Estaca *et al.*, 2009a). The incorporation of the borage extract into the films gave rise to a pronounced increase of their antioxidant properties irrespective of the gelatin origin from sole skin gelatin or commercial fish skin gelatin. Decreased breaking force and increased film opacity were formed with the addition of borage extract (Gómez-Estaca *et al.*, 2009b). The addition of murta yielded films from tuna-fish gelatin with increased protection of UV light as well as antioxidant activity (Gómez-Guillén *et al.*, 2007).

1.2.6.4 Uses of oils and fatty acids

Hydrophobic plasticizer can be used to improve water vapor barrier property of films. However, it may yield films with different properties. The incorporation of hydrophobic substances such as lipid, fatty acid, wax, etc has been implemented to improve water barrier property (Limpisophon *et al.*, 2010; Prodpran *et al.*, 2007; Soazo *et al.*, 2011). Due to hydrophilic character, protein based films have poor water vapor barrier property, thereby restricting their use as packing materials. To improve the barrier property, the incorporation of hydrophobic materials such as essential oils, fatty acids or waxs have been implemented (Atarés *et al.*, 2010a; Benavides *et al.*, 2012; Fabra *et al.*, 2009; Limpisophon *et al.*, 2010). Andreuccetti *et al.* (2009) reported that gelatin-based films using hydrophobic

plasticizers derived from citric acid and soy lecithin as an emulsifier had the increase in TS from 36 to 103 MPa. However, the increase in the concentration of plasticizers (acetyltributyl citrate and tributyl citrate) reduced TS by 57% and no relation was observed between plasticizer quantities and the elongation. Addition of hydrophobic substances including amaranth oil, rapeseed oil, lanolin, beeswax and ozococerite at a concentration of 10% decreased WVP of gelatin films by 42, 15, 37, 53 and 36%, respectively. Increasing concentration of these substances up to 60% as emulsifiers caused further improvement of the water barrier properties. Addition of lecithin into film-forming emulsions prevented separation of lipid layer on the film surface. Among films with lecithin and 60% of lipids, the highest decrease of WVP was found in case of amaranth oil and beeswax in which the decreases by 73 and 87% were found respectively, in comparison to the control gelatin film (Sztuka and Kołodziejska, 2009).

Fatty acids have received substantial attention as plasticizers (Santosa and Padua, 1999). The long chain hydrocarbon segments of organic acid might act as plasticizer for gelatin. Oleic acid was used to modify zein sheets, in which TS of zein sheets decreased but EB increased (Santosa and Padua, 1999). 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 than the addition of palmitic acid. The addition of the blend (stearic/palmitic, 1:1) signifi cantly decreased WVP of the films as compared to those without lipid addition or containing palmitic acid. However, it showed similar effect the film containing stearic acid. Additionally, TS of film generally decreased with the addition of fatty acid, while gradually increased with increasing amount of fatty acid sucrose esters (Jongjareonrak *et al.*, 2006c). Limpisophon *et al.* (2010) also studied the effect of incorporation of fatty acids (stearic and oleic) on the properties of edible films based on blue shark (*Prionace glauca*) skin gelatin. Increasing fatty acid concentration significantly decreased WVP.

Addition of stearic acid to the gelatin film reduced WVP more effectively than did oleic acid when the same fatty acid concentration was used. Increasing concentrations of both fatty acids decreased tensile strength, but increased elongation at break due to their plasticizing effect. At the same concentration, oleic

acid gave a greater plasticizing effect than did stearic acid. Faster homogenisation improved properties of the gelatin–stearic acid emulsion film (Limpisophon *et al.*, 2010). The differences of WVP observed between gelatin films could depend on differences in the protein composition, emulsifying protein composition, and emulsifying properties of the gelatins, while hydrophobicity (lower WVP) increased with the addition of long-chain fatty acids (Jongjareonrak *et al.*, 2006b). The incorporation of fatty acids and oil into gelatin based film could be an excellent barrier preventing UV light-induced lipid oxidation (Jongjareonrak *et al.*, 2006b; Pérez-Mateos *et al.*, 2009).

Effects of olive oil content on lipid droplet distributions in the film-forming dispersions and emulsified films based on gelatin were evaluated (Ma *et al.*, 2012b). The water barrier capability and tensile strength showed an enhanced trend as the lipid droplets in the films decreased. WVP and tensile property were mainly dependent on the lipid droplet size or distribution in the films. The presence of large agglomerates of lipid droplets within film matrix may weaken mechanical resistance and water barrier ability of the films (Ma *et al.*, 2012b). In order to obtain protein-lipid composite films with improved water barrier ability, a lipid can be incorporated into the protein matrix by dispersing a lipid in the protein aqueous solutions to obtain an emulsified film. 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). Ma *et al.* (2012a) studied the homogenization techniques (rotor–stator homogenizer and microfluidizer) and conditions of gelatin-olive oil composite films on lipid droplets distributions and film properties. The rotor–stator homogenizer provided a lower energy input and produced the largest particles and olive oil molecules tended probably to form bi-layer on air side of films. These films exhibited excellent water barrier ability, but poor mechanical resistance, extensibility and transparency. The microfluidizer can provide filmogenic dispersions with narrower particle size distributions, promoting mechanical resistance, extensibility and transparency of the films. Concomitantly, the WVP decreased upon increasing pressure or cycle (Ma *et al.*, 2012a).

1.2.7 Essential oils

Essential oils, also called volatile odoriferous oil, are aromatic oily liquids extracted from different parts of plants, e.g. leaves, peels, barks, flowers, buds, seeds, etc. They can be extracted from plant materials by several methods, steam distillation, expression, etc. Among all methods, steam distillation method has been widely used, especially for commercial scale production (Cassel and Vargas, 2006; Di Leo Lira *et al.*, 2009). Essential oils have been widely used as food flavors (Burt, 2004). Essential oils found in many different plants, especially the aromatic plants, vary in odor and flavor, which are governed by the types and amount of constituents present in oils. Additionally, the amount of essential oil from different plants is varied and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigments contribute to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods. Essential oils have been known to possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils, can be improved. Almost parts of a plant may be the source of the oil, which could be extracted and fully explored for food applications or others. Modern technologies have been continuously developed to conquer the limitation of conventional methods, and to enhance the extraction efficacy. Due to the increasing attention in natural additives, essential oils from several plants have been used more widely, especially in conjunction with other preservations under concept of 'hurdle technology'. Thus, essential oils can serve as the alternative additives or processing aid as green technology.

1.2.7.1 Sources and chemical composition

Several plants contain essential oils, however parts of plants, which serve as the major source of essential oil can be different (Table 4). Those include roots, peels, leaves, seeds, fruits, barks, etc. Plant essential oils are usually the complex mixture of natural compounds. The mixture, that constitute the essential oil,

comprises polar and non-polar compounds (Masango, 2005). Dominant compounds in various essential oils are presented in Table 5. In general, the constituents in essential oils are terpenes (monoterpenes and sesquerpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, etc.) and terpenoids (isoprenoids) (Bakkali *et al.*, 2008; Mohamed *et al.*, 2010a). Compounds and aroma of essential oils can be divided into 2 major groups: terpene hydrocarbons and oxygenated compounds.

1.2.7.1.1 Terpene hydrocarbons

The hydrocarbons are the molecule, constituting of H and C atoms arranged in chains. These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic or tricyclic) or aromatic. Terpenes are the most common class of chemical compounds found in essential oils. Terpenes are made from isoprene units (several 5 carbon base units, C_5), which are the combinations of two isoprene units, called a "terpene unit". Essential oils consist of mainly monoterpenes (C_{10}) and sesquiterpenes (C_{15}), which are hydrocarbons with the general formula $(C_5H_8)_n$. The diterpenes (C_{20}), triterpenes (C_{30}) and tetraterpenes (C_{40}) are exist in essential oils at low concentration (Mohamed *et al.*, 2010a). Terpenoids (a terpene containing oxygen) is also found in essential oils (Burt, 2004).

Essential oils mostly contain monoterpenes and sesquiterpenes, which are $C_{10}H_{16}$ (MW 136 amu) and $C_{15}H_{24}$ (MW 204 amu), respectively. Although sesquiterpenes are larger in molecules and structure but functional properties of sesquiterpenes are similar to the monoterpenes (Ruberto and Baratta, 2000). For diterpenes, triterpenes and tetraterpenes, they have the larger molecule than monoterpenes and sesquiterpenes, however they are present at very low concentration in essential oils (Bakkali *et al.*, 2008).

1.2.7.1.2 Oxygenated compounds

These compounds are the combination of C, H and O, and there are a variety of compounds found in essential oils. Oxygenated compounds can be derived from the terpenes, in which they are termed "terpenoids". Some oxygenated compound compounds prevalent in plant essential oils are shown as follows:

- *Phenols*: thymol, eugenol, carvacrol, chavicol, thymol, etc.

- *Alcohols*:

Monoterpene alcohol: borneol, isopulegol, lavanduol, α-terpineol, etc.

Sesquiterpenes alcohol: elemol, nerolidol, santalol, α-santalol, etc.

- *Aldehydes*: citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde, etc.

- *Ketones*: carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone, etc.

- *Esters*: bomyl acetate, linalyl acetate, citronellyl acetate, geranyl acetate, etc.

- *Oxides*: 1,8-cineole, bisabolone oxide, linalool oxide, sclareol oxide, etc.

-*Lactones*: bergaptene, nepetalactone, psoralen, aesculatine, citroptene, etc.

- *Ethers*: 1,8-cineole, anethole, elemicin, myristicin, etc.

Different constituents in essential oils exhibit varying smell or flavor (Burt, 2004). Also, the perception of individual volatile compounds depends on their threshold.

Table 4. Parts of plant material containing essential oils.

Parts	Plants
Leaves	Basil, bay leaf, cinnamon, common sage, eucalyptus, lemon grass, citronella, melaleuca, mint, oregano, patchouli, peppermint, pine, rosemary, spearmint, tea tree, thyme, wintergreen, kaffir lime, laurel, savory, tarragon, cajuput, lantana, lemon myrtle, lemon teatree, niaouli, may chang, petitgrain, laurel, cypress
Seeds	Almond, anise, cardamom , caraway, carrot celery, coriander, cumin, nutmeg, parsley, fennel
Wood	Amyris, atlas cedarwood, himalayan cedarwood, camphor, rosewood, sandalwood, myrtle, guaiac wood
Bark	Cassia, cinnamon, sassafras, katrafay
Berries	Allspice, juniper
Resin	Frankincense, myrrh
Flowers	Blue tansy, chamomile, clary sage, clove, cumin, geranium, helichrysum hyssop, jasmine, lavender, manuka, marjoram, orange, rose, baccharises, palmarosa, patchouli, rhododendron anthopogon, rosalina, ajowan, ylang-ylang, marjoram sylvestris, tarragon, immortelle, neroli
Peel	Bergamot, grapefruit, kaffir lime, lemon, lime, orange, tangerine, mandarin
Root	Ginger, plai, turmeric, valerian, vetiver, spikenard, angelica
Fruits	Xanthoxylum, nutmeg, black pepper

Table 5. Chemical composition of volatiles in essential oils**.**

Essential oils	Chemical compounds						References
	Monoterpene hydrocarbons	Oxygenated monoterpenes	Sesquiterpene hydrocarbons	Oxygenated sesquiterpenes	Esters	Others	
Basil	β-Pinene, β-Limonene, γ-Terpinene	endo-5,5,6-Trimethyl-2-norbornanone	β-Elemene, 2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene, γ-Cadinene, γ-Muurolene,	Methyleugenol	-	Methylchavicol, 3- Methoxycinnamaldehyde	Teixeira *et al.* (2013)
Lemon	α-Pinene, β-Pinene, Cymene, α-Limonene, α-Fellandrene	-	*trans*-Caryophyllene	-	-	1,2,3,5-Tetramethyl-benzene, 1-(1,5-Dimethylhexyl)-4-methylbenzene	Teixeira *et al.* (2013)
Citronella	S-3-Carene, Mentha-1,4,8-triene, Δ^2-Carene, *cis*-2,6-Dimethyl-2,6-octadiene, γ-Terpinene	(−)-Isopulegol, β-Citronellal, β- Citronellol	β-Elemene, β-Selinene, α-Selinene, α-Muurolene, (+)-δ-Cadinene, Eremophilene, γ-Selinene, (+)-δ-Selinene, (−)-α-Amorphene	(−)-Cedreanol	m-(Trimethylsiloxy) -cinnamic acid methyl ester	-	Teixeira *et al.* (2013)
Garlic	1(7),5,8-*o*-Menthatriene	*trans*-Limone oxide, endo-5,5,6-Trimethyl-2-norbornanone,	-	-	-	di-2-Propenyldisulfide, Dimethyl tetrasulphide, di-2-Propenyltetrasulfide, 3,3'-Thiobis-1-propene, Sulfur	Teixeira *et al.* (2013)

Table 5. Chemical composition of volatiles in essential oils (cont.).

Essential oils	Chemical compounds						References
	Monoterpene hydrocarbons	Oxygenated monoterpenes	Sesquiterpene hydrocarbons	Oxygenated sesquiterpenes	Esters	Others	
Clove	-	-	*trans*-Caryophyllene, α-Humulene	Methyleugenol	Aceteugenol	p-Eugenol	Teixeira *et al.* (2013)
Sage	α-Pinene, Camphene, β-Pinene, Cymene, α-Fellandrene, m-Cymene, Mentha-1,4,8-triene, Δ^2-Carene, 1,3,8-*p*-Menthatriene, α-Terpinolene	Eucalyptol, (E)-2,3-Epoxycarane, (−)-Camphor, *endo*-Borneol, *endo*-5,5,6-Trimethyl-2-norbornanone	*trans*-Caryophyllene, β-Selinene, β-Bisabolene	-	(−)-Bornylacetate	-	Teixeira *et al.* (2013)
Rosemary	α-Pinene, Camphene, β-Pinene, Cymene, α-Fellandrene, S-3-Carene, *m*-Cymene, Mentha-1,4,8-triene	Eucalyptol, (E)-2,3-Epoxycarane, (−)-Camphor, *endo*-Borneol, *endo*-5,5,6-Trimethyl-2-norbornanone	*trans*-Caryophyllene	-	(−)-Bornylacetate	-	Teixeira *et al.* (2013)
Thyme	Camphene, β-Pinene, Cymene, α-Fellandrene, *m*-Cymene	Eucalyptol, (E)-2,3-Epoxycarane, *endo*-5,5,6-Trimethyl-*m*-Thymol, Carvacrol	*trans*-Caryophyllene	-	-	(3E,5E,8E)-3,7,11-Trimethyl-1,3,5,8,10-dodecapentaene	Teixeira *et al.* (2013)

Table 5. Chemical composition of volatiles in essential oils (cont.).

Essential oils	Chemical compounds						References
	Monoterpene hydrocarbons	Oxygenated monoterpenes	Sesquiterpene hydrocarbons	Oxygenated sesquiterpenes	Esters	Others	
Thymus longicaulis subsp. *longicaulis* var.*longicaulis*	α-Thujene, α-Pinene, Myrcene, Camphene, β-Pinene, α-Phellandrene, α-Terpinene, p-Cymene, (E)- β-Ocimene, γ-Terpinene, *cis*-Sabinene hydrate, Terpinolene	Camphor, Borneol, Terpinen-4-ol, α-Terpineol, Thymol, Carvacrol, β-Caryophyllene	α-Humulene, δ-Cadinene , Germacrene D	-	-	-	Sarikurkcu *et al.* (2010)
Lemongrass	α-Pinene, 3-Carene, Camphene	β-Citral, α-Citral, α-Cyclocitral, Terpineol, 2,3-Dehydro-1,8-cineole	β-Caryophyllene	-	-	*m*-Eugenol, Geranyl N-butyrate, Isogeraniol	Leimann *et al.* (2009)
Mint (*Satureja Montana*)	α-Thujene, α-Pinene, Myrcene, α-Terpinene , γ-Terpinene, p-Cymene	Linalool, α-Terpineol, Borneol, Thymol , Carvacrol	β-Cubebene, δ-Cadinene	Caryophyllene oxide, Spathulenol	-	1-Octen-3-ol, Thymol methyl ether, Carvacrol methyl ether, Thymyl acetate	Bezić *et al.* (2005)

Table 5. Chemical composition of volatiles in essential oils (cont.).

Essential oils	Chemical compounds						References
	Monoterpene hydrocarbons	Oxygenated monoterpenes	Sesquiterpene hydrocarbons	Oxygenated sesquiterpenes	Esters	Others	
Mint (*Satureja cuneifolia*)	α-Pinene, Myrcene, Limonene, *cis*-β-Ocimene, p-Cymene, allo-Ocimene	Thymol, Carvacrol, Camphor, Linalool, Terpinen-4-ol , Neral, α-Terpineol, Borneol, Geranial, Geraniol	β-Bourbonene, β-Caryophyllene, Aromadendrene, β-Cubebene, δ-Cadinene,	Caryophyllene oxide , Spathulenol, Viridiflorol,	-	-	Bezić *et al.* (2005)
Plai-Dam *(Zingiber ottensii)*	α-Pinene, β-Pinene, Sabinene, Myrcene, α-Terpinene , Limonene , E-β-Ocimene, p-Cymene , Terpinolene , γ-Terpinene	1,8-Cineole, Linalool, Terpinen-4-ol, *cis*-Menth-2-en-1-ol, Borneol , *trans*-Piperitol	β-Elemene, β-Caryophyllene, Humulene	Caryophyllene oxide, Humulene oxide, α-Eudesimol, β-Eudesimol, Zerumbone	-	Bornyl acetate, Sabinene hydrate, 4-phenylbutan-2-one	Thubthimthed *et al.* (2005)
Mandarin	α-Pinene, di-Limonene, Allo-Ocimene, Camphene, Sabinene	Neo-Dihydrocaveol, *cis*-Limonene oxide, Linalool, Borneol, Limonene glycol, Carvone	Farnesene, α-Farnesene	-	-	Linalyl acetate, Undecanoic acid, Methly-anthranilate, Benzaldehyde	Mohamed *et al.* (2010b)

Table 5. Chemical composition of volatiles in essential oils (cont.).

Essential oils	Chemical compounds						References
	Monoterpene hydrocarbons	Oxygenated monoterpenes	Sesquiterpene hydrocarbons	Oxygenated sesquiterpenes	Esters	Others	
Orange	Myrcene, β-Phellandrene, α-Terpinolene, Menthatriene	*cis*-Limonene oxide, Decanal, Linalool, Verbenol, Carvone, Perilladehyde, *cis*-Carveol, Citronellol	Farnesene	-	-	Nonyl-aldehyde, Caprylic acid, Cinnamic-aldehyde, Heptadecanol	Mohamed *et al.* (2010b)
Lemon	α-Pinene, α-Fenchene, Limonene, Camphene	Citronellal, *cis*-Carveol, α-Citral, Carvacol, Terpniol, Thymol, Carvacrol, Citral	-	-	-	Cyclohexane, Heptanal, Dihydroiso-pimaric , Dihydro-abitec	Mohamed *et al.* (2010b)
Tangerine	α-Pinene, Limonene, α-Terpinene, *trans*- Menthadiene, *trans*-Ocimene, *trans*-Decalone	Citronellal, Linalool, *cis*-Limonene oxide, *trans*-Carveol, Limonene dioxide, Perillyl alcohol	-	Ledol, Globulol	-	Aloxiprin, Heptadiene, Methyl- heptadiene, Cyclooctanone, Benzyl-dicarboxylic	Mohamed *et al.* (2010b)
Oregano	α-Terpinene, Limonene, γ-Terpinene	1,8-Cineole, Terpinen-4-ol, α-Terpineol, Thymol, Carvacrol,	β-Caryophyllene, *cis*-Hydrate sabinene, *trans*-Hydrate sabinene	-	-	-	Aguirre *et al.*(2013)

1.2.7.2 Role of essential oils as food additives

Essential oils from plants have been known to act as natural additives, e.g. antimicrobial agents, antioxidant, etc. Their activities vary with source of plants, chemical composition, extraction methods, etc. Due to the unique smell associated with the volatiles, this may limit the use of essential oil in some foods since it may alter the typical smell/flavor of foods.

1.2.7.2.1 Antimicrobial activity

The ability of plant essential oils to protect foods against pathogenic and spoilage microorganisms has been reported (Friedman, 2006; Lis-Balchin *et al.*, 1998; Rojas-Graü *et al.*, 2007). Among chemical components in several essential oils, carvacrol has been shown to exert a distinct antimicrobial action (Veldhuizen *et al.*, 2006). Carvacrol is the major component of essential oil from oregano (60 to 74% carvacrol) and thyme (45% carvacrol) (Arrebola *et al.*, 1994; Lagouri *et al.*, 1993). It has a broad spectrum of antimicrobial activity against most gram-positive and gram-negative bacteria (Friedman *et al.*, 2002). Carvacrol disintegrates the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (Burt, 2004). For gram-positive bacteria, it is able to interact with the membranes of bacteria and alter the permeability for cations like H^+ and K^+ (Veldhuizen *et al.*, 2006). In general, the higher antimicrobial activity of essential oils is observed on gram positive bacteria than gram negative bacteria (Kokoska *et al.*, 2002; Okoh *et al.*, 2010). Lipophilic ends of lipoteichoic acids in cell membrane of gram possitive bacteria may facilitate the penetration of hydrophobic compounds of essential oils (Cox *et al.*, 2000). On the other hand, the resistance of gram-negative bacteria to essential oils is associated with the protecting role of extrinsic membrane proteins or cell wall lipopolysaccharides, which limits the diffusion rate of hydrophobic compounds through the lipopolysaccharide layer (Burt, 2004). The dissipation of ion gradients leads to impairment of essential processes in the cell and finally to cell death (Ultee *et al.*, 1999). The cytoplasmic membrane of bacteria generally has two principal functions: (i) barrier function and energy transduction, which allow the membrane to form ion

gradients that can be used to drive various processes, and (ii) formation of a matrix for membrane-embedded proteins (such as the membrane-integrated F_0 complex of ATP synthase) (Hensel *et al.*, 1996; Sikkema *et al.*, 1995). Antimicrobial mechanism of essential oil is proposed as shown in Figure 4. The activity of the essential oils is related to composition, functional groups and synergistic interactions between components (Dorman and Deans, 2000). The removal of the aliphatic ring substituent of carvacrol slightly decreased the antimicrobial activity. 2-Amino-*ρ*-cymene has similar structure to cavacrol, except hydroxyl group (Figure 5). The lower activity by 3-fold of 2-amino-*ρ*-cymene, as compared to carvacrol, indicates the essential role of hydroxyl group in antimicrobial activity of carvacrol (Veldhuizen *et al.*, 2006). The hydroxyl group present in the structure of phenolic compounds confers antimicrobial activity and its relative position is very crucial for the effectiveness of these natural components; this can explain the superior antimicrobial activity of carvacrol, compared to other plant phenolics (Veldhuizen *et al.*, 2006).

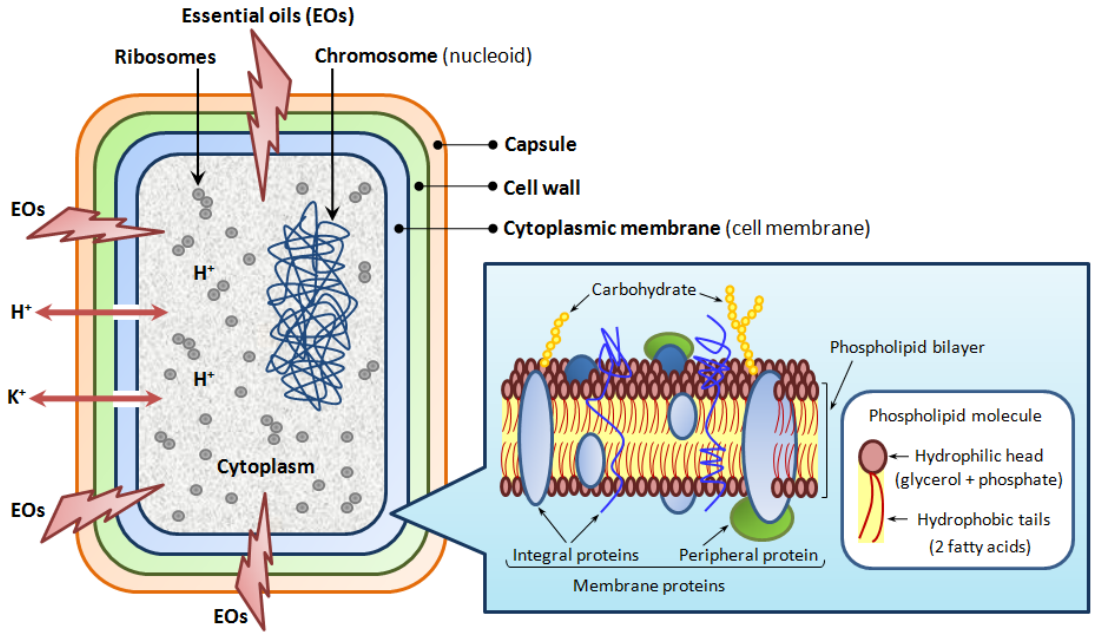


Figure 4. Schematic illustration the effect of essential oils on bacteria cell.

Plant essential oils have been known as antimicrobial agents. Essential oil of rosemary (*R. officinalis*) exhibited both gram possitive (*S. aureus* and *B. subtilis*) and gram negative (*E. coli* and *K. pneumoniae*) bacteria (Okoh *et al.*, 2010). The major components of rosemary oil are monoterpenes such as α-pinene, β-pinene,

myrcene 1,8-cineole, borneol, camphor and verbinone (Okoh *et al.*, 2010; Santoyo *et al.*, 2005), which possess strong antimicrobial activity by the disruption of bacteria membrane integrity (Knobloch *et al.*, 1989). Aguirre *et al.* (2013); Burt (2004) and Pelissari *et al.* (2009) also reported that oregano essential oil had higher antimicrobial activity against the gram positive bacteria (*Staphylococcus aureus*) than gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*). The main constituents of oregano essential oil are thymol, carvacrol, γ-therpinene and *ρ*-cymene (Aguirre *et al.*, 2013; Burt, 2004; Lambert *et al.*, 2001). However, *P. putida* was resistant to carrot seed and parsley essential oils (Teixeira *et al.*, 2013). *E. coli* and *S. typhimurium* were also tolerant to carrot seed, grapefruit, lemon, onion, and parsley essential oils. The greater resistance of gram negative bacteria toward essential oils may be attributed to the complexity of their double layer cell membrane, compared with the single layer membrane of gram positive bacteria (Hogg, 2005).

Antimicrobial activity of *Callistemon comboynensis* essential oil was observed against gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), gram negative (*Proteus vulgaris* and *Pseudomonas aeruginosa*) and a pathogenic fungus *Candida albicans*. This might be associated with the high content of oxygenated constituents (Abdelhady and Aly, 2012). Essential oil of *Callistemon comboynensis* leave consisted of 1,8-cineole (53.03%), eugenol (12.1%), methyl eugenol (8.3%), α-terpineol (4.3%) and carveol (3.4%) (Abdelhady and Aly, 2012). Teixeira *et al.* (2013) found that the highest reduction (8.0 log CFU mL^{-1}) was obtained when coriander, origanum and rosemary essential oils at a level of 20 μL was used to inhibit *L. innocua*. Thyme essential oil (20 μL) was able to inhibit both *L. innocua* and *L. monocytogenes*. However, rosemary essential oil exhibited the highest MIC (90.8 mg mL^{-1}) against *B. thermosphacta* and *S. typhimurium.* Thus, essential oils from the selected plants can be used as antimicrobial agents for food applications as well as other purposes, however their activity depends on types of essential oil used.

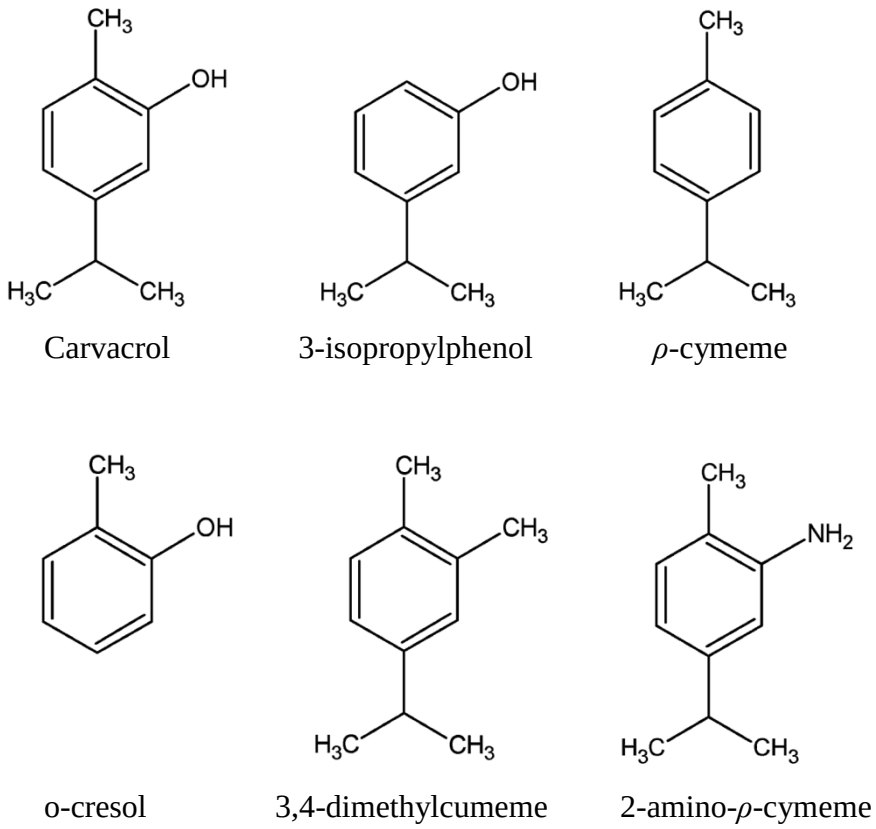


Figure 5. Structure of carvacrol and carvacrol-related compounds.
Source: Veldhuizen *et al.* (2006).

1.2.7.2.2 Antioxidant activity

Several compounds in essential oils have the structure mimicing the well known plant phenols with antioxidant activity. Among the major compounds available in the oil, thymol and carvacrol were reported to possess the highest antioxidant activity (Dapkevicius *et al.*, 1998). Essential oils have several modes of actions as antioxidant, such as prevention of chain initiation, free radical scavengers, reducing agents, termination of peroxides, prevention of continued hydrogen abstraction as well as quenchers of singlet oxygen formation and binding of transition metal ion catalysts (Mao *et al.*, 2006; Yildirim *et al.*, 2000). Phenolics are organic compounds consisting of hydroxyl group (-OH) attached directly to a carbon atom that is a part of aromatic ring. The hydrogen atom of hydroxyl group can be donated to free radicals, thereby preventing other compounds to be oxidized (Nguyen *et al.*, 2003). Teixeira *et al.* (2013) reported that the highest scavenging activity of DPPH

radical was observed for clove and origanum essential oils with the EC_{50} values of 35.7 ± 1.2 μg/ml^{-1} and 46.8 ± 0.4 μg/ml^{-1}, respectively. Clove and origanum essential oils also showed the high ferric reducing power (Teixeira *et al.*, 2013). The antioxidant capability of phenolic compounds is mainly due to their redox properties, which permit them to act as hydrogen donors, reducing agents, singlet oxygen quenchers as well as metal chelators (Kumar *et al.*, 2005). The antioxidant activity is generally related with the major active compounds in essential oils such as eugenol in clove (Wei and Shibamoto, 2010), carvacrol in origanum (Bounatirou *et al.*, 2007), *m*-thymol in thyme (Bozin *et al.*, 2006) and β-citronellol or β-citronellal in citronella (Ruberto and Baratta, 2000). However, the other antioxidant compounds in essential oils such as terpinene, (−)-camphor, (−)-bornylacetate, eucalyptol, and methylchavicol have been reported to exhibit antioxidant activity, but their amounts were probably too low to exhibit antioxidant activity (Mitić-Ćulafić *et al.*, 2009; Ruberto and Baratta, 2000; Teixeira *et al.*, 2013). Antioxidant activity varies with source of essential oils. The differences in antioxidative activity of different essential oils were mostly due to the differences in types and amounts of antioxidative components presented in essential oils (Burt, 2004; Kordali *et al.*, 2005). Antioxidative activity of essential oil is also affected by extraction method or solvents used. Sarikurkcu *et al.* (2010) reported that free radical scavenging activity (DPPH assay) and reducing power of essential oil from *T. longicaulis* subsp. *longicaulis* var. *longicaulis* extracted using hydrodistillation method was lower than those extracted using methanol or water. Methanol extract of *Salvia tomentosa* exhibited superior radical scavenging activity to other extracts (IC_{50} =18.7l μg/ml) (Tepe *et al.*, 2005). Non-polar extracts showed less effective activities than polar extracts. Therefore, antioxidative activity of essential oil is strictly related with the polarities of their phytochemicals. Additionally, the harvesting period of plant also determines the concentration of the major oil components such as phenolic compounds, which are directly related with the antioxidant activity of essential oils (Malatova *et al.*, 2011; Wu *et al.*, 2013; Zheljazkov *et al.*, 2012). The antioxidant activity of essential oil from *T. longicaulis* subsp. *longicaulis var. longicaulis* extracted by hydrodistillation method at 2.0 mg/ml showed similar antioxidative activity to synthetic antioxidants BHT and BHA when tested by *β*-carotene-linoleic acid model system and was higher

than those extracted with other solvents (Sarikurkcu *et al.*, 2010). In contrast, the inhibition of linoleic acid oxidation of model system by essential oil of *Salvia tomentosa* (Miller) was lower than those extracted using solvents with different polarities and BHT (Tepe *et al.*, 2005). Abdelhady and Aly (2012) reported that *Callistemon comboynensis* essential oil exhibited the antioxidant activity at a concentration of 1000 µg/ml (91.1 ± 0.3% inhibition), comparable to 100 µg/ml gallic acid (95.7 ± 2% inhibition). Non-phenolic antioxidants of plant extracts might also contribute to the antioxidant activity (Hassimotto *et al.*, 2005; Newman *et al.*, 2002).

1.2.7.3 Active packaging containing essential oils and applications

1.2.7.3.1 Development of active packaging

Nowadays, smart packaging has gained increasing attention, e.g. antimicrobial packaging, which can be applied to extend the shelf-life of food and products (Appendini and Hotchkiss, 2002; Quintavalla and Vicini, 2002). To enhance the property of those packaging, the extracts with selected bioactivity are incorporated. Thus, several approaches have been introduced, not only for increasing bioactivity but also modifying the property of biomaterials used for packaging. Among biomaterials, proteins have gained attention, due to their variety in compositions, properties, as well as nutritive value. However, protein based material for packaging is still encountering the poor property, especially poor barrier property toward water vapor. Chemical and enzyme treatment can be applied to modify polymer network through the cross-linking of the polymer chains to improve the properties of protein film (De Carvalho and Grosso, 2004; Mahmoud and Savello, 1993; Yildirim and Hettiarachchy, 1997). Hydrophobic plasticizer can be used to improve water vapor barrier property of films. However, it may yield films with different properties. The incorporation of hydrophobic substances such as lipid, fatty acid, wax, etc has been implemented to improve water vapor barrier property (Limpisophon *et al.*, 2010; Prodpran *et al.*, 2007; Soazo *et al.*, 2011). Hydrophobic materials such as essential oils has been incorporated to improve water vapor barrier property of protein based films, e.g. film from fish muscle protein, film from fish gelatin, etc (Atarés *et al.*, 2010b). Monoterpenes are highly hydrophobic substances

found in essential oils, in which the content varied with types of essential oils (Turina *et al.*, 2006). Hydrophobic essential oil could increase the hydrophobicity of films, thereby reducing the water vapor migration through the film. Atarés *et al.* (2010b) studied the mechanical properties of soy protein isolate incorporated with cinnamon and ginger essential oil at different concentrations (protein to oil mass ratios: 1:0.025, 1:0.050, 1:0.075 and 1:0.100). A slight decreasing trend of elastic modulus (EM) was observed as the oil content increased. The water vapor permeability was slightly reduced by both essential oils. The oil type significantly affected both tensile strength (resistance to elongation) and EM (capacity for stretching) (Atarés *et al.*, 2010b). Essential oils may cause some degree of rearrangement in the protein network, thus strengthening and increasing the film resistance to elongation. Moreover, Pires *et al.* (2011) studied the effect of thyme essential oil incorporated in hake protein film. The addition of thyme oil significantly reduced the water vapor permeability. Nevertheless, the addition of essential oil had impact on the transparency of film, depending on type and concentration of essential oils. The addition of thyme oil decreased the transparency value of hake proteins films (Pires *et al.*, 2011). Table 6 presents the properties of protein based films containing various essential oils.

The ability of plant essential oils to protect foods against pathogenic and spoilage microorganisms have been reported by several researchers (Friedman, 2006; Lis-Balchin *et al.*, 1998; Rojas-Graü *et al.*, 2007). Film or packaging incorporated with essential oils can be employed as active packaging due to their antimicrobial or antioxidant activities. Seydim and Sarikus (2006) evaluated antimicrobial activity of whey protein isolate-based edible films incorporated with oregano essential oil. Oregano essential oil added films exhibited the larger inhibitory zone on *S. aureus* with increasing levels of essential oil added. Gómez-Estaca *et al.* (2010) reported that clove essential oil presented the highest antimicrobial activity against both pathogenic and spoilage bacteria, which was maintained the activity when it was incorporated in bovine-hide gelatin and gelatin-chitosan blend films. Salgado *et al.* (2013) also reported that the sunflower protein film incorporated with clove essential oil exhibited the inhibitory effect with varying degree against selected 26 strains of microorganisms (probiotics, pathogens and spoilage bacteria), which was probably corresponded to the presence of eugenol as active compound in clove

essential oil. The highest antimicrobial activity of sunflower protein film containing clove essential oil against gram-positive and gram-negative bacteria was observed for *Photobacterium phosphoreum* and *Brochothrix thermophacta*, respectively, whereas the largest antimicrobial activity against yeast and molds was found in *Debaryomyces hansenii* and *Aspergillus niger,* respectively. Moreover, oregano and thyme essential oil containing soy protein edible films had the similar antibacterial activity against all test microorganisms. The higher antibacterial activities of films added both essential oils were corresponded against *Staphylococcus aureus*, *Escherichia coli* and *Escherichia coli* O157:H7, compared with *Pseudomanas aeruginosa* and *Lactobacillus plantarum* (Emiroğlu *et al.*, 2010). Wu *et al.* (2014) reported that oregano essential oil had larger inhibitory effect than that of cinnamon essential oil except the tested microorganism *Salmonella enteritidis,* whereas anise essential oil exhibited no inhibitory effect to all tested microorganisms. Thus, oregano essential oil had the highest potential to prepare antimicrobial film. The inhibition effect of gelatin-chitosan films containing oregano essential oil against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) was greater than that of gram-negative bacteria (*Escherichia coli*, *Salmonella enteritidis* and *Shigabacillus*). Jouki *et al.* (2014) also reported the similar results that oregano essential oil containing quince seed mucilage films exhibited more effective against gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) than gram-negative bacteria (*Salmonella typhimurium* and *Pseudomonas aeruginosa*).

Films added with essential oils are shown to possess antioxidant activities, which can vary with type and amount of essential oil incorporated. Gómez-Estaca *et al.* (2009a) reported that bovine-hide and tuna skin gelatin films supplemented with oregano and rosemary extracts exhibited the reducing ability and free radical-scavenging capacity. Antioxidant power was generally being proportional to the amount of extract added. Teixeira *et al*. (2014) reported that the addition of essential oils significantly yielded the fish protein films with higher antioxidative activity than those without essential oil incorporated. In general, antioxidant activity of fish protein was due to the free sulfhydryl groups and peptides containing particular amino acids such as such as tryptophan, methionine and tyrosine (Faraji *et al.*, 2004). DPPH radical-scavenging activity of films incorporated with clove and

garlic essential oils was the highest, followed by film added with origanum essential oil. For the reducing power, film containing with garlic essential oil showed the highest activity, followed by film added with clove and origanum essential oils, respectively (Teixeira *et al.*, 2014). The different compounds with antioxidant activity as well as their amounts in essential oils composition exhibit varying antioxidant activity in films containing essential oils (Burt, 2004). Kavoosi *et al.* (2014) also reported that the incorporation of *Zataria multiflora* essential oil could enhance the antioxidant activity of gelatin film via ABTS radical scavenging assey, especially with increasing concentrations, whereas gelatin film exhibited slightly activity. Gelatin possesses the antioxidant activity due to the peptides containing particular amino acids such as glycine and proline (Kim *et al.*, 2001). Moreover, Salgado *et al.* (2013) evaluated the antioxidant activities of sunflower protein film incorporated with clove essential oil. In general, sunflower protein film without clove essential oil incorporated exhibited antioxidant capacity, which was due to the natural phenolic compounds of sunflower. The increased in both the radical scavenging and reducing capacities in sunflower protein film were observed when clove essential oil was incorporated, however those activies were due to phenolic compounds contain in both sunflower and clove essential oil. The major phenolic compounds which possessed the antioxidant activity in clove oil were chlorogenic acid, caffeic acid and eugenol (Salgado *et al.*, 2013). Therefore, the antioxidant of protein based films incorporated with essential oils governed by types of proteins and essential oils as well as active compounds or their amount containing in essential oils. Antioxidant activities of protein based films containing various essential oils are shown in Table 7.

Table 6. Properties of protein based films containing various types of essential oils.

Protein type, Conc.	Plasticizer conc.	Essential oils, Conc.	Thickness (mm)	Mechanical properties		WVP ($x10^{-10}$g m^{-1} s^{-1} Pa^{-1})	Transparency (%)	References
				TS (MPa)	EAB (%)			
Hake muscle protein, 1.5% (w/w) of FFS	Glycerol, 59% (w/w) of protein	Thyme (*Thymus vulgaris* L.), 0.025, 0.05, 0.1 and 0.25 ml oil/g protein	0.022 - 0.025	4.13 - 6.67, 3.30 - 8.49 N (Breaking force)	111.2 - 129.8, 87.87 - 115.41 (Puncture deformation)	0.35 - 0.43	1.8 - 6.5	Pires *et al.* (2011)
Soy protein isolate, 8% (w/w) of FFS	Glycerol, 30% (w/w) of protein	Cinnamon *(Cinnamomum verum)*, 0.025, 0.05, 0.075 and 0.1 ml oil/g protein	-	11.0 - 17.6	3.4 - 7.5	0.46 - 0.64 [a]	-	Atarés *et al.* (2010b)
		Ginger *(Zingiber officinale)*, 0.025, 0.05, 0.075 and 0.1 ml oil/g protein	-	4 - 8	1.7 - 3	0.56 - 0.68 [a]	-	
Sodium caseinate, 8% (w/w) of FFS	Glycerol, 30% (w/w) of protein	Cinnamon *(Cinnamomum verum)*, 0.025 and 0.075 ml oil/g protein	-	22 and 24 [b] 10.2 and 11.4 [c]	13 and 22 [b] 67 and 76 [c]	0.64 and 0.57 [d] 2.14 and 1.7 [e]	-	Atarés *et al.* (2010a)
		Ginger *(Zingiber officinale)*, 0.025 and 0.075 ml oil/g protein	-	22 and 22 [b] 10 and 11.6 [c]	18 and 16 [b] 57 and 72 [c]	0.57 and 0.52 [d] 2.1 and 1.8 [e]	-	

Table 6. Properties of protein based films containing various types of essential oils (cont.).

Protein type, Conc.	Plasticizer conc.	Essential oils, Conc.	Thickness (mm)	Mechanical properties		WVP ($x10^{-10}$g m^{-1} s^{-1} Pa^{-1})	Transparency (%)	References
				TS (MPa)	EAB (%)			
Chicken feet protein, 5% (w/v) of FFS	Glycerol-sorbitol (3:2, w/w), 40% (w/w) of protein	Marjoram (*Origanum majorana*), 0.01 g oil/g FFS	-	7.59 ± 0.62	11.92 ± 1.44	2.59 ± 0.11 †	-	Lee *et al.* (2015)
		Coriander (*Coriandrum sativum*), 0.01 g oil/g FFS	-	10.00 ± 1.06	6.43 ± 1.37	2.71 ± 0.22	-	
		Clove (*Syzygium aromaticum*) ,0.01 g oil/g FFS	-	6.49 ± 0.72	20.91 ± 1.75	2.93 ± 0.15	-	
Sunflower protein concentrate, 5% (w/v) of FFS	Glycerol, 1.5% (w/v) of FFS	Clove (*Syzygium aromaticum*) 0.75 mL/g sunflower protein	0.080 ± 0.01	2.5 ± 0.2	24.9 ± 1.7	1.16 ± 0.09 aa	-	Salgado *et al.* (2013)
Silver carp skin gelatin-chitosan (3:1, w/w), 4% (w/v) of FFS	Glycerol, 25% (w/w) of gelatin plus chitosan	Oregano (*Origanum vulgare*), 1%, 2%, 3% and 4% (v/v) of FFS	0.027 - 0.029	30.69 - 36.78	37.36 - 52.00	9.58 - 10.00 ††	-	Wu *et al.* (2014)

Table 6. Properties of protein based films containing various types of essential oils (cont.).

Protein type, Conc.	Plasticizer conc.	Essential oils, Conc.	Thickness (mm)	Mechanical properties		WVP ($x10^{-10}$g m^{-1} s^{-1} Pa^{-1})	Transparency (%)	References
				TS (MPa)	EAB (%)			
Cold water fish skin gelatin-chitosan (1:0.75, w/w), 1% (w/v) of FFS	Glycerol, 30% (w/w) of gelatin plus chitosan	Oregano (*Origanum vulgare*), 0.4, 0.8 and 1.2% (w/v) of FFS	-	18.49 - 38.82	35.31 - 41.25	0.531 - 0.760 [a]	1.72 – 2.14	Hosseini *et al.* (2015)
Hake protein, 1.5% (w/v) of FFS	Glycerol, 59% (w/w) of protein	Garlic (*Allium sativum* L.)	0.012 ± 0.007	6.6 ± 2.7	53.3 ± 21.1	4.3 ± 1.0 [††]	22.9 ± 2.3	Teixeira *et al.* (2014)
		Clove (*Syzygium aromaticum* L.)	0.012 ± 0.004	7.3 ± 2.3	55.7 ± 31.7	3.8 ± 0.9	7.0 ± 3.3	
		Origanum (*Thymus capitatus*)	0.017 ± 0.006	6.4 ± 4.0	83.2 ± 50.3	7.8 ± 0.8	2.3 ± 0.2	
Bovine gelatin, 10% (w/v) of FFS	Glycerol, 25% (w/w) of gelatin	Zataria (*Zataria multiflora*), 2, 4, 6 and 8% (w/w) of FFS	-	2.7 - 3.7	140 - 172	0.23 - 0.31 [a]	-	Kavoosi *et al.* (2014)

[*] FFS= Film forming solution; WVP= Water vapor permeability; [a] WVP unit (g mm/m^2 h kPa); [aa] WVP unit (10^{10} g H_2O/Pa.m.s); [†] WVP unit (10^{-9} g m/m^2 s Pa); [††] WVP unit (10^{-11} g/(m Pa s)); [b,c] Final moisture content in the film: 5 and 10 g water/100 g film, respectively; [d,e] WVP of films tested at 25 °C and two range of relative humidity (RH) (33-53% and 53-75, respectively).

Table 7. Antioxidative effect of protein based films containing various types of essential oils.

Film forming materials, Conc.	Plasticizer, Conc.	Essential oils, Conc	Antioxidant activity						References
			DPPH	ABTS	Reducing power	FRAP	PCL		
							PCL-ACW	PCL-ACL	
Hake protein, 1.5% (w/v) of FFS	Glycerol, 59% (w/w) of protein	Thyme (*Thymus vulgaris* L.), 0.025, 0.05, 0.1 and 0.25 ml oil/g protein	18.5 - 40.1 [a]	-	17.5 - 25.4 [b]	-	-	-	Pires *et al.* (2011)
Sunflower protein concentrate, 5% (w/v) of FFS	1.5% (w/v) of FFS	Clove (*Syzygium aromaticum* L.) , 0.75 ml oil/g SPC	-	1194.1 ± 77.0 [c]	-	5733.3 ± 92.5 [d]	229.0 ± 6.6 [e]	1767.0 ± 11.8 [f]	Salgado *et al.* (2013)
Hake protein, 1.5% (w/v) of FFS	Glycerol, 59% (w/w) of protein	Garlic (*Allium sativum* L.)	72.00 ± 0.65 [a]	8.29 ± 0.46 [c]	-	-	-	-	Teixeira et al. (2014)
		Clove (*Syzygium aromaticum* L.)	72.00 ± 1.43	4.11 ± 0.62	-	-	-	-	
		Origanum (*Thymus capitatus*)	35.15 ± 2.35	1.63 ± 0.23	-	-	-	-	
Bovine gelatin, 10% (w/v) of FFS	Glycerol, 25% (w/w) of gelatin	Zataria (*Zataria multiflora*), 2, 4, 6 and 8% (w/w) of FFS	-	2.6 - 5.5 [c]	-	-	-	-	Kavoosi *et al.* (2014)

* FFS = Film forming solution; DPPH = 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity; ABTS = 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) radical scavenging activity, FRAP = *Ferric reducing antioxidant power*; PCL-ACW = *Photochemiluminescence*-antiradical *capacity of water soluble substances*; PCL-ACL = *Photochemiluminescence*-antiradical *capacity of* lipid-soluble substances

[a] DPPH unit (%); [b] Reducing power unit (mg Ascorbic acid/g film); [c] ABTS unit (mg Ascorbic acid/g film); [d] FRAP unit (mmol $FeSO_4.7H_2O$ equivalents/g film); [e] PCL-ACW unit (µmol Ascorbic acid/g film); [f] PCL-ACL unit (µmol Trolox/g film). 35.15 ± 2.35 [a]

However, film or packaging may have the smell of essential oils due to its volatilization. The smell intensity of essential oil in films increased with increasing essential oil levels. This might limit the application of the film in food when it was incorporated at the high amount.

1.2.7.3.2 Use of packaging for meat and fish products

Active film containing essential oil can be applied to extend the shelf-life and maintain the quality of foods, such as meat, fish and their products. Films can serve as carriers for various antimicrobial agent and antioxidant that can maintain fresh quality, extend product shelf life and reduce the risk of pathogen growth. The intrinsic properties of the food (fat, protein, pH, etc.), as well as the environment in which the food is maintained (storage temperature, packaging, etc.), may influence the prevention effect of essential oils (Burt, 2004; Tassou *et al.*, 1995). Low pH and storage temperature, decrease O_2 concentrations and high salt content enhances the antimicrobial effect of essential oils, while high levels of protein and fat and low water activity seem to protect bacteria from the inhibition by essential oils (Gómez-Estaca *et al.*, 2010). However, soy protein film with oregano, thyme and mixture of those did not have significant effects on total viable counts, lactic acid bacteria and *Staphylococcus* spp. when applied on ground beef patties. Nevertheless, the reduction in coliform and *Pseudomonas* spp. counts was observed. Table 8 presents the antimicrobial effect of active films containing various essential oils in food systems. The effectiveness of bioactive films containing essential oils against the spoilage or pathogenic bacteria in food system has been studied. Zinoviadou *et al.* (2009) studied the antibacterial effects of WPI film containing oregano oil (0.5% and 1.5% w/w of FFS) against total variable bacteria, *Pseudomonas* spp. and lactic acid bacteria on beef cuts. The use of films containing the highest level of oregano oil (1.5% w/w of FFS) resulted in a significant reduction of total variable bacteria count and *Pseudomonas* spp. population during 12 days of refrigeration storage (5 °C). The total variable bacteria population of the samples wrapped with films containing the high essential oil content at day 8 was 5.1 log CFU/cm^2, while the control had population of 8.4 log CFU/cm^2. Since microbial loads higher than 10^7 CFU/cm^2 are usually associated with off-odors (Ercolini *et al.*, 2006), it may be suggested that the use of WPI films

containing 1.5% (w/w) oregano oil could double the shelf-life of fresh beef stored under refrigerated condition. Oussalah *et al.* (2004) reported the application of milk protein films incorporated with essential oils (oregano, pimento and mixed) on meat surfaces containing 10^3 CFU/cm^2 of *Escherichia coli* O157:H7 and *Pseudomonas* spp. Film containing oregano was the most effective in inhibition both bacteria, whereas film with pimento oils seemed to be the least effective against these two bacteria. The reduction of around 1 log unit of *Escherichia coli* O157:H7 and *Pseudomonas* spp. was observed at the end of storage (day 7, at 4 °C) when film containing oregano extracts was used, compared to samples without film coated. Ouattara *et al.* (2000) reported that chitosan film incorporated with cinnamaldehyde reduced the growth of *Lactobacillus sakei, Serratia liquefaciens* and *Enterobacteriaceae*, on the surface of meat products (bologna, cooked ham and pastrami). However, the films had no effect or little effect on the numbers of lactic acid bacteria on bologna or pastrami, after 21 days of storage at 4 or 10 °C. Zivanovic *et al.* (2005) tested the impact of chitosan film containing oregano essential oil (1 and 2% of FFS) on microbial growth of the inoculated bologna samples and stored for 5 days at 10 °C. The higher activity was obtained in films with 1% and 2% oregano, which decreased the numbers of *Listeria monocytogenes* by 3.6 to 4 logs and *Escherichia coli* O157:H7 by 3 logs, whereas the pure chitosan films reduced *Listeria monocytogenes* by 2 logs.

Essential oils are able to extend shelf-life of foods by lowering lipid oxidation (Oussalah *et al.*, 2004; Zivanovic *et al.*, 2005). Therefore the incorporation of essential oils into the biodegradable films could provide antioxidant activity for resulting films. Oussalah *et al.* (2004) reported that the incorporation of oregano essential oil into milk protein based film increased the ability to stabilize lipid oxidation in beef muscle samples during refrigerated storage. Moradi *et al.* (2011) studied the antioxidant effects of chitosan film containing *Zataria multiflora* Boiss essential oil (ZEO) wrapped on mortadella sausage during 21 days of refrigeration storage (4 °C). Lipid oxidation of samples decreased markedly within the first 6 days when compared to samples wrapped with control film (without ZEO incorporated) and unwrapped samples up to the end of storage. The highest effectiveness was observed when samples were packed with film containing 10 g/kg ZEO in combination with 10 g/kg grape seed extract.

Fish and fish products are perishable and also prone to chemical deterioration, especially caused by lipid oxidation. Essential oils possessing both antimicrobial and antioxidant activities, can be used to maintain the quality of those products. Gómez-Estaca *et al.* (2010) reported that the complex gelatin-chitosan film incorporated with clove essential oil was applied to cover orwrap fish during chilled storage. The growth of gram-negative bacteria, especially enterobacteria was retarded, and corresponded with the delay in TVB production. Lactic acid bacteria remained practically constant during 11 days of storage. H_2S-producing bacteria were also inhibited since their growth was interrupted with the application of the film. This microbial inhibition could be attributed to the hydrophobic nature of essential oil, which enable them and their components to partition in the lipids of the bacteria cell membrane and mitochondria while disturbing the structures and rendering it more permeable (Sikkema *et al.,* 1995). Gomez-Estaca *et al*. (2007) tested the antibacterial effects of gelatin-based films added with an extract of oregano or rosemary against microbial spoilage in preserving cold-smoked sardine. Coating the fish with films enriched with oregano or rosemary extract lowered the microbial growth by 1.99 and 1.54 log cycles, respectively, on day 16.

Films containing essential oil can be used to retard lipid oxidation in fish and fish products. Salgado *et al.* (2013) tested the antioxidant activity of sunflower protein films enriched with clove essential oil in preserving fish patties 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 exerted by the clove essential oil components. This allowed TBARS remaining at the lowest values during storage. Use of natural plant extracts to prevent lipid oxidation in fish has been reported (Giménez *et al.*, 2004; Serdaroglu and Felekoglu, 2005). Gomez-Estaca *et al.* (2007) developed gelatin based film enriched with oregano or rosemary essential oils to prevent lipid oxidation in cold-smoked sardine during 20 days of storage at 5 °C. Coating the muscle with the films enriched with both essential oils, particularly oregano oil, lowered the lipid oxidation rate (as measured by the peroxide and TBARS indices) of the muscle. Therefore, the edible films with the added plant extracts could lower lipid oxidation levels in food systems.

Table 8. Antimicrobial effect of active films containing various essential oils in food systems.

Film forming materials	Essential oils, Conc	Food samples	Tested organisms	References
Chitosan	Cinnamaldehyde, 1% (w/w) of FFS	Bologna, Regular cooked ham, Pastrami	*Enterobacteriaceae*, *Lactobacillus sakei*, *Serratia liquefaciens*, Lactic acid bacteria	Ouattara *et al.* (2000)
Milk protein	Oregano (OR), Pimento (PI), Mixture (OR+PI, 1:1), 1% (w/v) of FFS	Whole beef muscle	*Escherichia coli* O157:H7, *Pseudomonas* spp.	Oussalah *et al.* (2004)
Chitosan	Oregano, 1 and 2% of FFS	Bologna slices	*Listeria monocytogenes*, *Escherichia coli* O157:H7	Zivanovic *et al.* (2005)
Pigskin gelatin	Oregano, Rosemary, 1.25% and 20% of FFS, respectively	Cold-smoked sardine	Total viable bacteria, H_2S-producing microorganisms	Gomez-Estaca *et al.* (2007)
Whey protein isolate	Oregano, 1.5% (w/w) of FFS	Fresh beef	Total viable bacteria, *Pseudomonas* ssp., Lactic acid bacteria	Zinoviadou *et al.* (2009)

Table 8. Antimicrobial effect of active films containing various essential oils in food systems (cont.).

Film forming materials	Essential oils, Conc	Food samples	Tested organisms	References
Soy protein	Oregano (OR), Thyme (TH), Mixture (OR+TH, 1:1), 5% (v/v) of FFS	Fresh ground beef patties	*Pseudomanas* spp., *Staphylococcus* spp. Coliform	Emiroğlu *et al.* (2010)
Bovine-hide gelatin-Chitosan	Clove, 0.75 ml/g biopolymer	Cod fillets	Total viable bacteria, H_2S-producing microorganisms, Lactic acid bacteria, *Pseudomonas* ssp., *Enterobacteriaceae*	Gómez-Estaca *et al.* (2010)
Sunflower protein concentrate	Clove, 0.75 ml/g biopolymer	Sardine patties	Total viable bacteria, Total mesophiles, H_2S-producing microorganisms, Luminescent colonies, Lactic bacteria, *Pseudomonas* spp. Enterobacteriaceae	Salgado *et al.* (2013)
Silver carp skin gelatin-chitosan	Oregano (*Origanum vulgare*), 1%, 2%, 3% and 4% (v/v) of FFS	Grass carp muscle	Total aerobic plate count	Wu *et al.* (2014)

1.3 Objectives

1.3.1 To elucidate the impact of different citrus essential oils and levels of glycerol on properties of fish skin gelatin film.

1.3.2 To investigate the impact of different root essential oils at different concentrations on properties and antioxidative activity of fish skin gelatin film.

1.3.3 To study the effect of various surfactants on the properties of films added with leaf essential oils.

1.3.4 To study the effect of palm oil concentrations on properties of fish skin gelatin film.

1.3.5 To investigate the impact of different oils and surfactants on the mechanical and thermal properties as well as heat seal ability of fish skin gelatin film.

1.3.6 To investigate moisture migration and oxidative stabilities of dried chicken powder and chicken skin oil packaged in pouches from gelatin films incorporated without and with essential oil or palm oil or mixed oils during storage.

1.4 References

Abdelhady, M.I. and Aly, H.A.H. 2012. Antioxidant antimicrobial activities of *callistemon comboynensis* essential oil. Free Radical. Antioxidants. 2: 37-41.

Abdorreza, M.N., Cheng, L.H. and Karim, A.A. 2011. Effects of plasticizers on thermal properties and heat sealability of sago starch films. Food Hydrocolloid. 25: 56-60.

Abdorreza, M.N., Nassiri, R., Sheibani, S., Ariffin, F. and Karim, A.A. 2013. Preparation and characterization of bionanocomposite films filled with nanorod-rich zinc oxide. Carbohyd. Polym. 96: 233–239.

Abu Diak, O., Bani-Jaber, A., Amro, B., Jones, D. and Andrews, G.P. 2007. The manufacture and characterization of casein films as novel tablet coatings. Food Bioprod. Process. 85: 284-290.

Adebiyi, A.P., Adebiyi, A.O., Jin, D.-H., Ogawa, T. and Muramoto, K. 2008. Rice bran protein-based edible films. Int. J. Food Sci.Tech. 43: 476-483.

Aguirre, A., Borneo, R. and León, A.E. 2013. Antimicrobial, mechanical and barrier properties of triticale protein films incorporated with oregano essential oil. Food Biosci. 1: 2-9.

Alves, P.M.A., Carvalho, R.A., Moraes, I.C.F., Luciano, C.G., Bittante, A.M.Q.B. and Sobral, P.J.A. 2011a. Development of films based on blends of gelatin and poly(vinyl alcohol) cross linked with glutaraldehyde. Food Hydrocolloid. 25: 1751-1757.

Alves, V.D., Castelló, R., Ferreira, A.R., Costa, N., Fonseca, I.M. and Coelhoso, I.M. 2011b. Barrier properties of carrageenan/pectin biodegradable composite films. Procedia Food Sci. 1: 240-245.

Amarante, C. and Banks, N.H. 2001. Post harvest physiology and quality of coated fruits and vegetables. Hortic. Rev. 26: 161-238.

Andreuccetti, C., Carvalho, R. and Grosso, C.R.F. 2009. Effect of hydrophobic plasticizers on functional properties of gelatin-based films. Food Res. Int. 42: 1113-1121.

Andreuccetti, C., Carvalho, R.A., Galicia-García, T., Martínez-Bustos, F. and Grosso, C.R.F. 2011. Effect of surfactants on the functional properties of gelatin-based edible films. J. Food Eng. 103: 129-136.

Anker, M., Berntsen, J., Hermansson, A.-M. and Stading, M. 2002. Improved water vapor barrier of whey protein films by addition of an acetylated monoglyceride. Innov. Food Sci Emerg. 3: 81-92.

Anonymous. 1992. Comprehensive dictionary of physical chemistry. Ellis Horwood. Chichester, UK.

Appendini, P. and Hotchkiss, J.H. 2002. Review of antimicrobial food packaging. Innov. Food Sci. Emerg. 3: 113-126.

Arrebola, M.L., Navarro, M.C., Jimenez, J. and Ocana, F.A. 1994. Yield and composition of the essential oil of *Thymus serpylloides* subsp. *serpylloides*. Phytochemistry. 36: 67-72.

Artharn, A., Benjakul, S., Prodpran, T. and Tanaka, M. 2007. Properties of a protein-based film from round scad (*Decapterus maruadsi*) as affected by muscle types and washing. Food Chem. 103: 867-874.

Artharn, A., Benjakul, S. and Prodpran, T. 2008. The effect of myofibrillar/sarcoplasmic protein ratios on the properties of round scad muscle protein based film. Eur. Food Res.Technol. 227: 215-222.

Arvanitoyannis, I., Nakayama, A. and Aiba, S.-i. 1998. Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water. Carbohyd. Polym. 36: 105-119.

Arvanitoyannis, I.S. 2002. Formation and properties of collagen and gelatin films and coatings. *In* Protein-based films and coatings. (Gennadios, A., ed.). p. 275-304. CRC Press. Boca Raton.

Atarés, L., Bonilla, J. and Chiralt, A. 2010a. Characterization of sodium caseinate-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 100: 678-687.

Atarés, L., De Jesús, C., Talens, P. and Chiralt, A. 2010b. Characterization of SPI-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 99: 384-391.

Avena-Bustillos, R.J., Olsen, C.W., Olson, D.A., Chiou, B., Yee, E., Bechtel, P.J. and McHugh, T.H. 2006. Water vapor permeability of mammalian and fish gelatin films. J. Food Sci. 71: 202-207.

Ayranci, E. and Tunc, S. 2003. A method for the measurement of the oxygen permeability and the development of edible films to reduce the rate of oxidative reactions in fresh foods. Food Chem. 80: 423-431.

Bae, H.J., Darby, D.O., Kimmel, R.M., Park, H.J. and Whiteside, W.S. 2009. Effects of transglutaminase-induced cross-linking on properties of fish gelatin-nanoclay composite film. Food Chem. 114: 180-189.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils - A review. Food Chem. Toxicol. 46: 446-475.

Baldwin, E.A., Nispero-Carriedo, M. and Baker, R.A. 1995. Edible coatings for lightly processed fruits and vegetables. Hortic. Sci. 30: 35-38.

Baldwin, E.A., Nispero-Carriedo, M.O., Hagenmaier, R.D. and Baker, R.A. 1997. Use of lipids in coatings for food products. Food Technol. 51: 56-64.

Banejee, R., Chen, H. and Wu, J. 1996. Milk protein-based edible film mechanical strength changes due to ultrasound process. J. Food Sci. 61: 824-828.

Bao, S., Xu, S. and Wang, Z. 2009. Antioxidant activity and properties of gelatin films incorporated with tea polyphenol-loaded chitosan nanoparticles. J. Sci. Food Agr. 89: 2692-2700.

Baziwane, D. and He, Q. 2003. Gelatin: the paramount food additive. Food Rev. Int. 9: 423-435.

Benavides, S., Villalobos-Carvajal, R. and Reyes, J.E. 2012. Physical, mechanical and antibacterial properties of alginate film: Effect of the crosslinking degree and oregano essential oil concentration. J. Food Eng. 110: 232-239.

Benbettaïeb, N., Kurek, M., Bornaz, S. and Debeaufort, F. 2014. Barrier, structural and mechanical properties of bovine gelatin–chitosan blend films related to biopolymer interactions. J. Sci. Food Agr. 94: 2409-2419.

Benjakul, S., Artharn, A. and Prodpran, T. 2008. Properties of protein-based film from round scad (*Decapterus maruadsi*) muscle as influenced by fish quality. LWT - Food Sci. Technol. 41: 753-763.

Bergo, P. and Sobral, P.J.A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. Food Hydrocolloid. 21: 1285-1289.

Bergo, P., Moraes, I.C.F. and Sobral, P.J.A. 2013. Effects of plasticizer concentration and type on moisture content in gelatinfilms. Food Hydrocolloid. 32: 412-415.

Bertan, L.C., Tanada-Palmu, P.S., Siani, A.C. and Grosso, C.R.F. 2005. Effect of fatty acids and 'Brazilian elemi' on composite films based on gelatin. Food Hydrocolloid. 19: 73-82.

Bezić, N., Skočibušić, M. and Dunkić, V. 2005. Phytochemical composition and antimicrobial activity of *Satureja montana* L. and *Satureja cuneifolia*Ten. essential oils. Acta Bot. Croat. 64: 313-322.

Białopiotrowicz, T. and Jańczuk, B. 2001. Wettability and surface free energy of bovine serum albumin films. J. Surfactants Deterg. 4: 287-292.

Bigi, A., Cojazzi, G., Panzavolta, S., Rubini, K. and Roveri, N. 2001. Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking. Biomaterials. 22: 763-768.

Bigi, A., Cojazzi, G., Panzavolta, S., Roveri, N. and Rubini, K. 2002. Stabilization of gelatin films by crosslinking with genipin. Biomaterials. 23: 4827-4832.

Bigi, A., Panzavolta, S. and Rubini, K. 2004. Relationship between triple-helix content and mechanical properties of gelatin films. Biomaterials. 25: 5675-5680.

Bosquez-Molina, E., Guerrero-Legarreta, I. and Vernon-Carter, E.J. 2003. Moisture barrier properties and morphology of mesquite gum-candelilla wax based edible emulsion coatings. Food Res. Int. 36: 885-893.

Bounatirou, S., Smiti, S., Miguel, M.G., Faleiro, L., Rejeb, M.N., Neffati, M., Costa, M.M., Figueiredo, A.C., Barroso, J.G. and Pedro, L.G. 2007. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link. Food Chem. 105: 146-155.

Bozin, B., Mimica-Dukic, N., Simin, N. and Anackov, G. 2006. Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. J. Agr. Food Chem. 54: 1822-1828.

Bravin, B., Peressini, D. and Sensidoni, A. 2004. Influence of emulsifier type and content on functional properties of polysaccharide lipid-based edible films. J. Agr. Food Chem. 52: 6448-6455.

Buonocore, G.G., Conte, A., Corbo, M.R., Sinigaglia, M. and Del Nobile, M.A. 2005. Mono- and multilayer active films containing lysozyme as antimicrobial agent. Innov. Food Sci. Emerg. 6: 459-464.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods - A review. Int. J. Food Microbiol. 94: 223-253.

Cao, N., Fu, Y. and He, J. 2007. Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. Food Hydrocolloid. 21: 575-584.

Cao, N., Yang, X. and Fu, Y. 2009. Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films. Food Hydrocolloid. 23: 729-735.

Carvalho, R.A., Sobral, P.J.A., Thomazine, M., Habitante, A.M.Q.B., Gimenez, B., Gomez-Guillen, M.C. and Montero, P. 2008. Development of edible films based on differently processed Atlantic halibut (*Hippoglossus hippoglossus*) skin gelatin. Food Hydrocolloid. 22: 1117-1123.

Cassel, E. and Vargas, R.M.F. 2006. Experiments and modeling of the *Cymbopogon winterianus* essential oil extraction by steam distillation. J. Mex. Chem. Soc. 50: 126-129.

Chambi, H. and Grosso, C. 2006. Edible films produced with gelatin and casein cross-linked with transglutaminase. Food Res. Int. 39: 458-466.

Chandrasekaran, R. and Radha, A. 1995. Molecular architectures and functional properties of gellan gum and related polysaccharides. Trends Food Sci. Tech. 6: 143-148.

Chen, C.-H., Kuo, W.-S. and Lai, L.-S. 2009. Effect of surfactants on water barrier and physical properties of tapioca starch/decolorized hsian-tsao leaf gum films. Food Hydrocolloid. 23: 714-721.

Chen, C.-H., Kuo, W.-S. and Lai, L.-S. 2010. Water barrier and physical properties of starch/decolorized hsian-tsao leaf gum films: impact of surfactant lamination. Food Hydrocolloid. 24: 200-207.

Chen, C., Pearson, A.M. and Gray, J.I. 1992. Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. Food Chem. 43: 177-183.

Chen, J., Liu, C., Chen, Y., Chen, Y. and Chang, P.R. 2008. Structural characterization and properties of starch/konjac glucomannan blend films. Carbohyd. Polym. 74: 946-952.

Chen, Y. and Hagerman, A.E. 2004. Quantitative examination of oxidized polyphenol-protein complexes. J. Agr. Food Chem. 52: 6061-6067.

Chiou, B.-S., Avena-Bustillos, R.J., Bechtel, P.J., Jafri, H., Narayan, R., Imam, S.H., Glenn, G.M. and Orts, W.J. 2008. Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. Eur. Polym. J. 44: 3748-3753.

Cho, S.-W., Ullsten, H., Gällstedt, M. and Hedenqvist, M.S. 2007. Heat-sealing properties of compression-molded wheat gluten films. J. Biobased Mater. Bio. 1: 56-63.

Choa, S.Y., Leeb, S.Y. and Rheec, C. 2010. Edible oxygen barrier bilayer film pouches from corn zein and soy protein isolate for olive oil packaging. LWT - Food Sci. Technol. 43: 1234–1239.

Choi, S.S. and Regenstein, J.M. 2000. Physicochemical and Sensory Characteristics of Fish Gelatin. J Food Sci. 65: 194-199.

Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R. and Wyllie, S.G. 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J. Appl. Microbiol. 88: 170-175.

Cuq, B., Aymard, C., Cuq, J.L. and Guilbert, S. 1995a. Edible packaging films based on fish myofibrillar proteins: formulation and functional properties. J. Food Sci. 60: 1369-1374.

Cuq, B., Gontard, N. and Guilbert, S. 1995b. Edible films and coatings as active layers. *In* Active food packaging. (Rooney, M., ed.). p. 11 11-142. Blackie, Glasgow, UK.

Cuq, B. 1996. Technologie des Proteines. *In* Université Montpellier II Ed. p. 41-84.

Cuq, B., Gontard, N., Cuq, J.L. and Guilbert, S. 1996. Functional properties of myofibrillar protein-based biopackaging as affected by film thickness. J. Food Sci. 61: 580-584.

Cuq, B., Gontard, N. and Guilbert, S. 1997. Thermal properties of fish myofibrillar protein-based films as affected by moisture content. Polymer. 38: 2399-2405.

da Matta, M.D., Sarmento, S.B.S., de Oliveira, L.M. and Zocchi, S.S. 2011. Mechanical properties of pea starch films associated with xanthan gum and glycerol. Starch Starke. 63: 274-282.

Dapkevicius, A., Venskutonis, R., van Beek, T.A. and Linssen, J.P.H. 1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J. Sci. Food Agr. 77: 140-146.

De Carvalho, R.A. and Grosso, C.R.F. 2004. Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. Food Hydrocolloid. 18: 717-726.

De Carvalho, R.A. and Grosso, C.R.F. 2006. Properties of chemically modified gelatin films. Braz. J. Chem. Eng. 23: 45 - 53.

Debeaufort, F., Martin-Polo, M. and Voilley, A. 1993. Polarity homogeneity and structure affect water vapor permeability of model edible films. J. Food Sci. 58: 426-429.

Debeaufort, F. and Voilley, A. 1995. Methyl cellulose-based edible films and coatings I. Effect of plasticizer content on water and 1-octen-3-ol sorption and transport. Cellulose. 2: 205-213.

Debeaufort, F., Quezada-Gallo, J.A. and Voilley, A. 1998. Edible films and coatings: tomorrow's packaging: a review. Cr. Rev. Food Sci. 38: 299-313.

Deiber, J.A., Peirotti, M.B. and Ottone, M.L. 2011. Rheological characterization of edible films made from collagen colloidal particle suspensions. Food Hydrocolloid. 25: 1382-1392.

Denavi, G.A., Pérez-Mateos, M., Añón, M.C., Montero, P., Mauri, A.N. and Gómez-Guillén, M.C. 2009. Structural and functional properties of soy protein isolate and cod gelatin blend films. Food Hydrocolloid. 23: 2094-2101.

Di Gioia, L. and Guilbert, S. 1999. Corn protein-based thermoplastic resins: effect of some polar and amphiphilic plasticizers. J. Agr. Food Chem. 47: 1254-1261.

Di Leo Lira, P., Retta, D., Tkacik, E., Ringuelet, J., Coussio, J.D., van Baren, C. and Bandoni, A.L. 2009. Essential oil and by-products of distillation of bay leaves (*Laurus nobilis* L.) from Argentina. Ind. Crop. Prod. 30: 259-264.

Di Pierro, P., Chico, B., Villalonga, R., Mariniello, L., Masi, P. and Porta, R. 2007. Transglutaminase-catalyzed preparation of chitosan-ovalbumin films. Enzyme Microb. Tech. 40: 437-441.

Diab, T., Biliaderis, C.G., Gerasopoulos, D. and Sfakiotakis, E. 2001. Physicochemical properties and application of pullulan edible films and coatings in fruit preservation. J. Sci. Food Agr. 81: 988-1000.

Donhowe, I.G. and Fenema, O. 1994. Edible coatings and films to improve food quality. (Krochta, J. M. *et al.*, eds.). p. 1-25. Technomic Publishing. Lancaster.

Dorman, H.J.D. and Deans, S.G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88: 308-316.

Emiroğlu, Z.K., Yemiş□, G.P., Coşkun, B.K. and Candoğan, K. 2010. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Sci. 86: 283-288.

Ercolini, D., Ruso, F., Torrieri, E., Masi, P. and Villani, F. 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Appl. Environ. Microbiol. 72: 4663-4671.

Fabra, M.J., Jiménez, A., Atarés, L., Talens, P. and Chiralt, A. 2009. Effect of fatty acids and beeswax addition on properties of sodium caseinate dispersions and films. Biomacromolecules. 10: 1500-1507.

Fadini, A.L., Rocha, F.S., Alvim, I.D., Sadahira, M.S., Queiroz, M.B., Alves, R.M.V. and Silva, L.B. 2013. Mechanical properties and water vapour permeability of hydrolysed collagen-cocoa butter edible films plasticised with sucrose. Food Hydrocolloid. 30: 625-631.

Fang, Y., Tung, M.A., Britt, I.J., Yada, S. and Dalgleish, D.G. 2002. Tensile and barrier properties of edible films made from whey proteins. J. Food Sci. 67: 188-193.

Faraji, H., McClements, D.J. and Decker, E.A. 2004. Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. J. Agr. Food Chem. 52: 4558-4564.

Fishman, M.L., Coffin, D.R., Onwulata, C.I. and Konstance, R.P. 2004. Extrusion of pectin and glycerol with various combinations of orange albedo and starch. Carbohyd. Polym. 57: 401-413.

Folk, J.E. 1980. Transglutaminases. Annu. Rev. Biochem. 49: 517-531.

Frazier, R.A., Papadopoulou, A., Mueller-Harvey, I., Kissoon, D. and Green, R.J. 2003. Probing protein-tannin interactions by isothermal titration microcalorimetry. J. Agr. Food Chem. 51: 5189-5195.

Friedman, M., Henika, P.R. and Mandrell, R.E. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. J. Food Protect. 65: 1545-1560.

Friedman, M. 2006. Antibiotic activities of plant compounds against non-resistant and antibiotic-resistant foodborne human pathogens. *In* Advances in microbial food safety ACS Symposium Series. (Juneja, V. K. *et al.*, eds.). p. 167−183. American Chemical Society. Washington, DC.

Galietta, G., Di Gioia, L., Guilbert, S. and Cuq, B. 1998. Mechanical and thermomechanical properties of films based on whey proteins as affected by plasticizer and crosslinking agents. J. Dairy Sci. 81: 3123-3130.

Galus, S. and Lenart, A. 2013. Development and characterization of composite edible films based on sodium alginate and pectin. J. Food Eng. 115: 459-465.

Garg, S. and Jana, A.K. 2007. Studies on the properties and characteristics of starch-LDPE blend films using cross-linked, glycerol modified, cross-linked and glycerol modified starch. Eur. Polym. J. 43: 3976-3987.

Gennadios, A., McHugh, T.H., Weller, C.L. and Krochta, J.M. 1994. Edible coatings and films based on proteins. *In* Edible coatings and films to improve food quality. (Krochta, J. M. *et al.*, eds.). p. 201-277. Technomic Publishing Company, Inc. Lancaster, PA.

Gennadios, A., Weller, C.L., Hanna, M.A. and Froning, G.W. 1996. Mechanical and barrier properties of egg albumen films. J. Food Sci. 61: 585-589.

Gennadios, A., Hanna, M.A. and Kurth, L.B. 1997. Application of edible coatings on meats, poultry and seafoods: a review. LWT - Food Sci. Technol. 30: 337-350.

Gennadios, A., Handa, A., Froning, G.W., Weller, C.L. and Hanna, M.A. 1998. Physical properties of egg white-dialdehyde starch films. J. Agr. Food Chem. 46: 1297-1302.

Ghanbarzadeh, B., Oromiehie, A.R., Musavi, M., D-Jomeh, Z.E., Rad, E.R. and Milani, J. 2006. Effect of plasticizing sugars on rheological and thermal properties of zein resins and mechanical properties of zein films. Food Res. Int. 39: 882-890.

Giménez, B., Roncalés, P. and Beltrán, J.A. 2004. The effects of natural antioxidants and lighting conditions on the quality characteristics of gilt-head sea bream fillets (*Sparus aurata*) packaged in a modified atmosphere. J. Sci. Food Agr. 84: 1053-1060.

Giménez, B., Alemán, A., Montero, P. and Gómez-Guillén, M.C. 2009a. Antioxidant and functional properties of gelatin hydrolysates obtained from skin of sole and squid. Food Chem. 114: 976-983.

Giménez, B., Gómez-Estaca, J., Alemán, A., Gómez-Guillén, M.C. and Montero, M.P. 2009b. Improvement of the antioxidant properties of squid skin gelatin films by the addition of hydrolysates from squid gelatin. Food Hydrocolloid. 23: 1322-1327.

Giménez, B., Gómez-Estaca, J., Alemán, A., Gómez-Guillén, M.C. and Montero, M.P. 2009c. Physico-chemical and film forming properties of giant squid (*Dosidicus gigas*) gelatin. Food Hydrocolloid. 23: 585-592.

Gomez-Estaca, J., Montero, P., Gimenez, B. and Gomez-Guillen, M.C. 2007. Effect of functional edible films and high pressure processing on microbial and oxidative spoilage in cold-smoked sardine (*Sardina pilchardus*). Food Chem. 105: 511-520.

Gómez-Estaca, J., Bravo, L., Gómez-Guillén, M.C., Alemán, A. and Montero, P. 2009a. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chem. 112: 18-25.

Gómez-Estaca, J., Giménez, B., Montero, P. and Gómez-Guillén, M.C. 2009b. Incorporation of antioxidant borage extract into edible films based on sole skin gelatin or a commercial fish gelatin. J. Food Eng. 92: 78-85.

Gómez-Estaca, J., López de Lacey, A., López-Caballero, M.E., Gómez-Guillén, M.C. and Montero, P. 2010. Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiol. 27: 889-896.

Gomez-Guillen, M.C., Perez-Mateos, M., Gomez-Estaca, J., Lopez-Caballero, E., Gimenez, B. and Montero, P. 2009. Fish gelatin: a renewable material for developing active biodegradable films. Trends Food Sci. Tech. 20: 3-16.

Gómez-Guillén, M.C., Ihl, M., Bifani, V., Silva, A. and Montero, P. 2007. Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae Turcz*). Food Hydrocolloid. 21: 1133-1143.

Gontard, N., Thibault, R., Cuq, B. and Guilbert, S. 1996. Influence of relative humidity and film composition on oxygen and carbon dioxide permeabilities of edible films. J. Agr. Food Chem. 44: 1064-1069.

Gounga, M.E., Xu, S.-Y. and Wang, Z. 2007. Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. J. Food Eng. 83: 521-530.

Grondahl, M., Eriksson, L. and Gatenholm, P. 2004. Material properties of plasticized hardwood xylans for potential application as oxygen barrier films. Biomacromolecules. 5: 1528-1535.

Guerrero, P., Retegi, A., Gabilondo, N. and de la Caba, K. 2010. Mechanical and thermal properties of soy protein films processed by casting and compression. J. Food Eng. 100: 145-151.

Guilbert, S. 1986. Technology and application of edible protective films. *In* Food Packaging and Preservation. (Mathlouthi, M., ed.). p. 371–394. Elsevier Applied Science. London.

Guilbert, S. and Gontard, N. 1995. Edible and biodegradable food packaging. *In* Foods and packaging materials - chemical interactions. (Ackermann, P. *et al.*, eds.). p. 159-168. The Royal Society of Chemistry. Cambridge, England.

Guilbert, S., Gontard, N. and Gorris, L.G.M. 1996. Prolongation of the shelf life of perishable food products using biodegradable films and coatings. LWT - Food Sci. Technol. 29: 10-17.

Guilbert, S., Cuq, B. and Gontard, N. 1997. Recent innovations in edible andlor biodegradable packaging materials. Food Addit. Contam. 14: 741-751.

Guilbert, S. 2002. Edible and biodegradable coating/film systems. *In* Active food packaging. (Han, J. H., ed.). p. 4-10. SCI Publication and Communication Services. Winnipeg, Canada.

Hagerman, A.E., Rice, M.E. and Ritchard, N.T. 1998. Mechanisms of protein precipitation for two tannins, pentagalloyl glucose and epicatechin16 (4→8) catechin (procyanidin). J. Agr. Food Chem. 46: 2590-2595.

Hamaguchi, P.Y., Weng, W., Kobayashi, T., Runglertkreingkrai, J. and Tanaka, M. 2007. Effect of fish meat quality on the properties of biodegradable protein films. Food Sci. Technol. Res. 13: 200-204.

Han, J.H. and Floros, J.D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. J. Plast. Film Sheet. 13: 287-298.

Han, J.H. and Floros, J.D. 1998. Potassium sorbate diffusivity in American processed and Mozzarella cheeses. J. Food Sci. 63: 435-437.

Han, J.H. 2002. Protein-based edible films and coatings carrying antimicrobial agents. *In* Protein-based Films and Coatings. (Gennadios, A., ed.). p. 485-499. CRC Press. Boca Raton, FL.

Han, J.H. 2003. Antimicrobial food packaging. *In* Novel Food Packaging Techniques. (Ahvenainen, R., ed.). p. 50-70. Woodhead Publishing Ltd. Cambridge, UK.

Han, J.H., Aristippos, G. and Jung, H.H. 2005. Edible films and coatings: A review. *In* Innovations in Food Packaging. p. 239-262. Academic Press. London.

Hassimotto, N.M.A., Genovese, M.I. and Lajolo, F.M. 2005. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. J. Agr. Food Chem. 53: 2928-2935.

Hauser, C. and Wunderlich, J. 2011. Antimicrobial packaging films with a sorbic acid based coating. Procedia Food Sci. 1: 197-202.

Hensel, M., Achmus, H., Deckers-Hebestreit, G. and Altendorf, K. 1996. The ATP synthase of *Streptomyces lividans*: characterization and purification of the F_1F_0 complex. Biochim. Biophys. Acta. 1274: 101-108.

Hernandez-Izquierdo, V.M. and Krochta, J.M. 2009. Thermal transitions and heat-sealing of glycerol-plasticized whey protein films. Packag. Technol. Sci. 22: 255-260.

Hernández-Muñoz, P., Villalobos, R. and Chiralt, A. 2004. Effect of cross-linking using aldehydes on properties of glutenin-rich films. Food Hydrocolloid. 18: 403-411.

Hernandez, R.J. 1997. Polymer properties. *In* The Wiley encyclopedia of packaging technology. 2nd Ed. (Brody, A. L. and Marsh, K. S., eds.). p. 758-765. John Wiley and Sons Inc. New York.

Hogg, S. 2005. Essential Microbiology. John Wiley & Sons, Ltd. Chichester, West Sussex, England.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2010. Effect of heat treatment of film-forming solution on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. J. Food Eng. 96: 66-73.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011a. Effects of partial hydrolysis and plasticizer content on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. Food Hydrocolloid. 25: 82-90.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011b. Properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin incorporated with cinnamon, clove and star anise extracts. Food Hydrocolloid. 25: 1085-1097.

Hoque, M.S., Benjakul, S., Prodpran, T. and Songtipya, P. 2011c. Properties of blend film based on cuttlefish (*Sepia pharaonis*) skin gelatin and mungbean protein isolate. Int. J. Biol. Macromol. 49: 663-673.

Hosseini, M.H., Razavi, S.H. and Mousavi, M.A. 2009. Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. J. Food Process. Pres. 33: 727-743.

Hosseini, S.F., Rezaei, M., Zandi, M. and Farahmandghavi, F. 2015. Bio-based composite edible films containing *Origanum vulgare* L. essential oil. Ind. Crop. Prod. 67: 403-413.

Howard, L.R. and Gonzales, A.R. 2001. Food safety and produce operations: what is the future? Hortic. Sci. 36: 33-39.

Irissin-Mangata, J., Bauduin, G., Boutevin, B. and Gontard, N. 2001. New plasticizers for wheat gluten films. Eur. Polym. J. 37: 1533-1541.

Jangchud, A. and Chinnan, M.S. 1999. Peanut protein film as affected by drying temperature and pH of film forming solution. J. Food Sci. 64: 153-157.

Jeya Shakila, R., Jeevithan, E., Varatharajakumar, A., Jeyasekaran, G. and Sukumar, D. 2012. Comparison of the properties of multi-composite fish gelatin films with that of mammalian gelatin films. Food Chem. 135: 2260-2267.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006a. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid. 20: 492-501.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006b. Effects of plasticizers on the properties of edible films from skin gelatin of bigeye snapper and brownstripe red snapper. Eur. Food Res. Technol. 222: 229-235.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006c. Fatty acids and their sucrose esters affect the properties of fish skin gelatin-based film. Eur. Food Res. Technol. 222: 650-657.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2008. Antioxidative activity and properties of fish skin gelatin films incorporated with BHT and α-tocopherol. Food Hydrocolloid. 22: 449-458.

Jouki, M., Yazdi, F.T., Mortazavi, S.A. and Koocheki, A. 2014. Quince seed mucilage films incorporated with oregano essential oil: Physical, thermal, barrier, antioxidant and antibacterial properties. Food Hydrocolloid. 36: 9-19.

Kavoosi, G., Rahmatollahi, A., Mohammad Mahdi Dadfar, S. and Mohammadi Purfard, A. 2014. Effects of essential oil on the water binding capacity, physico-mechanical properties, antioxidant and antibacterial activity of gelatin films. LWT - Food Sci. Technol. 57: 556-561.

Kester, J.J. and Fennema, O.R. 1986. Edible films and coatings: a review. Food Technol. 40: 47-59.

Kim, D.-H., Na, S.-K. and Park, J.-S. 2003. Preparation and characterization of modified starch-based plastic film reinforced with short pulp fiber. I. Structural properties. J. Appl. Polym. Sci. 88: 2100-2107.

Kim, S.J. and Ustunol, Z. 2001. Thermal properties, heat sealability and seal attributes of whey protein isolate/ lipid emulsion edible films. J. Food Sci. 66: 985-990.

Kim, S.K., Kim, Y.T., Byun, H.G., Nam, K.S., Joo, D.S. and Shahidi, F. 2001. Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska pollack skin. J. Agr. Food Chem. 49: 1984-1989.

Knobloch, K., Pauli, P., Iberl, B., Weigand, H. and Weis, N. 1989. Antibacterial and antifungal properties of essential oil components. J. Essent. Oil Res. 1: 603-608.

Kokoska, L., Polesny, Z., Rada, V., Nepovim, A. and Vanek, T. 2002. Screening of some Siberian medicinal plants for antimicrobial activity. J. Ethnopharmacol. 82: 51-53.

Kolodziejska, I. and Piotrowska, B. 2007. The water vapour permeability, mechanical properties and solubility of fish gelatin-chitosan films modified with transglutaminase or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and plasticized with glycerol. Food Chem. 103: 295-300.

Kołodziejska, I., Piotrowska, B., Bulge, M. and Tylingo, R. 2006. Effect of transglutaminase and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide on the solubility of fish gelatin-chitosan films. Carbohyd. Polym. 65: 404-409.

Kołodziejska, I. and Piotrowska, B. 2007. The water vapour permeability, mechanical properties and solubility of fish gelatin-chitosan films modified with transglutaminase or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and plasticized with glycerol. Food Chem. 103: 295-300.

Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A. and Yildirim, A. 2005. Determination of the chemical composition and antioxidant activity of the essential oil of artemisia dracunculus and of the antifungal and antibacterial activities of turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. J. Agr. Food Chem. 53: 9452-9458.

Kowalczyk, D. and Baraniak, B. 2011. Effects of plasticizers, pH and heating of film-forming solution on the properties of pea protein isolate films. J. Food Eng. 105: 295-305.

Krishna, M., Nindo, C.I. and Min, S.C. 2012. Development of fish gelatin edible films using extrusion and compression molding. J. Food Eng. 108: 337-344.

Krochta, J.M., Baldwin, E.A. and Nisperos-Carriedo, M.O. 1994. Edible coatings and films to improve food quality. Technomic Publishing Company. Lancaster, PA.

Krochta, J.M. 1997. Edible protein films and coatings. *In* Food proteins and their applications. (Damodaran, S. and Paraf, A., eds.). p. 529-549. Marcel Dekker. New York.

Krochta, J.M. and De Mulder-Johnston, C. 1997. Edible and biodegradable polymer films: challenges and opportunities. Food Technol. 51: 61-74.

Krochta, J.M. 2002. Protein as raw materials for films and coatings: definitions, current status, and opportunities. *In* Protein-Based Films and Coating (Gennadios, A., ed.). p. 1-39. CRC Press. New York.

Kumar, R.S., Sivakumar, T., Sunderam, R.S., Gupta, M., Mazumdar, U.K., Gomathi, P., Rajeshwar, Y., Saravanan, S., Kumar, M.S., Murugesh, K. and Kumar, K.A. 2005. Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. Braz J. Med. Biol. Res. 38: 1015-1024.

Kurian, M., Dasgupta, A., Galvin, M.E., Ziegler, C.R. and Beyer, F.L. 2006. A novel route to inducing disorder in model polymer-layered silicate nanocomposites. Macromolecules. 39: 1864-1871.

Lacroix, M. and Ouattara, B. 2000. Combined industrial processes with irradiation to assure innocuity and preservation of food products-A review. Food Res. Int. 33: 719-724.

Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S. and Boskou, D. 1993. Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. Z. Lebensm. Unters. Forsch. 197: 20-23.

Lambert, R.J.W., Skandamis, P.N., Coote, P.J. and Nychas, G.J.E. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 91: 453-462.

Lee, J.-H., Lee, J. and Song, K.B. 2015. Development of a chicken feet protein film containing essential oils. Food Hydrocolloid. 46: 208-215.

Leimann, F.V., Gonçalves, O.H., Machado, R.A.F. and Bolzan, A. 2009. Antimicrobial activity of microencapsulated lemongrass essential oil and the effect of experimental parameters on microcapsules size and morphology. Mater. Sci. Eng: 29: 430-436.

Leuangsukrerk, M., Phupoksakul, T., Tananuwong, K., Borompichaichartkul, C. and Janjarasskul, T. 2014. Properties of konjac glucomannan–whey protein isolate blend films. LWT - Food Sci. Technol. 59: 94-100.

Limpan, N., Prodpran, T., Benjakul, S. and Prasarpran, S. 2010. Properties of biodegradable blend films based on fish myofibrillar protein and polyvinyl alcohol as influenced by blend composition and pH level. J. Food Eng. 100: 85-92.

Limpisophon, K., Tanaka, M., Weng, W., Abe, S. and Osako, K. 2009. Characterization of gelatin films prepared from under-utilized blue shark (*Prionace glauca*) skin. Food Hydrocolloid. 23: 1993-2000.

Limpisophon, K., Tanaka, M. and Osako, K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionace glauca*) skin gelatin. Food Chem. 122: 1095-1101.

Lis-Balchin, M., Deans, S.G. and Eaglesham, E. 1998. Relationship between bioactivity and chemical composition of commercial essential oils. Flavour Frag. J. 13: 98-104.

Liu, C.-C., Tellez-Garay, A.M. and Castell-Perez, M.E. 2004. Physical and mechanical properties of peanut protein films. LWT - Food Sci. Technol. 37: 731-738.

Liu, G. and Xiong, Y.L. 2000. Electrophoretic pattern, thermal denaturation, and *in vitro* digestibility of oxidized myosin. J. Agr. Food Chem. Phys. Lipids. 48: 624-630.

Liu, L., Liu, C.K., Fishman, M.L. and Hicks, K.B. 2007. Composite films from pectin and fish skin gelatin or soybean flour protein. J. Agr. Food Chem. 55: 2349−2355.

Liu, L., Jin, T., Liu, C.-K., Hicks, K., Mohanty, A.K., Bhardwaj, R. and Misra, M. 2008. A preliminary study on antimicrobial edible films from pectin and other food hydrocolloids by extrusion method. J. Nat. Fibers. 5: 366-382.

López, O.V., Lecot, C.J., Zaritzky, N.E. and García, M.A. 2011. Biodegradable packages development from starch based heat sealable films. J. Food Eng. 105: 254-263.

Ma, W., Tang, C.-H., Yin, S.-W., Yang, X.-Q., Qi, J.-R. and Xia, N. 2012a. Effect of homogenization conditions on properties of gelatin-olive oil composite films. J. Food Eng. 113: 136-142.

Ma, W., Tang, C.-H., Yin, S.-W., Yang, X.-Q., Wang, Q., Liu, F. and Wei, Z.-H. 2012b. Characterization of gelatin-based edible films incorporated with olive oil. Food Res. Int. 49: 572-579.

Mahmoud, R. and Savello, P.A. 1993. Solubility and hydrolyzability of films produced by transglutaminase catalytic crosslinking of whey protein. J. Dairy Sci. 76: 29-35.

Malatova, K., Hitimana, N., Niyibizi, T., Simon, J.E. and Juliani, H.R. 2011. Optimization of harvest regime and post-harvest handling in geranium production to maximize essential oil yield in Rwanda. Ind. Crop. Prod. 34: 1348-1352.

Mao, L.-C., Pan, X., Que, F. and Fang, X.-H. 2006. Antioxidant properties of water and ethanol extracts from hot air-dried and freeze-dried daylily flowers. Eur. Food Res. Technol. 222: 236-241.

Marquie, C., Aymard, C., Cuq, J.L. and Guilbert, S. 1995. Biodegradable packaging made from cottonseed flour: formation and improvement by chemical treatments with gossypol, formaldehyde, and glutaraldehyde. J. Agr. Food Chem. 43: 2762-2767.

Marquie, C., Tessier, A.M., Aymard, C. and Guilbert, S. 1997. HPLC determination of the reactive lysine content of cottonseed protein films to monitor the extent of cross-linking by formaldehyde, glutaraldehyde, and glyoxal. J. Agr. Food Chem. 45: 922-926.

Marquié, C. and Guilbert, S. 2002. Formation and properties of cottonseed protein films and coating. *In* Protein-based films and coating. (Gennadios, A., ed.). p. 143-149. CRC Press. New York.

Martínez-Camachoa, A.P., Cortez-Rocha, M.O., Ezquerra-Brauer, J.M., Graciano-Verdugo, A.Z., Rodriguez-Félix, F., Castillo-Ortega, M.M., Yépiz-Gómez, M.S. and Plascencia-Jatomea, M. 2010. Chitosan composite films: Thermal, structural, mechanical and antifungal properties. Carbohyd. Polym. 82: 305-315.

Masango, P. 2005. Cleaner production of essential oils by steam distillation. J. Clean. Prod. 13: 833-839.

Mate, J.I. and Krochta, J.M. 1998. Oxygen uptake model for uncoated and coated peanuts. J. Food Eng. 35: 299-312.

McHugh, T.H. and Krochta, J.M. 1994. Sorbital - versus glycerol - plasticized edible films: integrated oxygen permeability and tensile property evaluation. J. Agr. Food Chem. 42: 841-845.

Mecitoglu, C., Yemenicioglu, A., Arslanoglu, A., ElmacI, Z.S., Korel, F. and Cetin, A.E. 2006. Incorporation of partially purified hen egg white lysozyme into zein films for antimicrobial food packaging. Food Res. Int. 39: 12-21.

Meka, P. and Stehling, F.C. 1994. Heat sealing of semicrystalline polymer films. I. Calculation and measurement of interfacial temperatures: Effect of process variables on seal properties. J. Appl. Polym. Sci. 51: 89-103.

Micard, V., Belamri, R., Morel, M.H. and Guilbert, S. 2000a. Properties of chemically and physically treated wheat gluten films. J. Agr. Food Chem. 48: 2948-2953.

Micard, X., Belamri, R., Morel, M.H. and Guilbert, S. 2000b. Properties of chemically and physically treated wheat gluten films. J. Agr. Food Chem. 48: 2948-2953.

Miller, K.S., Chiang, M.T. and Krochta, J.M. 1997. Heat curing of whey protein films. J. Food Sci. 62: 1189-1193.

Miller, K.S., Upadhyaya, S.K. and Krochta, J.M. 1998. Permeability of d-limonene in whey protein films. J. Food Sci. 63: 244-247.

Mitić-Ćulafić, D., Žegura, B., Nikolić, B., Vuković-Gačić, B., Knežević-Vukčević, J. and Filipič, M. 2009. Protective effect of linalool, myrcene and eucalyptol against *t*-butyl hydroperoxide induced genotoxicity in bacteria and cultured human cells. Food Chem. Toxicol. 47: 260-266.

Mohamed, A.A., El-Emary, G.A. and Ali, H.F. 2010a. Influence of some citrus essential oils on cell viability, glutathione-s-transferase and lipid peroxidation in *Ehrlich ascites* Carcinoma Cells. J. Am. Sci. 6: 820-826.

Mohamed, A.A., El-Emary, G.A. and Ali, H.F. 2010b. Influence of some citrus essential oils on cell viability, glutathione-s-transferase and lipid peroxidation in Ehrlich ascites Carcinoma Cells. J. Am. Sci. 6: 820-826.

Montero, P. and Gómez-Guillén, M.C. 2000. Extracting conditions for megrim (*Lepidorhombus boscii*) skin collagen affect functional properties of the resulting gelatin. J. Food Sci. 65: 434-438.

Moradi, M., Tajik, H., Razavi Rohani, S.M. and Oromiehie, A.R. 2011. Effectiveness of *Zataria multiflora* Boiss essential oil and grape seed extract impregnated chitosan film on ready-to-eat mortadella-type sausages during refrigerated storage. J. Sci. Food Agr. 91: 2850-2857.

Mueller, C., Capaccio, G., Hiltner, A. and Baer, E. 1998. Heat sealing of LLDPE: relationships to melting and interdiffusion. J. Appl. Polym. Sci. 70: 2021-2030.

Nagarajan, M., Benjakul, S., Prodpran, T., Songtipya, P. and Nuthong, P. 2013. Film forming ability of gelatins from splendid squid (*Loligo formosana*) skin bleached with hydrogen peroxide. Food Chem. 138: 1101-1108.

Newman, D.J., Gragg, G.M., Holbeck, S. and Sausville, E.A. 2002. Natural products as leads to cell cycle pathway targets in cancer chemotherapy. Curr. Cancer Drug Targets. 2: 279-308.

Nguyen, M.T., Kryachko, E.S. and Vanquickenborne, L.G. 2003. General and theoretical aspects of phenols. *In* The Chemistry of Phenols. (Rappoport, Z., ed.). p. 1-198. John Wiley & Sons Ltd. Chichester, West Sussex, UK.

Nishio, Y. and Takahashi, T. 1984. Morphological Study of hydroxypropyl cellulose films prepared from thermotropic melt under shear. J. Macromol. Sci. 23: 483-495.

Núñez-Flores, R., Giménez, B., Fernández-Martín, F., López-Caballero, M.E., Montero, M.P. and Gómez-Guillén, M.C. 2012. Role of lignosulphonate in properties of fish gelatin films. Food Hydrocolloid. 27: 60-71.

Núñez-Flores, R., Giménez, B., Fernández-Martín, F., López-Caballero, M.E., Montero, M.P. and Gómez-Guillén, M.C. 2013. Physical and functional characterization of active fish gelatin films incorporated with lignin. Food Hydrocolloid. 30: 163-172.

Nur Hanani, Z.A., Roos, Y.H. and Kerry, J.P. 2012. Use of beef, pork and fish gelatin sources in the manufacture of films and assessment of their composition and mechanical properties. Food Hydrocolloid. 29: 144-151.

Nur Hanani, Z.A., McNamara, J., Roos, Y.H. and Kerry, J.P. 2013. Effect of plasticizer content on the functional properties of extruded gelatin-based composite films. Food Hydrocolloid. 31: 264-269.

Nur Hanani, Z.A., O'Mahony, J.A., Roos, Y.H., Oliveira, P.M. and Kerry, J.P. 2014. Extrusion of gelatin-based composite films: Effects of processing temperature and pH of film forming solution on mechanical and barrier properties of manufactured films. Food Packag. Shelf Life. 2: 91-101.

Nussinovitch, A. 2009. CHAPTER 10 - Biopolymer films and composite coatings. *In* Modern Biopolymer Science. (Stefan, K. *et al.*, eds.). p. 295-326. Academic Press. San Diego.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009a. Effect of some factors and pretreatment on the properties of porcine plasma protein-based films. LWT - Food Sci. Technol. 42: 1545-1552.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009b. Effect of phenolic compounds on the properties of porcine plasma protein-based film. Food Hydrocolloid. 23: 736-741.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009c. Characterization of porcine plasma protein-based films as affected by pretreatment and cross-linking agents. Int. J. Biol. Macromol. 44: 143-148.

Okoh, O.O., Sadimenko, A.P. and Afolayan, A.J. 2010. Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. Food Chem. 120: 308-312.

Ouattara, B., Simard, R.E., Piette, G., Begin, A. and Holley, R.A. 2000. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. Int. J. Food Microbiol. 62: 139-148.

Oussalah, M., Caillet, S., Salmieri, S., Saucier, L. and Lacroix, M. 2004. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. J. Agr. Food Chem. 52: 5598-5605.

Park, H.J. and Chinnan, M.S. 1995. Gas and water vapor barrier properties of edible films from protein and cellulosic materials. J. Food Eng. 25: 497-507.

Pelissari, F.M., Grossmann, M.V.E., Yamashita, F. and Pineda, E.A.G. 2009. Antimicrobial, mechanical, and barrier properties of cassava starch-chitosan films incorporated with oregano essential oil. J. Agr. Food Chem. 57: 7499-7504.

Perez-Gago, M.B. and Krochta, J.M. 2002. Formation and properties of whey protein films and coatings. *In* Protein-based Films and Coatings. (Gennadios, A., ed.). p. 159-180. CRC Press. Boca Raton, FL.

Pérez-Mateos, M., Montero, P. and Gómez-Guillén, M.C. 2009. Formulation and stability of biodegradable films made from cod gelatin and sunflower oil blends. Food Hydrocolloid. 23: 53-61.

Piotrowska, B., Sztuka, K., Kołodziejska, I. and Dobrosielska, E. 2008. Influence of transglutaminase or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) on the properties of fish-skin gelatin films. Food Hydrocolloid. 22: 1362-1371.

Pires, C., Ramos, C., Teixeira, G., Batista, I., Mendes, R., Nunes, L. and Marques, A. 2011. Characterization of biodegradable films prepared with hake proteins and thyme oil. J. Food Eng. 105: 422-428.

Pommet, M., Redl, A., More, M., Domenek, S. and Guilbert, S. 2003. Thermoplastic processing of protein-based bioplastics: chemical engineering aspects of mixing, extrusion and hot molding. Macromol. Symp. 197: 207-217.

Pranoto, Y., Salokhe, V.M. and Rakshit, S.K. 2005. Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. Food Res. Int. 38: 267-272.

Pranoto, Y., Lee, C.M. and Park, H.J. 2007. Characterizations of fish gelatin films added with gellan and α-carrageenan. LWT - Food Sci. Technol. 40: 766-774.

Prigent, S.V.E., Gruppen, H., Visser, A.J.W.G., van Koningsveld, G.A., de Jong, G.A.H. and Voragen, A.G.J. 2003. Effects of non-covalent interactions with 5-*O*-caffeoylquinic acid (chlorogenic acid) on the heat denaturation and solubility of globular proteins. J. Agr. Food Chem. 51: 5088-5095.

Prodpran, T., Benjakul, S. and Artharn, A. 2007. Properties and microstructure of protein-based film from round scad (*Decapterus maruadsi*) muscle as affected by palm oil and chitosan incorporation. Int. J. Biol. Macromol. 41: 605-614.

Qiu, L., Hu, F. and Peng, Y. 2013. Structural and mechanical characteristics of film using modified corn starch by the same two chemical processes used in different sequences. Carbohyd. Polym. 91: 590-596.

Quintavalla, S. and Vicini, L. 2002. Antimicrobial food packaging in meat industry. Meat Sci. 62: 373-380.

Ralston, B.E. and Osswald, T.A. 2008. Viscosity of soy protein plastics determined by screw-driven capillary rheometry. J. Polym. Environ. 16: 169-176.

Rattaya, S., Benjakul, S. and Prodpran, T. 2009. Properties of fish skin gelatin film incorporated with seaweed extract. J. Food Eng. 95: 151-157.

Rawel, H.M., Rohn, S. and Kroll, J. 2003. Influence of a sugar moiety (rhamnosylglucoside) at 3-*O* position on the reactivity of quercetin with whey proteins. Int. J. Biol. Macromol. 32: 109-120.

Rhim, J.-W. 2004. Physical and mechanical properties of water resistant sodium alginate films. LWT - Food Sci. Technol. 37: 323-330.

Rhim, J.W., Gennadios, A., Handa, A., Weller, C.L. and Hanna, M.A. 2000. Solubility, tensile, and color properties of modified soy protein isolate films. J. Agr. Food Chem. 48: 4937-4941.

Rhim, J.W., Shellhammer, T.H. and Jung, H.H. 2005. 21 - Lipid-based edible films and coatings. *In* Innovations in Food Packaging. p. 362-383. Academic Press. London.

Rhim, J.W., Mohanty, K.A., Singh, S.P. and Ng, P.K.W. 2006. Preparation and properties of biodegradable multilayer films based on soy protein isolate and poly(lactide). Ind. Eng. Chem. Res. 45: 3059-3066.

Rhim, J.W. and Ng, P.K.W. 2007. Natural biopolymer-based nanocomposite films for packaging applications. Cr. Rev. Food Sci. 47: 411-433.

Rivero, S., García, M.A. and Pinotti, A. 2013. Correlations between structural, barrier, thermal and mechanical properties of plasticized gelatin films. Innov. Food Sci. Emerg. 11: 369–375.

Rodríguez, M., Osés, J., Ziani, K. and Maté, J.I. 2006. Combined effect of plasticizers and surfactants on the physical properties of starch based edible films. Food Res. Int. 39: 840-846.

Rojas-Graü, M.A., Avena-Bustillos, R.J., Olsen, C., Friedman, M., Henika, P.R., Martín-Belloso, O., Pan, Z. and McHugh, T.H. 2007. Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films. J. Food Eng. 81: 634-641.

Rouhi, J., Mahmud, S., Naderi, N., Ooi, C. and Mahmood, M. 2013. Physical properties of fish gelatin-based bio-nanocomposite films incorporated with ZnO nanorods. Nanoscale Res. Lett. 8: 364.

Ruberto, G. and Baratta, M.T. 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem. 69: 167-174.

Salgado, P.R., Molina Ortiz, S.E., Petruccelli, S. and Mauri, A.N. 2010. Biodegradable sunflower protein films naturally activated with antioxidant compounds. Food Hydrocolloid. 24: 525-533.

Salgado, P.R., López-Caballero, M.E., Gómez-Guillén, M.C., Mauri, A.N. and Montero, M.P. 2013. Sunflower protein films incorporated with clove essential oil have potential application for the preservation of fish patties. Food Hydrocolloid. 33: 74–84.

Santosa, F.X.B. and Padua, G.W. 1999. Tensile properties and water absorption of zein sheets plasticized with oleic and linoleic acids. J. Agr. Food Chem. 47: 2070-2074.

Santoyo, S., Cavero, S., Jaime, L., Ibanez, E., Senorans, F.J. and Reglero, G. 2005. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. J. Food Protect. 68: 790-795.

Sarikurkcu, C., Sabih Ozer, M., Eskici, M., Tepe, B., Can, Ş. and Mete, E. 2010. Essential oil composition and antioxidant activity of *Thymus longicaulis* C. Presl subsp. *longicaulis* var. *longicaulis*. Food Chem. Toxicol. 48: 1801-1805.

Sayanjali, S., Ghanbarzadeh, B. and Ghiassifar, S. 2011. Evaluation of antimicrobial and physical properties of edible film based on carboxymethyl cellulose containing potassium sorbate on some mycotoxigenic Aspergillus species in fresh pistachios. LWT - Food Sci. Technol. 44: 1133-1138.

Schrieber, R. and Gareis, H. 2007. Gelatine handbook. Wiley-VCH GmbH & Co. Weinhem.

Sears, J.K. and Darby, J.R. 1982. Mechanism of plasticizer action. *In* The Technology of Plasticizers. (Sears, J. K. and Darby, J. R., eds.). p. 35-77. Wiley-Interscience. New York, USA.

Selling, G.W., Woods, K.K., Biswas, A. and Willett, J.L. 2009. Reactive extrusion of zein with glyoxal. J. Appl. Polym. Sci. 113: 1828-1835.

Seol, K.-H., Lim, D.-G., Jang, A., Jo, C. and Lee, M. 2009. Antimicrobial effect of k-carrageenan-based edible film containing ovotransferrin in fresh chicken breast stored at 5°C. Meat Sci. 83: 479-483.

Serdaroglu, M. and Felekoglu, E. 2005. Effects of using rosemary extract and onion juice on oxidative stability of sardine (*Sardina pilchardus*) mince. J. Food Quality. 28: 109-120.

Seydim, A.C. and Sarikus, G. 2006. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food Res. Int. 39: 639-644.

Shahidi, F., Botta, J.R. and Shahidi, F. 1994. Seafood processing by-products. *In* Seafoods: Chemistry, Processing Technology and Quality. p. 320-334. Springer US.

Shen, X.L., Wu, J.M., Chen, Y. and Zhao, G. 2010. Antimicrobial and physical properties of sweet potato starch films incorporated with potassium sorbate or chitosan. Food Hydrocolloid. 24: 285-290.

Shih, F.F., Daigle, K.W. and Champagne, E.T. 2011. Effect of rice wax on water vapour permeability and sorption properties of edible pullulan films. Food Chem. 127: 118-121.

Siebert, K.J., Troukhanova, N.V. and Lynn, P.Y. 1996. Nature of polyphenol-protein interactions. J. Agr. Food Chem. 44: 80-85.

Sikkema, J., de Bont, J.A.M. and Poolman, B. 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 59: 201-222.

Siripatrawan, U. and Harte, B.R. 2010. Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. Food Hydrocolloid. 24: 770-775.

Sivarooban, T., Hettiarachchy, N.S. and Johnson, M.G. 2008. Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. Food Res. Int. 41: 781-785.

Soazo, M., Rubiolo, A.C. and Verdini, R.A. 2011. Effect of drying temperature and beeswax content on physical properties of whey protein emulsion films. Food Hydrocolloid. 25: 1251-1255.

Sobral, P.J.D.A., Dos Santos, J.S. and Garcia, F.T. 2005. Effect of protein and plasticizer concentrations in film forming solutions on physical properties of edible films based on muscle proteins of a Thai Tilapia. J. Food Eng. 70: 93-100.

Soradech, S., Limatvapirat, S. and Luangtana-anan, M. 2013. Stability enhancement of shellac by formation of composite film: Effect of gelatin and plasticizers. J. Food Eng. 116: 572-580.

Sothornvit, R. and Krochta, J.M. 2000. Plasticizer effect on oxygen permeability of β-lactoglobulin films. J. Agr. Food Chem. 48: 6298-6302.

Sothornvit, R. and Krochta, J.M. 2001. Plasticizer effect on mechanical properties of β-lactoglobulin films. J. Food Eng. 50: 149-141 155.

Sothornvit, R. and Krochta, J.M. 2005. Plasticizers in edible films and coatings. *In* Innovations in food packaging. Vol. 403-433. (Han JH, ed.). Elsevier Academic Press. San Diego, Calif.

Sothornvit, R., Rhim, J.-W. and Hong, S.-I. 2009. Effect of nano-clay type on the physical and antimicrobial properties of whey protein isolate/clay composite films. J. Food Eng. 91: 468-473.

Stadtman, E.R. 2001. Protein oxidation in aging and age-related diseases. Annals of the New York Academy of Sciences. 928: 22-38.

Staroszczyk, H., Pielichowska, J., Sztuka, K., Stangret, J. and Kołodziejska, I. 2012. Molecular and structural characteristics of cod gelatin films modified with EDC and TGase. Food Chem. 130: 335-343.

Stevens, M.P. 1999. Polymer chemistry: an introduction. p. 551. Oxford University Press. New York.

Su, J.-F., Yuan, X.-Y., Huang, Z., Wang, X.-Y., Lu, X.-Z., Zhang, L.-D. and Wang, S.-B. 2012. Physicochemical properties of soy protein isolate/carboxymethyl cellulose blend films crosslinked by Maillard reactions: Color, transparency and heat-sealing ability. Mater. Sci. Eng. 32: 40-46.

Suryaprakash, P., Kumar, R.P. and Prakash, V. 2000. Thermodynamics of interaction of caffeic acid and quinic acid with multisubunit proteins. Int. J. Biol. Macromol. 27: 219-228.

Sztuka, K. and Kołodziejska, I. 2009. The influence of hydrophobic substances on water vapor permeability of fish gelatin films modified with transglutaminase or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Food Hydrocolloid. 23: 1062-1064.

Tabasi, R.Y., Najarzadeh, Z. and Ajji, A. 2015. Development of high performance sealable films based on biodegradable/compostable blends. Ind. Crop. Prod. 72: 206-213.

Talens, P. and Krochta, J.M. 2005. Plasticizing Effects of Beeswax and Carnauba Wax on Tensile and Water Vapor Permeability Properties of Whey Protein Films. J. Food Sci. 70: 239-243.

Tanner, K.E., Getz, J.J., Burnett, S., Youngblood, E. and Draper, P.R. 2001. Film forming compositions comprising modified starches and iota-carrageenan and methods for manufacturing soft capsules using same. US Patent 6-582-727.

Tassou, C.C., Drosinos, E.H. and Nychas, G.J.E. 1995. Effects of essential oil from mint (*Mentha piperita*) on Salmonella enteritidis and Listeria monocytogenes in model food systems at 4° and 10°C. J. Appl. Microbiol. 78: 593-600.

Teixeira, B., Marques, A., Ramos, C., Neng, N.R., Nogueira, J.M.F., Saraiva, J.A. and Nunes, M.L. 2013. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Ind. Crop. Prod. 43: 587-595.

Teixeira, B., Marques, A., Pires, C., Ramos, C., Batista, I., Saraiva, J.A. and Nunes, M.L. 2014. Characterization of fish protein films incorporated with essential oils of clove, garlic and origanum: Physical, antioxidant and antibacterial properties. LWT - Food Sci. Technol. 59: 533-539.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M. and Polissiou, M. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem. 90: 333-340.

Thubthimthed, S., Limsiriwong, P., Rerk-am, U. and Suntorntanasat, T. 2005. Chemical composition and cytotoxic activity of the essential oil of *Zingiber ottensii*. Acta Hort. 675: 107-109.

Turina, A.V., Nolan, M.V., Zygadlo, J.A. and Perillo, M.A. 2006. Natural terpenes: self-assembly and membrane partitioning. Biophys. Chem. 122: 101-113.

Ultee, A., Kets, E.P.W. and Smid, E.J. 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 65: 4606-4610.

Uriarte-Montoya, M.H., Arias-Moscoso, J.L., Plascencia-Jatomea, M., Santacruz-Ortega, H., Rouzaud-Sández, O., Cardenas-Lopez, J.L., Marquez-Rios, E. and Ezquerra-Brauer, J.M. 2010. Jumbo squid (*Dosidicus gigas*) mantle collagen: Extraction, characterization, and potential application in the preparation of chitosan-collagen biofilms. Bioresource Technol. 101: 4212-4219.

Vanin, F.M., Sobral, P.J.A., Menegalli, F.C., Carvalho, R.A. and Habitante, A.M.Q.B. 2005. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. Food Hydrocolloid. 19: 899-907.

Vargas, M., Pastor, C., Chiralt, A., McClements, D.J. and González-Martínez, C. 2008. Recent advances in edible coatings for fresh and minimally processed fruits. Cr. Rev. Food Sci. 48: 496-511.

Veldhuizen, E.J.A., Tjeerdsma-van Bokhoven, J.L.M., Zweijtzer, C., Burt, S.A. and Haagsman, H.P. 2006. Structural requirements for the antimicrobial activity of carvacrol. J. Agr. Food Chem. 54: 1874-1879.

Wainewright, F.W. 1977. Physical tests for gelatin and gelatin products. *In* The science and technology of gelatin. Vol. 507-534. (Ward, A. G. and Courts, A., eds.). Academic Press. New York.

Wan, V.C.-H., Kim, M.S. and Lee, S.-Y. 2005. Water vapor permeability and mechanical properties of soy protein isolate edible films composed of different plasticizer combinations. J. Food Sci. 70: 387-391.

Wang, Y. and Padua, G.W. 2003. Tensile properties of extruded zein sheets and extrusion blown films. Macromol. Mater. Eng. 288: 886-893.

Wasswa, J., Tang, J. and Gu, X. 2007. Utilization of fish processing by-products in the gelatin industry. Food Rev. Int. 23: 159-174.

Wei, A. and Shibamoto, T. 2010. Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. J. Agr. Food Chem. 58: 7218-7225.

Weng, W., Hamaguchi, P.Y. and Tanaka, M. 2006. Effect of propylene glycol alginate on the properties of edible film prepared from Alaska pollack surimi. J. Jpn. Soc. Food Sci. 53: 542-547.

Were, L., Hettiarachcky, N.S. and Coleman, M. 1999. Properties of cysteine-added soy protein-wheat gluten films. J. Food Sci. 64: 514-518.

Wu, J., Ge, S., Liu, H., Wang, S., Chen, S., Wang, J., Li, J. and Zhang, Q. 2014. Properties and antimicrobial activity of silver carp (*Hypophthalmichthys molitrix*) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation. Food Packag. Shelf Life. 2: 7-16.

Wu, Y., Weller, C.L., Hamouz, F., Cuppett, S.L. and Schnepf, M. 2002. Development and application of multicomponent edible coatings and films: A review. Adv. Food Nutr. Res. 44: 347-394.

Wu, Z., Li, H., Yang, Y., Zhan, Y. and Tu, D. 2013. Variation in the components and antioxidant activity of *Citrus medica* L. var. *sarcodactylis* essential oils at different stages of maturity. Ind. Crop. Prod. 46: 311-316.

Xu, X., Li, B., Kennedy, J.F., Xie, B.J. and Huang, M. 2007. Characterization of konjac glucomannan-gellan gum blend films and their suitability for release of nisin incorporated therein. Carbohyd. Polym. 70: 192-197.

Yang, L. and Paulson, A.T. 2000. Effects of lipids on mechanical and moisture barrier properties of edible gellan film. Food Res. Int. 33: 571-578.

Yildirim, A., Mavi, A., Oktay, M., Kara, A.A., Algur, O. and Bilaloglu, V. 2000. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea Desf ex DC*), sage (*Salvia triloba* L.), and black tea (*Camellia sinensis*) extracts. J. Agr. Food Chem. 48: 5030-5034.

Yildirim, M. and Hettiarachchy, N.S. 1997. Biopolymers produced by cross-linking soybean 11S globulin with whey proteins using transglutaminase. J. Food Sci. 62: 270-275.

Yue, H.B., Cui, Y.D., Shuttleworth, P.S. and Clark, J.H. 2012. Preparation and characterisation of bioplastics made from cottonseed protein. Green Chem. 14: 2009-2016.

Zheljazkov, V.D., Astatkie, T. and Hristov, A.N. 2012. Lavender and hyssop productivity, oil content, and bioactivity as a function of harvest time and drying. Ind. Crop. Prod. 36: 222-228.

Zhong, Q.-P. and Xia, W.-S. 2008. Physicochemical properties of chitosan-based films. Food Technol. Biotechnol. 46: 262-269.

Ziani, K., Osés, J., Coma, V. and Maté, J.I. 2008. Effect of the presence of glycerol and Tween 20 on the chemical and physical properties of films based on chitosan with different degree of deacetylation. Food Sci. Technol. Int. 41: 2159-2165.

Zinoviadou, K.G., Koutsoumanis, K.P. and Biliaderis, C.G. 2009. Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. Meat Sci. 82: 338-345.

Zivanovic, S., Chi, S. and Draughon, A.F. 2005. Antimicrobial activity of chitosan films enriched with essential oils. J. Food Sci. 70: 45-51.

CHAPTER 2

PROPERTIES AND ANTIOXIDANT ACTIVITY OF FISH SKIN GELATIN FILM INCORPORATED WITH CITRUS ESSENTIAL OILS

2.1 Abstract

Properties of protein-based film from fish skin gelatin incorporated with different citrus essential oils, including bergamot, kaffir lime, lemon and lime (50% based on protein) in the presence of 20 and 30% glycerol were investigated. Films containing 20% glycerol had higher tensile strength (TS) but lower elongation at break (EAB), compared with those prepared with 30% glycerol, regardless of essential oils incorporated ($p < 0.05$). Films incorporated with essential oils, especially from lime, at both glycerol levels showed the lower TS but higher EAB than the control films ($p < 0.05$). Water vapor permeability (WVP) of films containing essential oils was lower than that of control films for both glycerol levels ($p < 0.05$). Film with essential oils had varying ΔE, where the highest value was observed in that added with bergamot essential oil ($p < 0.05$). Higher glycerol content increased EAB and WVP but decreased TS of films. Fourier transforms infrared (FTIR) spectra indicated that films added with essential oils exhibited higher hydrophobicity with higher amplitude at wavenunber of 2874-2926 cm^{-1} and 1731-1742 cm^{-1} than control film. Film incorporated with essential oils exhibited slightly lower thermal degradation resistance, compared to the control film. Varying effect of essential oil on thermal degradation temperature and weight loss was noticeable, but all films prepared using 20% glycerol had higher thermal degradation temperature with lower weight loss, compared with those containing 30% glycerol. Films added with all types of essential oils had rougher cross-section, compared with control films, irrespective of glycerol levels. Nevertheless, smooth surface was observed in all film samples. Film incorporated with lemon essential oil showed the highest ABTS radical scavenging activity and ferric reducing antioxidant power (FRAP) ($p < 0.05$), while the other films had lower activity. Thus, the incorporation of different essential oils and glycerol levels directly affected the properties of gelatin-based film from fish skin.

2.2 Introduction

Development of biodegradable packaging materials is breakthrough alternative to synthetic material packaging from petrochemical products, which are non-biodegradable and have the negative impact on environment. Moreover, biodegradable materials are eco-friendly, non-toxic and have been used to prepare biodegradable films and coating for food preservation and protection. The biodegradable film can be made from neutral biopolymers, including polysaccharides, proteins and lipids or the combination of these materials (Huber *et al.*, 2009; Tharanathan, 2003). Among these biopolymers, proteins have been impressively 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, 1999). The protein-based films have excellent oxygen, carbon dioxide and volatile compounds barrier properties, in comparison with synthetic film under low relative humidity condition (Limpan *et al.*, 2010).

Gelatin is a natural biopolymer, which can be derived from collagen. It has gained more attention as a new material for edible films (Arvanitoyannis, 2002; Jongjareonrak *et al.*, 2006a). Gelatin edible films prepared from bovine and porcine skin with high puncture strength, low puncture deformation and high water vapor permeability were reported (Sobral *et al.*, 2001). Fish skin gelatin, which had poor gelling property, can be used as film forming material, to wider its application. Generally, properties of film vary depending on the source of gelatin, plasticizer and other factors (Gomez-Guillen *et al.*, 2009). Mechanical properties of film from brownstripe red snapper skin gelatin were superior to those of bigeye snapper skin gelatin film at any protein and plasticiser concentrations tested (Jongjareonrak *et al.*, 2006b). However, gelatin films have poor water barrier vapor property, thereby limiting its use as potential packaging (Gomez-Guillen *et al.*, 2009; Krochta, 1997). This is due to its hydrophilicity in nature (Gennaidios *et al.*, 1993; Krochta, 2002). To tackle this problem, the incorporation of hydrophobic substances such as lipid, fatty acid, wax, etc has been implemented to improve water barrier property (Limpisophon *et al.*, 2010; Prodpran *et al.*, 2007; Soazo *et al.*, 2011).

Essential oils from various plants have been known to exhibit antioxidant property, which can extend shelf-life by lowering lipid oxidation in foods (Oussalah *et al.*, 2004; Zivanovic *et al.*, 2005). Therefore the incorporation of essential oils, especially from various citrus, into the films could be an approach to lower water vapor permeability. Simultaneously, smart edible film with antioxidant activity can be gained. However, rare information regarding the improvement of water barrier property of fish gelatin-based film, using citrus essential oils has been reported. This study aimed to elucidate the impact of different citrus essential oils on properties of fish skin gelatin film.

2.3 Materials and Methods

2.3.1 Fish gelatin and chemicals

Fish skin gelatin (~240 bloom) was purchased from Lapi Gelatine S.p.A (Empoli, Italy). Essential oils from bergamot, kaffir lime, lemon and lime were purchased from Botanicessence (Bangkok, Thailand).

2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), glycerol and Tween 20 were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.3.2 Preparation of film from gelatin incorporated with different essential oils

To prepare film forming solution (FFS), 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 concentrations of 20% and 30% (w/w) of protein content was used as a plasticizer. Different essential oils previously mixed with Tween 20 at 25% (w/w, based on essential oil) were added into FFS at a level of 50% (w/w, based on protein content). FFS was homogenized at 22,000 rpm for 3 min using a homogeniser (IKA Labortechnik homogeniser, Selangor, Malaysia). The dissolved air in the FFS was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim Germany) for 30 min at room temperature.

To prepare the film, FFS (4 g) 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). The resulting films were manually peeled off and subjected to analyses. Control films were prepared from FFS without essential oils added.

2.3.3 Determination of film properties

2.3.3.1 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 average thickness determination.

2.3.3.2 Mechanical properties

Prior to testing the mechanical properties, 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.

2.3.3.3 Water vapor permeability (WVP)

WVP was measured using a modified ASTM method (American Society for Testing & Materials, 1989) as described by Shiku *et al.* (2004). The film was sealed on an aluminum permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and a rubber gasket to hold the film 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:

$$WVP\ (gm^{-1}s^{-1}Pa^{-1}) = wlA^{-1}t^{-1}(P_2 - P_1)^{-1}$$

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 (Pa).

2.3.3.4 Color differences

Color differences of film samples were determined using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA). D_{65} (day light) and a measure cell with opening of 30 mm was used. 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 the color parameter of the white standard (L^* = 93.45, a^* = - 0.81, b^* = 0.33).

2.3.3.5 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):

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

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.

2.3.3.6 Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy

Prior to analysis, films were conditioned in a desiccator containing P_2O_5 for 2 weeks at room temperature to obtain the most dehydrated films (Sobral *et*

al., 2001). Films were scanned with a Bruker Model Equinox 55 FTIR spectrometer (Bruker Co., Ettlingen, Germany) equipped with a horizontal ATR Trough plate crystal cell (45º ZnSe; 80 mm long, 10 mm wide and 4 mm thick) (PIKE Technology Inc., Madison, WI, USA) at 25 ºC as described by Nuthong *et al.* (2009). Films were placed onto the crystal cell and the cell was 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 were ratioed against a background spectrum recorded from the clean empty cell at 25 ºC.

2.3.3.7 Thermo-gravimetric analysis (TGA)

Prior to testing, films were conditioned in a desiccator containing P_2O_5 for 2 weeks at room temperature. Conditioned films were scanned using a thermo-gravimetric analyzer (TGA7, PerkinElmer, Norwalk, CT, USA) from 50 to 600 ºC at a rate of 10 ºC/min (Nuthong *et al*., 2009). Nitrogen was used as the purge gas at a flow rate of 20 ml/min.

2.3.3.8 Scanning electron microscopy (SEM)

Morphology of surface and cross-section of film samples were 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.

2.3.4 Determination of antioxidative activity of fish skin gelatin films incorporated with essential oils

Films were solidified using liquid nitrogen in mortar and ground with a pestle. Ground film (0.25 g) was mixed with 5 ml of methanol and stirred vigorously for 3 h. The mixture was centrifuged at 2700 xg for 10 min using a centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Beckman Coulter, Inc., Palo Alto, CA, USA). The supernatant obtained was analyzed for DPPH radical scavenging activity, ABTS radical scavenging activity and ferric reducing antioxidant power.

2.3.4.1 DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Wu *et al.* (2003) with a slight modification. Sample (1.5 ml) was added with 1.5 ml of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in 95% ethanol. The mixture was mixed vigorously and allowed to stand at room temperature in dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. Sample blank was prepared in the same manner except that 95% methanol was used instead of DPPH solution. A standard curve was prepared using Trolox in the range of 10 to 60 μM. The activity was calculated after the sample blank substraction and expressed as μmol Trolox equivalents (TE)/g dried film.

2.3.4.2 ABTS radical scavenging activity

ABTS radical scavenging activity was assayed as per the method of Arnao *et al.* (2001) with a slight modification. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS solution with 50 ml of methanol in order to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using spectrophotometer. Fresh ABTS solution was prepared for each assay. Sample (150 μl) was mixed with 2850 μl of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm using a spectrophotometer. Sample blank was prepared in the same manner except that methanol was used instead of ABTS solution. A standard curve of Trolox ranging from 50 to 600 μM was prepared. The activity was calculated after sample blank subtraction and was expressed as μmol Trolox equivalents (TE)/g dried film.

2.3.4.3 Ferric reducing antioxidant power (FRAP)

FRAP was assayed according to the method of Benzie and Strain (1996). Stock solutions included 30 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. A

working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3 \cdot 6H_2O$ solution. The mixed solution was incubated at 37 °C for 30 min in a water bath (Memmert, D-91126, Schwabach, Germany) and was referred to as FRAP solution. A sample (150 µl) was mixed with 2850 µl of FRAP solution and kept for 30 min in dark at room temperature. The ferrous tripyridyltriazine complex (colored product) was measured by reading the absorbance at 593 nm. Sample blank was prepared by omitting $FeCl_3$ from FRAP solution and distilled water was used instead. The standard curve was prepared using Trolox ranging from 50 to 600 µM. The activity was calculated after sample blank subtraction and was expressed as µmol Trolox equivalents (TE)/g dried film.

2.3.5 Statistical analysis

All experiments were run in triplicate. 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, 1980). Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL, USA).

2.4 Results and Discussion

2.4.1 Mechanical properties and thickness

Mechanical properties and thickness of fish skin gelatin films incorporated with different essential oils (bergamot, kaffir lime, lemon and lime) along with 20 or 30% glycerol are shown in Table 9. The thickness of fish skin gelatin films incorporated with essential oils was higher than that of the control film (without incorporated essential oils), regardless of types of essential oil incorporated ($p < 0.05$) (Table 9). When the same type of essential oil was used, there were no differences in thickness between films containing 20 and 30% glycerol ($p > 0.05$). Negligible differences in thickness of gelatin-based films with the different levels of glycerol were reported (Hoque *et al.*, 2011; Vanin *et al.*, 2005).

At the same level of glycerol used, the control film showed higher TS, but lower EAB, compared with those incorporated with essential oils ($p < 0.05$). At

the same level of glycerol, film containing bergamot essential oil had the highest TS with coincidentally lowest EAB, compared with those incorporated with other essential oils ($p < 0.05$). On the other hand, that added with lime essential oil exhibited the lowest TS but highest EAB ($p < 0.05$). Films prepared using 20% glycerol had higher TS, but lower EAB than those with 30% glycerol ($p < 0.05$). Similar results have been reported for gelatin film from cuttlefish skin and, where increasing glycerol content enhanced the flexibility of film (Hoque *et al.*, 2011). Addition of essential oils at 50% possibly resulted in the lowered interaction between gelatin molecules. As a consequence, the decrease in rigidity with the concomitant increase in extensibility/elasticity of film was gained. 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). Hoque *et al.* (2010) reported that gelatin film was mainly stabilized by the weak bond including hydrogen bond and hydrophobic interaction. Interactions between polar polymer molecules are much stronger than those between non-polar molecules (lipid) and between polar polymer and non-polar molecules (Yang and Paulson, 2000). Among all essential oils tested, lime essential oil showed the highest plasticizing effect than others. This was most likely governed by the different components in different essential oils. However, strengthening effect of essential oil were reported in various protein-based film. Atarés *et al.* (2010) reported that the incorporation of cinnamon essential oil into soybean protein isolate (SPI) increased the tensile strength of film. Essential oil might induce rearrangement in protein network in the way which strengthened the films. Some compounds in essential oil might be able to cross-link gelatin chain, thereby increasing the rigidity of film. Essential oils consist of various compounds and high concentration of phenolic compounds such as carvacrol, eugenol and thymol were reported (Burt, 2004). The phenolic compounds were able to react with more than one protein site and led to protein cross-links (Haslam, 1989). Therefore, different compounds had varying properties, affecting the film matrix differently.

2.4.2 Water vapor permeability (WVP)

WVP of fish skin gelatin films prepared using 20 and 30% glycerol and incorporated with different essential oils is shown in Table 9. When comparing the effect of glycerol on WVP of control films, that containing 20% glycerol had lower WVP than did that with 30% glycerol ($p < 0.05$). Hydrophilic glycerol could increase the hydrophillicity of films. When all essential oils were incorporated, the decrease in WVP was obtained, compared with the control ($p < 0.05$), regardless of glycerol content. There were no differences in WVP among films incorporated with different essential oils when 20% glycerol was used ($p > 0.05$). For films containing 30% glycerol, the lowest WVP was observed in that incorporated with lemon essential oil ($p < 0.05$). It was noted that no difference in WVP between films added with the same essential oil when different glycerol contents were used, except for films added with lemon essential oil. Therefore, the incorporation of essential oils into gelatin film directly affected WVP of resulting films. Monoterpene hydrocarbon was generally found in essential oils (Ikeda *et al.*, 1962) and contributed to increased hydrophobicity of film. The presence of a hydrophobic disperse phase, even at small ratio, limits water vapor transfer since it introduces the interfere in hydrophilic phase and increases the tortuosity factor of mass transfer (Atarés *et al.*, 2010). Furthermore, water vapor transfer normally occurs through the hydrophilic portion of film network and depends on hydrophilic/hydrophobic ratio of film constituent (Hernandez, 1994). Similar results were observed for thyme essential oil incorporated hake protein-based film, in which the decrease of WVP was obtained with increasing levels of essential oil (Pires *et al.*, 2011).

Table 9. Properties of films from fish skin gelatin containing 20 and 30% glycerol in the presence of different essential oils at 50% of protein.

Glycerol (%)	Essential oils	TS (MPa)	EAB (%)	WVP ($x10^{-11}$ $gm^{-1}s^{-1}Pa^{-1}$)	Thickness (mm)
20	Control	49.09 ± 2.34 ^{a,A}*	9.61 ± 2.06 ^{e,A}	3.62 ± 0.26 ^{a,B}	0.040 ± 0.001 ^{b}
	Bergamot	42.42 ± 2.03 ^{b,A}	15.29 ± 2.92 ^{d,B}	3.15 ± 0.31 ^{b,A}	0.046 ± 0.002 ^{a}
	Kaffir Lime	36.87 ± 1.33 ^{c,A}	31.43 ± 4.29 ^{c,A}	2.95 ± 0.23 ^{b,A}	0.046 ± 0.002 ^{a}
	Lemon	32.82 ± 1.17 ^{d,A}	39.06 ± 5.10 ^{b,B}	2.81 ± 0.04 ^{b,A}	0.047 ± 0.001 ^{a}
	Lime	27.32 ± 1.75 ^{e,A}	52.21 ± 5.62 ^{a,B}	2.91 ± 0.11 ^{b,B}	0.047 ± 0.003 ^{a}
30	Control	42.86 ± 4.97 ^{a,B}	13.25 ± 3.99 ^{d,A}	4.07 ± 0.17 ^{a,A}	0.041 ± 0.001 ^{b}
	Bergamot	36.52 ± 1.75 ^{b,B}	19.96 ± 3.72 ^{d,A}	3.22 ± 0.11 ^{b,A}	0.046 ± 0.002 ^{a}
	Kaffir Lime	34.22 ± 0.89 ^{b,B}	30.93 ± 5.46 ^{c,A}	3.38 ± 0.23 ^{b,A}	0.047 ± 0.001 ^{a}
	Lemon	31.06 ± 2.31 ^{c,B}	52.66 ± 6.14 ^{b,A}	2.85 ± 0.15 ^{c,A}	0.047 ± 0.003 ^{a}
	Lime	25.87 ± 4.48 ^{d,A}	69.79 ± 7.39 ^{a,A}	3.37 ± 0.08 ^{b,A}	0.046 ± 0.002 ^{a}

* Mean ± SD (n=3).

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

Different capital letters in the same column under the same essential oil indicate significant differences ($p < 0.05$).

2.4.3 Color differences, light transmittance and film transparency

Total color difference (ΔE^*) of films containing 20 and 30% glycerol, which were incorporated with bergamot, kaffir lime, lemon and lime essential oil are shown in Table 10. Among all samples, that incorporated with bergamot essential oil had the highest ΔE^* ($p < 0.05$). Higher ΔE^* in agreement with the higher b^*-value and lower a^*-value of films (data not shown). This might be due to the yellow color of bergamot essential oil. However, ΔE^* of film added with kaffir lime essential oil was lower than that of control film ($p < 0.05$). Therefore, incorporation of different essential oils had an impact on the color of resulting gelatin film.

Light transmission at selected wavelengths from 200 to 800 nm in UV and visible ranges and transparency value of films from fish skin gelatin containing glycerol (20 and 30%) and different essential oils (Bergamot, Kaffir Lime, Lemon and Lime) are shown in Table 10. Decreases in light transmission of films incorporated with essential oils for both glycerol levels (20 and 30%) were observed at all wavelengths, compared with control film. The result indicated that essential oils were able to impede the light transmission through the film. This decreased light transmission was possibly due to the scattering of light at the interface of essential oil droplets imbedded in the film matrix. All films from fish skin gelatin had a good barrier property in the UV-ranges (200-280 nm), especially for film incorporated with all types of essential oils. The higher UV light barrier capacity was reported for gelatin films from bigeye snapper and brownstripe red snapper skin (Jongjareonrak *et al.*, 2006a) and cuttlefish skin (Hoque *et al.*, 2011). For visible range, films added with essential oil, especially that containing lemon essential oil, showed the better barrier property for light transmission. This was more likely due to the increase in opaqueness of films containing essential oils, which were distributed throughout the films.

Table 10. Color difference, light transmittance and transparency value of films from fish skin gelatin containing 20 and 30% glycerol in the presence of different essential oils at 50% of protein.

Glycerol (%)	Essential oils	Light transmittance (%) at different wavelength (nm)								Transparency value	ΔE^*
		200	280	350	400	500	600	700	800		
20	Control	0.00	55.70	79.94	82.41	85.12	86.66	87.69	88.43	$2.14 \pm 0.21^{d,A}$*	2.31 ± 0.08^{c}
	Bergamot	0.00	15.17	40.34	61.12	66.98	69.51	71.23	72.76	$4.28 \pm 0.01^{c,A}$	2.92 ± 0.07^{a}
	Kaffir Lime	0.00	23.51	47.57	53.35	59.52	62.98	65.70	68.32	$5.48 \pm 0.01^{b,A}$	2.08 ± 0.11^{d}
	Lemon	0.00	16.45	42.35	48.79	55.46	59.52	62.54	65.14	$5.46 \pm 0.01^{b,A}$	2.45 ± 0.10^{b}
	Lime	0.00	26.01	49.25	54.90	60.95	64.26	66.78	69.19	$5.66 \pm 0.10^{a,A}$	2.30 ± 0.02^{c}
30	Control	0.00	53.67	81.33	83.67	85.89	86.99	87.72	88.14	$2.15 \pm 0.01^{d,A}$	2.39 ± 0.05^{c}
	Bergamot	0.00	19.34	40.74	63.47	69.01	71.32	73.04	74.71	$4.45 \pm 0.23^{c,A}$	3.19 ± 0.04^{a}
	Kaffir Lime	0.01	18.61	47.52	53.40	59.52	62.87	65.54	68.13	$5.65 \pm 0.19^{a,A}$	2.09 ± 0.08^{d}
	Lemon	0.01	22.26	46.31	51.91	57.61	61.21	64.06	66.58	$5.31 \pm 0.06^{b,B}$	2.45 ± 0.05^{b}
	Lime	0.01	20.78	50.14	55.89	61.83	64.93	67.20	69.37	$5.46 \pm 0.07^{ab,A}$	2.25 ± 0.04^{c}

* Mean $\pm$ SD (n=3).

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

Different capital letters in the same column under the same essential oil indicate significant differences ($p < 0.05$).

Based on transparency value (Table 10), films incorporated with all types of essential oils had the lower transparency than control film containing both glycerol levels (20 and 30%) as evidenced by the higher transparency value. Higher transparency value indicated that the film had the lower transparency. Essential oil droplets localized in the film matrix lowered the transparency of gelatin film more likely due to the light scattering effect. Film incorporated with bergamot essential oil was more transparent than those added with other essential oils ($p < 0.05$). Therefore, the incorporation of essential oil had the impact on appearance and light barrier properties of gelatin film.

2.4.4 Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra of films from fish skin gelatin containing 20 and 30% glycerol in the presence of different essential oils are illustrated in Figure 6A and 6B, respectively. Generally, films containing both glycerol levels, with and without the addition of different essential oils showed the similar major peaks but the amplitudes of peaks varied. The band situated at the wavenumber of 1033-1039 cm^{-1} was found in all film samples, corresponding to the glycerol (OH group) added as a plasticizer (Bergo and Sobral, 2007). All films had the similar spectra in the range of 1700-700 cm^{-1}, covering with amide-I, II and III. All films had the major bands at 1630 cm^{-1} (amide-I, illustrating C=O stretching/hydrogen bonding coupled with COO), 1538 cm^{-1}(amide-II, presenting the bending vibrations of N-H groups and stretching vibrations of C-N groups) and 1237 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.*, 2004a). Hoque *et al.* (2010) also found the similar spectra for cuttlefish skin gelatin film, where amide-I, II and III bands were found at the wavenumbers of 1630, 1539 and 1235 cm^{-1}, respectively. An amide-A band was observed at the wavenumber of 3272-3271 cm^{-1} for all film samples. Amide-B band was also found at 2923-2925 cm^{-1} for all film samples. The amide-A band represented the NH-stretching coupled with hydrogen bonding and amide-B band represented the CH stretching and $-NH_3^+$ at wavenumber of 2928 cm^{-1} (Muyonga *et al.*, 2004b). Amplitude of peaks at wavenumbers 2853.62 $cm^{-1}$and 2923.58 cm^{-1} increased when the films were incorporated with essential oils. Those peaks represent the methylene

asymmetrical and symmetrical stretching vibration of the aliphatic C-H in CH_2 and CH_3 groups, respectively (Guillén and Cabo, 1997; Guillén and Cabo, 2004; Muik *et al.*, 2007). Both the methylene asymmetrical stretching bands at approximately 2853 cm^{-1} and methylene symmetrical stretching band near 2924 cm^{-1} were obviously present in most lipids (Guillén and Cabo, 2004). The spectra of control films (without incorporated essential oil) for both 20 and 30% glycerol levels showed the highest amplitude of amide-A followed by films incorporated with kaffir lime, bergamot, lime and lemon essential oils, respectively. Film incorporated with lemon essential oil exhibited the highest amplitude for amide-B peak and peak with wavenumber of 2877 cm^{-1}, followed by film added with lime, bergamot, kaffir lime essential oil, respectively, irrespective of glycerol content. As a result, films incorporated with essential oils generally had the increases in amplitude of peaks at wavenumbers of 2854 $cm^{-1}$and 2924 cm^{-1}. This was in agreement with the decreases in amplitude of peaks at wavenumbers of 3284 cm^{-1} (amide-A). Among all types of essential oils, film incorporated with lemon essential oil more likely had the highest hydrophobicity of film, as evidenced by the highest amplitude of peaks at wavenumbers of 2854 $cm^{-1}$and 2924 cm^{-1}, corresponding to CH of CH_2, CH_3 hydrophobic moieties.

Furthermore, the strong peak was observed at about 1742 cm^{-1} for film incorporated with lime essential oils, regardless of glycerol levels, while the tiny peaks at wavenumbers of 1731-1734 cm^{-1} were found in films incorporated with bergamot, kaffir lime and lime essential oils. Those peaks more likely represented the C=O stretching vibration of aldehyde or ester carbonyl groups (Guillén and Cabo, 1997; Guillén and Cabo, 2004; Muik *et al.*, 2007). The difference in peak position would depend on the varying compounds constituted in difference essential oils. Nevertheless, there was no peak at around 1742-1734 cm^{-1} found in control film. Addition of essential oils in gelatin film could increase the hydrophobicity of film, as evidenced by existing peak at wavenumber of 2854 cm^{-1}, 2924 cm^{-1} and 1745 cm^{-1}, especially for film added with lemon essential oil. This was supported by the lowest WVP of film incorporated with lemon essential oil for both glycerol levels (Table 9). Therefore, the incorporation of essential oils into gelatin film could decrease WVP of fish skin gelatin film.

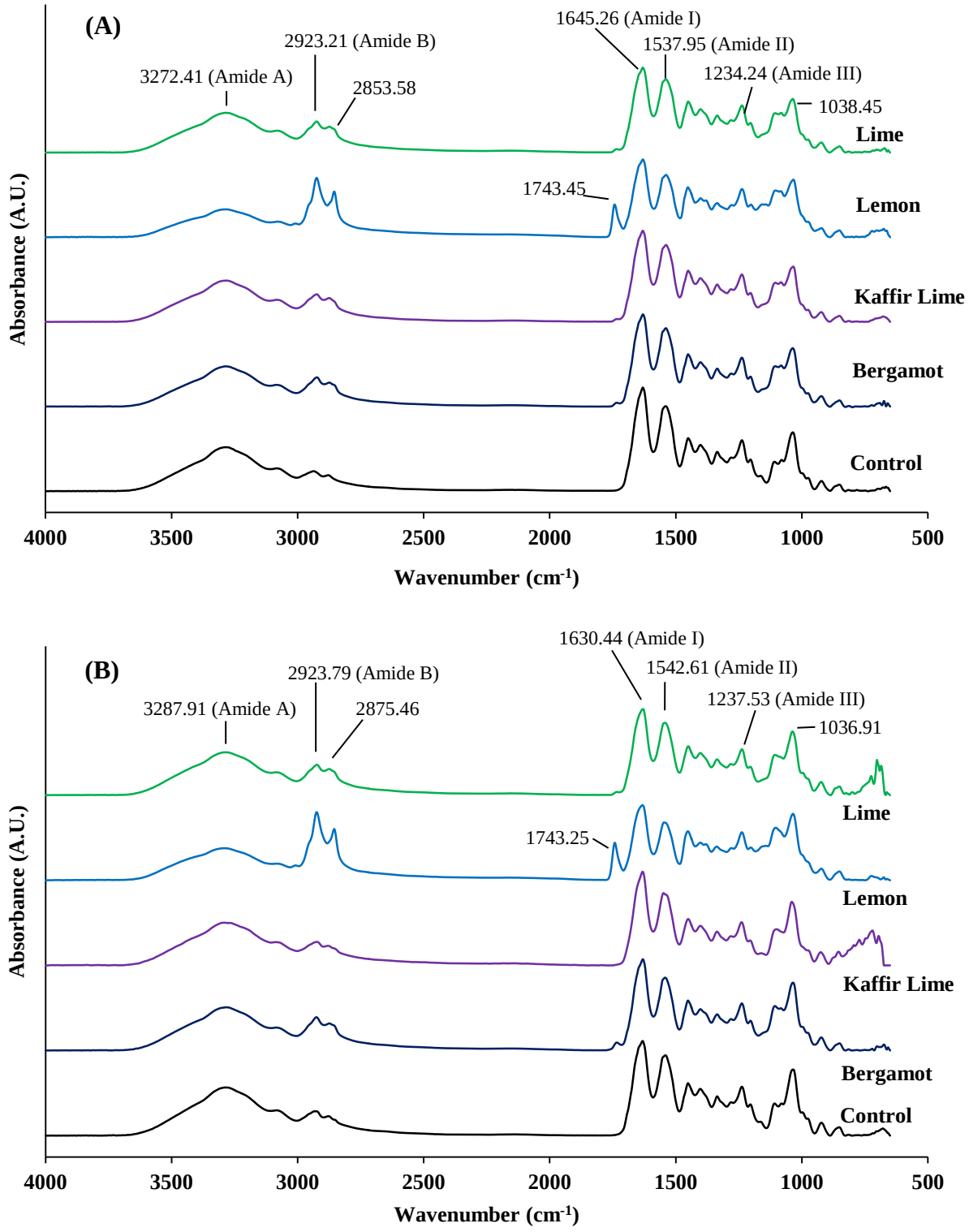


Figure 6. ATR-FTIR spectra of films from fish skin gelatin containing 20% (A) and 30% (B) glycerol in the presence of different essential oils at 50% of protein.

2.4.5 Thermal-gravimetric analysis (TGA)

TGA thermograms representing thermal degradation behavior of films containing 20 and 30% glycerol in the presence of different essential oils are presented in Figure 7. The degradation temperatures (T_d), weight loss (Δw) and residue of all film samples are showed in Table 11. Both control films with 20 and 30% glycerol exhibited three main stages of weight loss. However, four main stages of weight loss were found in films incorporated with all types of essential oils, regardless of glycerol levels. For all films, the first stage weight loss at 3.91-7.81% was observed with degradation temperature (T_{d1}) of 67.50-87.50 ºC. The weight loss at this temperature range was possibly due to the loss of free water as well as other volatile compounds absorbed in the film. It was noted that lower weight loss was found in film incorporated with essential oil, suggesting lower water absorbed in the film matrix, due to higher hydrophobicity of those films, compared to the control film. The similar result was observed in cuttlefish skin gelatin film (Hoque *et al.*, 2011) and porcine-plasma protein film (Nuthong *et al.*, 2009). The second stage of weight loss (Δw_2 = 14.28-28.37%) appeared at T_{d2} of 200.75-239.92 ºC. This transition revealed the degradation of film matrix. This result was in agreement with Hoque *et al.* (2011) who reported that the degradation temperature in the range of 196.30-216.71 ºC found in cuttlefish skin gelatin film was mostly associated with the loss of glycerol compound (plasticiser) and smaller size protein fraction as well as structurally bound water. For the third stage of weight loss, Δw_3 was 36.46-49.39% and T_{d3} of 305.48-322.69 ºC were obtained for all film samples, mostly associated with the degradation of the larger-size or associated protein fraction. For the fourth stage of weight loss, Δw_4 was 13.13-16.95% and 13.45-17.88% with T_{d4} of 401.92-417.64 ºC and 385.60-413.47 ºC, respectively, were obtained for films incorporated with different essential oils and added with 20 and 30% glycerol, respectively. Nevertheless, for both control films, the fourth stage of weight loss (Δw_4) disappeared. It was noted that the fourth stage of weight loss might be associated with the loss of thermally stable components constituted in essential oils incorporated in film matrix.

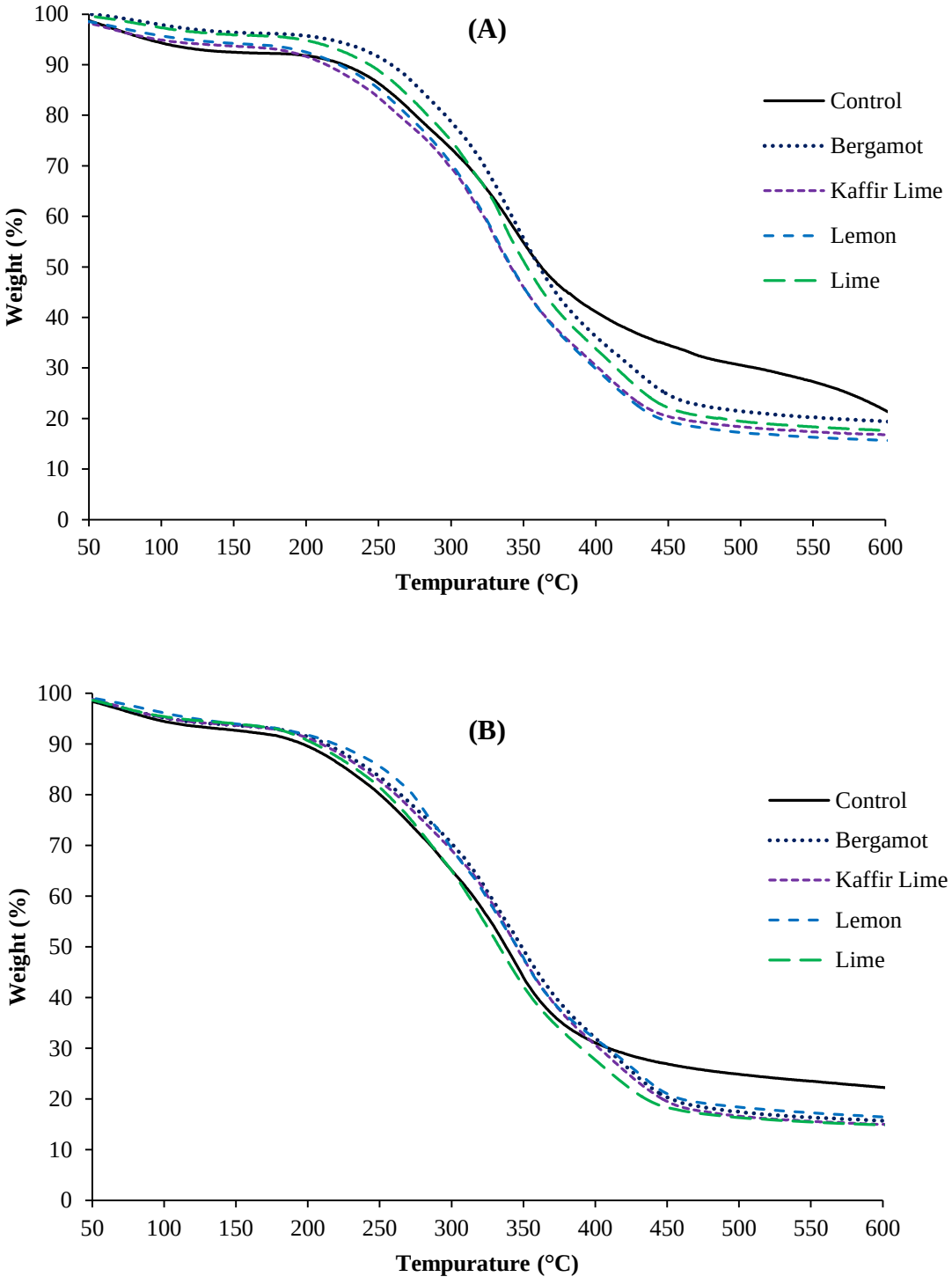


Figure 7. Thermo-gravimetric curves of films from fish skin gelatin containing 20% (A) and 30% (B) glycerol in the presence of different essential oils at 50% of protein.

Table 11. Thermal degradation temperatures (T_d, ºC) and weight loss (Δw, %) of films from fish skin gelatin containing 20 and 30% glycerol in the presence of different essential oils at 50% of protein.

Glycerol (%)	Essential oils	Δ_1		Δ_2		Δ_3		Δ_4		Residue (%)
		$Td_{1,onset}$	Δw_1	$Td_{2,onset}$	Δw_2	$Td_{3,onset}$	Δw_3	$Td_{4,onset}$	Δw_4	
20	Control	75.05	7.81	229.81	20.05	322.69	49.23	-	-	22.01
	Bergamot	84.21	3.91	239.92	14.28	318.79	48.84	417.64	13.13	19.13
	Kaffir Lime	70.00	6.25	211.49	15.03	319.28	47.11	401.92	14.83	16.79
	Lemon	77.50	5.76	221.68	14.52	310.24	49.39	405.01	13.35	15.62
	Lime	87.50	4.21	233.59	16.50	313.53	44.97	411.87	14.33	17.37
30	Control	77.50	7.12	215.65	28.37	318.89	42.79	-	-	21.72
	Bergamot	73.75	6.51	209.37	18.73	315.57	45.89	413.47	13.45	15.43
	Kaffir Lime	67.50	6.17	200.75	21.32	314.91	39.98	395.07	17.88	14.65
	Lemon	80.00	6.37	220.54	24.84	309.37	36.46	409.07	16.16	16.17
	Lime	72.50	5.69	218.14	26.46	305.48	36.75	385.60	16.69	14.41

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.

Additionally, lower T_{d3} was found in films incorporated with all essential oils, compared with the control films for both glycerol levels used. This was coincidental with the higher weight loss. This might be owing to the plasticizing effect in which incorporated essential oils might impede interaction between gelatin molecules in the film network. This result was in agreement with the lowered TS and higher EAB of films incorporated with essential oils (Table 9). Film prepared using 20% glycerol with and without incorporated essential oils showed the higher heat resistance than those prepared using 30% glycerol as evidenced by the higher T_{d2}, T_{d3} and T_{d4} and lower weight loss for Δw_2, Δw_3 and Δw_4. This reconfirmed that glycerol at higher content interfered protein interaction in film network. Hoque *et al.* (2011) reported that the higher content of plasticizer was responsible for hindering the protein-protein interaction, resulting in the higher heat sensitivity. In general, higher residue from thermal degradation was found in films incorporated with essential oils, compared with the control film, regardless of glycerol levels. Thus, both glycerol contents and essential oils had the marked impact on thermal stability of gelatin film.

2.4.6 Film morphology

SEM micrographs of the surface and freeze-fractured cross-section of films from fish skin gelatin containing glycerol at 20 and 30% and different essential oils are illustrated in Figure 8. The control film (without incorporated essential oils) had the smooth and continuous surface for both glycerol levels. Smooth surface was also observed in the films incorporated with essential oils, regardless of glycerol content. This result indicated that FFS had the stable emulsion system and no collapse of emulsion occurred during FFS dehydration. For cross-section, the control films prepared at 20 and 30% glycerol had smooth and compact structure. Nevertheless, the control film containing 20% glycerol had some discontinuous zone, compared with others. With the addition of essential oils, the cross-section of film became rougher, compared with the control film when the same glycerol level was used. The disruption of protein-protein interaction in the film matrix arose from droplets of essential oils would enhance roughness of film cross-section. Those oil droplets were more likely localised inside the film network as observed in cross-section, no dispersion of oil droplet on the surface of film was noticeable. The film microstructure might be

associated with the reduction in tensile strength and lower WVP of films incorporated with essential oils, compared with control film (without incorporated essential oil). Oil droplet could obstruct the transfer of water molecules through the film network (Karbowiak *et al.*, 2007). As a result, a homogeneous dispersion of oil droplets would give a lower WVP in the emulsion film.

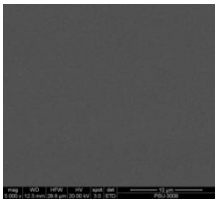
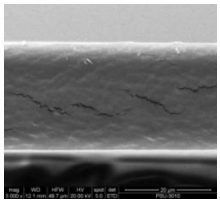
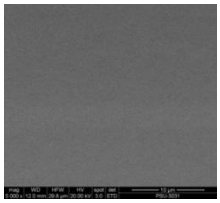
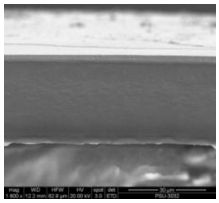
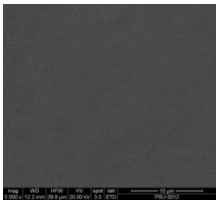
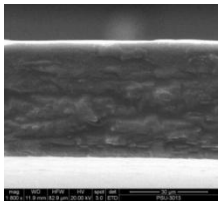
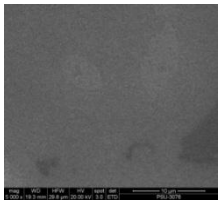
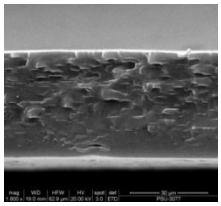
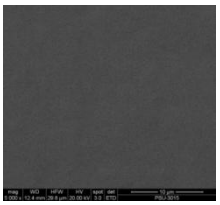
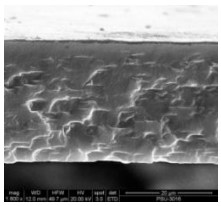
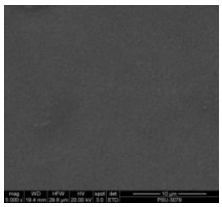
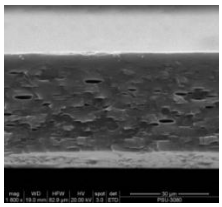
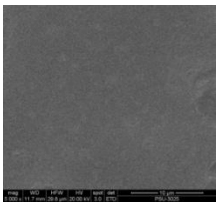
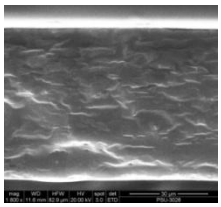
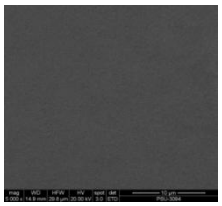
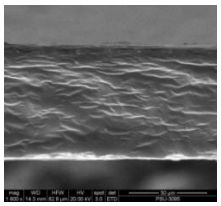
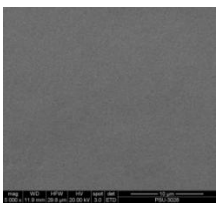
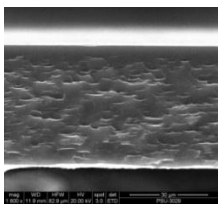
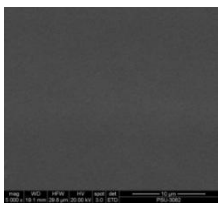
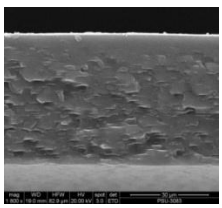
Essential oils	20% glycerol		30% glycerol	
	Surface	Cross-section	Surface	Cross-section
Control (Without essential oil)				
Bergamot				
Kaffir Lime				
Lemon				
Lime				

Figure 8. SEM micrographs of surface (5000 x) and cross-section (1800 x) of films from fish skin gelatin prepared containing 20% and 30% glycerol in the presence of different essential oils at 50% of protein.

2.4.7 Antioxidative activity of fish skin gelatin films incorporated with essential oils

Antioxidative activity expressed as DPPH radical scavenging activity, ABTS radical scavenging activity and ferric reducing antioxidant power (FRAP) of films from fish skin gelatin containing 20 and 30% glycerol in the presence of different essential oils is shown in Table 12. Control film without essential oils had no DPPH radical scavenging activity and possessed very low FRAP and ABTS radical scavenging activity. Gómez-Guillén *et al.* (2007) reported that films prepared from tuna skin gelatin showed antioxidative activity as determined by FRAP and ABTS assays. Control film containing 30% glycerol had slightly higher FRAP and ABTS radical scavenging activity, compared with that having 20% glycerol.

Generally, essential oils have been reported as the excellent source of antioxidant (Wu *et al.*, 1982). Film incorporated with all types of essential oils at both glycerol levels showed an antioxidant activity. ABTS radical-scavenging activity and FRAP of film incorporated with lemon essential oil was highest, followed by film added with bergamot essential oil. For DPPH radical-scavenging activity, film incorporated with bergamot essential oil showed the highest activity ($p < 0.05$), followed by film added with lemon essential oil. Films incorporated with different essential oils containing 30% glycerol mostly had the higher antioxidant activity analyzed by all assays than those with 20% glycerol ($p < 0.05$). This result suggested that more loosen structure of film network found in film containing 30% glycerol favored the release of essential oils, which had antioxidative activity. Furthermore, the interaction of antioxidant compounds in different essential oils might be different. As a consequence, the binding of those compounds with film matrix could be varied. Thus, the release of free antioxidant compounds could be varied.

Table 12. Antioxidant activity of films from fish skin gelatin containing 20 and 30% glycerol in the presence of different essential oils at 50% of protein.

Glycerol (%)	Essential oils	Antioxidant activity (µmol Trolox equivalents (TE)/g dried film)		
		DPPH	FRAP	ABTS
20	Control	0.00 ^{d,A}*	0.04 ± 0.01 ^{d,B}	0.14 ± 0.30 ^{d,B}
	Bergamot	0.25 ± 0.01 ^{a,B}	1.17 ± 0.14 ^{b,B}	4.33 ± 0.59 ^{b,B}
	Kaffir Lime	0.04 ± 0.02 ^{cd,B}	0.36 ± 0.11 ^{c,B}	1.98 ± 0.33 ^{c,B}
	Lemon	0.15 ± 0.01 ^{b,B}	2.76 ± 0.08 ^{a,B}	28.01 ± 2.07 ^{a,A}
	Lime	0.02 ± 0.02 ^{cd,B}	0.30 ± 0.08 ^{cd,A}	2.54 ± 0.33 ^{c,B}
30	Control	0.00 ^{d,A}	0.18 ± 0.02 ^{d,A}	0.64 ± 0.11 ^{c,A}
	Bergamot	0.42 ± 0.06 ^{a,A}	2.15 ± 0.08 ^{b,A}	6.25 ± 0.47 ^{b,A}
	Kaffir Lime	0.13 ± 0.01 ^{c,A}	0.68 ± 0.05 ^{c,A}	3.99 ± 0.64 ^{b,A}
	Lemon	0.27 ± 0.01 ^{b,A}	3.42 ± 0.08 ^{a,A}	31.18 ± 2.08 ^{a,A}
	Lime	0.09 ± 0.02 ^{c,A}	0.46 ± 0.02 ^{d,A}	4.24 ± 0.28 ^{b,A}

* Mean ± SD (n=3).

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

Different capital letters in the same column under the same essential oil indicate significant differences ($p < 0.05$).

2.5 Conclusion

The incorporation of citrus essential oils into fish skin gelatin film directly affected the mechanical properties, water vapor permeability, color and transparency of the film. The tested essential oils incorporated in fish skin gelatin film at 50% protein exhibited plasticizing effect, mostly owing to the decreased gelatin-gelatin interaction. Water vapor permeability was lowered when all types of essential oils were incorporated. Antioxidative activity of films incorporated with essential oils varied, depending on types of essential oils. Thus, an appropriate citrus essential oils could be potentially used to enhance flexibility and water vapor barrier property of fish skin gelatin film with antioxidative activity.

2.6 References

Aewsiri, T., Benjakul, S. and Visessanguan, W. 2009. Functional properties of gelatin from cuttlefish (*Sepia pharaonis*) skin as affected by bleaching using hydrogen peroxide. Food Chem. 115: 243-249.

Arnao, M.B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73: 239-244.

Arvanitoyannis, I.S. 2002. Formation and properties of collagen and gelatin films and coatings. *In* Protein-based films and coatings. (Gennadios, A., ed.). p. 275-304. CRC Press. Boca Raton.

Atarés, L., De Jesús, C., Talens, P. and Chiralt, A. 2010. Characterization of SPI-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 99: 384-391.

Benzie, I.F.F. and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. Anal. Biochem. 239: 70-76.

Bergo, P. and Sobral, P.J.A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. Food Hydrocolloid. 21: 1285-1289.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods - A review. Int. J. Food Microbiol. 94: 223-253.

Gennadios, A., Weller, C.L., Hanna, M.A. and Froning, G.W. 1996. Mechanical and barrier properties of egg albumen films. J. Food Sci. 61: 585-589.

Gennaidios, A., Brandenburg, A.H., Weller, C.L. and Testin, R.F. 1993. Effect of pH on properties of wheat gluten and soy protein isolate films. J. Agr. Food Chem. 41: 1835-1839.

Gomez-Guillen, M.C., Perez-Mateos, M., Gomez-Estaca, J., Lopez-Caballero, E., Gimenez, B. and Montero, P. 2009. Fish gelatin: a renewable material for developing active biodegradable films. Trends Food Sci. Technol. 20: 3-16.

Gómez-Guillén, M.C., Ihl, M., Bifani, V., Silva, A. and Montero, P. 2007. Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae Turcz*). Food Hydrocolloid. 21: 1133-1143.

Guillén, M.D. and Cabo, N. 1997. Infrared spectroscopy in the study of edible oils and fats. J. Sci. Food Agr. 75: 1-11.

Guillén, M.D. and Cabo, N. 2004. Study of the effects of smoke flavourings on the oxidative stability of the lipids of pork adipose tissue by means of Fourier transform infrared spectroscopy. Meat Sci. 66: 647-657.

Han, J.H. and Floros, J.D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. J. Plast. Film Sheet. 13: 287-298.

Haslam, E. 1989. Vegetable tannins revisited. *In* Plant polyphenols. (Phillipson, J. D. *et al.*, eds.). p. 230. Cambridge University Press. Cambridge.

Hernandez, E. 1994. Edible coatings for lipids and resins. *In* Edible coatings and films to improve food quality. (Krochta, J. M. *et al.*, eds.). p. 279-304. Technomic Publishing Company. Lancaster, PA.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2010. Effect of heat treatment of film-forming solution on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. J. Food Eng. 96: 66-73.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011. Effects of partial hydrolysis and plasticizer content on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. Food Hydrocolloid. 25: 82-90.

Huber, K.C., Embuscado, M.E., Pavlath, A.E. and Orts, W. 2009. Edible Films and Coatings: Why, What, and How? *In* Edible Films and Coatings for Food Applications. p. 1-23. Springer New York.

Ikeda, R.M., Stanley, W.L., Vannier, S.H. and Spitler, E.M. 1962. The Monoterpene Hydrocarbon Composition of Some Essential Oils. J. Food Sci. 27: 455-458.

Iwata, K., Ishizaki, S., Handa, A. and Tanaka, M. 2000. Preparation and characterization of edible films from fish water-soluble proteins. Fisheries Sci. 66: 372-378.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006a. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid. 20: 492-501.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006b. Effects of plasticizers on the properties of edible films from skin gelatin of bigeye snapper and brownstripe red snapper. Eur. Food Res. Technol. 222: 229-235.

Karbowiak, T., Debeaufort, F. and Voilley, A. 2007. Influence of thermal process on structure and functional properties of emulsion-based edible films. Food Hydrocolloid. 21: 879-888.

Krochta, J.M. 1997. Edible protein films and coatings. *In* Food proteins and their applications. (Damodaran, S. and Paraf, A., eds.). p. 529-549. Marcel Dekker. New York.

Krochta, J.M. 2002. Protein as raw materials for films and coatings: definitions, current status, and opportunities. *In* Protein-Based Films and Coating (Gennadios, A., ed.). p. 1-39. CRC Press. New York.

Limpan, N., Prodpran, T., Benjakul, S. and Prasarpran, S. 2010. Properties of biodegradable blend films based on fish myofibrillar protein and polyvinyl alcohol as influenced by blend composition and pH level. J. Food Eng. 100: 85-92.

Limpisophon, K., Tanaka, M. and Osako, K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionace glauca*) skin gelatin. Food Chem. 122: 1095-1101.

Muik, B., Lendl, B., Molina-Diaz, A., Valcarcel, M. and Ayora-Cañada, M.J. 2007. Two-dimensional correlation spectroscopy and multivariate curve resolution for the study of lipid oxidation in edible oils monitored by FTIR and FT-Raman spectroscopy. Anal. Chim. Acta. 593: 54-67.

Muyonga, J.H., Cole, C.G.B. and Duodu, K.G. 2004a. Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chem. 85: 81-89.

Muyonga, J.H., Cole, C.G.B. and Duodu, K.G. 2004b. Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (*Lates niloticus*). Food Chem. 86: 325-332.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009. Characterization of porcine plasma protein-based films as affected by pretreatment and cross-linking agents. Int. J. Biol. Macromol. 44: 143-148.

Oussalah, M., Caillet, S., Salmieri, S., Saucier, L. and Lacroix, M. 2004. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. J. Agr. Food Chem. 52: 5598-5605.

Pires, C., Ramos, C., Teixeira, G., Batista, I., Mendes, R., Nunes, L. and Marques, A. 2011. Characterization of biodegradable films prepared with hake proteins and thyme oil. J. Food Eng. 105: 422-428.

Prodpran, T., Benjakul, S. and Artharn, A. 2007. Properties and microstructure of protein-based film from round scad (*Decapterus maruadsi*) muscle as affected by palm oil and chitosan incorporation. Int. J. Biol. Macromol. 41: 605-614.

Shiku, Y., Hamaguchi, P.Y., Benjakul, S., Visessanguan, W. and Tanaka, M. 2004. Effect of surimi quality on properties of edible films based on Alaska pollack. Food Chem. 86: 493-499.

Soazo, M., Rubiolo, A.C. and Verdini, R.A. 2011. Effect of drying temperature and beeswax content on physical properties of whey protein emulsion films. Food Hydrocolloid. 25: 1251-1255.

Sobral, P.J.A., Menegalli, F.C., Hubinger, M.D. and Roques, M.A. 2001. Mechanical, water vapor barrier and thermal properties of gelatin based edible films. Food Hydrocolloid. 15: 423-432.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics. McGraw-Hill Book. New York.

Stevens, M.P. 1999. Polymer chemistry: an introduction. p. 551. Oxford University Press. New York.

Tharanathan, R.N. 2003. Biodegradable films and composite coatings: Past, present and future. Trends Food Sci. Technol. 14: 71-78.

Vanin, F.M., Sobral, P.J.A., Menegalli, F.C., Carvalho, R.A. and Habitante, A.M.Q.B. 2005. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. Food Hydrocolloid. 19: 899-907.

Wu, H.C., Chen, H.M. and Shiau, C.Y. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res. Int. 36: 949-957.

Wu, J., Lee, M.-H., Ho, C.-T. and Chang, S. 1982. Elucidation of the chemical structures of natural antioxidants isolated from rosemary. J. Am. Soc. 59: 339-345.

Yang, L. and Paulson, A.T. 2000. Effects of lipids on mechanical and moisture barrier properties of edible gellan film. Food Res. Int. 33: 571-578.

Zivanovic, S., Chi, S. and Draughon, A.F. 2005. Antimicrobial activity of chitosan films enriched with essential oils. J. Food Sci. 70: 45-51.

CHAPTER 3

PHYSICO-CHEMICAL PROPERTIES, MORPHOLOGY AND ANTIOXIDANT ACTIVITY OF FILM FROM FISH SKIN GELATIN INCORPORATED WITH ROOT ESSENTIAL OILS

3.1 Abstract

The influences of three root essential oils (ginger, turmeric and plai) at different levels (25, 50 and 100 %, based on protein content) on properties and antioxidative activity of fish skin gelatin-based film were investigated. Films incorporated with all essential oils showed the lower tensile strength (TS) but higher elongation at break (EAB) with increasing amount of essential oils, compared with the control film (without oil incorporated), regardless of types of essential oil ($p < 0.05$). Water vapor permeability (WVP) of films containing essential oils decreased as the amount of essential oils increased ($p < 0.05$). Decreases in L^*-value and increases in b^*-, ΔE^*- and transparency value were observed with increasing amount of essential oils incorporated ($p < 0.05$). FTIR spectra indicated that films added with essential oils, especially from plai root, exhibited higher hydrophobicity than the control film, as evidenced by higher amplitude at wavenumber of 2877-2922 cm^{-1} and 1732 cm^{-1}. Lower degradation temperature was obtained in films containing essential oils. Microstructural study revealed that bilayer films could be formed when essential oils at level above 50 % were incorporated. Film incorporated with plai and turmeric essential oils showed the higher DPPH and ABTS radical scavenging activity, respectively, ($p < 0.05$), compared with the control film and ginger essential oil added film. Thus, the incorporation of root essential oils directly affected properties of fish skin gelatin-based film, depending on types and levels incorporated.

3.2 Introduction

Biodegradable packaging includes films and coatings prepared from organic materials and biopolymers. It can be used as an alternative to plastic packaging, which is non- biodegradable and causes ecological imbalance and aesthetic deterioration of nature. The biodegradable films can be made from neutral biopolymers, including polysaccharides, proteins and lipids or the combination of these materials (Huber *et al.*, 2009; Tharanathan, 2003). Protein-based film has been received attention as a promising packaging, due to their good gas barrier properties (Cuq *et al.*, 1997; Park and Chinnan, 1995), biodegradation and environmental friendly aspect (Gennadios *et al.*, 1994).

Gelatin is a proteinaceous material derived from collagen and can form thermally reversible gels when the warm aqueous suspensions is cooled (Arvanitoyannis, 2002). It can be used as food additive, edible coating and film (Kester and Fennema, 1986) and as encapsulating agent (Moorhous and Grudon, 1994). Gelatin, especially fish gelatin, has been reported to have film forming ability; however, the gelatin film has poor water vapor barrier property (Gomez-Guillen *et al.*, 2009; Hoque *et al.*, 2010; Jongjareonrak *et al.*, 2006). This limits further application of gelatin-based film as food packaging. Therefore, the hydrophobic substances such as oils, fats, waxes and fatty acids, have been used to improve the water vapor barrier property (Limpisophon *et al.*, 2010; Prodpran *et al.*, 2007; Soazo *et al.*, 2011).

Essential oils, also called volatile odoriferous oil, are aromatic oily liquids extracted from different aromatic vegetable plant parts by physical means and widely used as food flavors (Burt, 2004a). Essential oils are the complex mixture of natural compounds. The main constituents in essential oils are terpenes (monoterpenes and sesquerpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, etc.) and terpenoids (isoprenoids) (Bakkali *et al.*, 2008; Mohamed *et al.*, 2010). Moreover, volatile oils have been reported to exhibit various antibacterial, antifungal, antiviral and antioxidant properties (Burt, 2004a; Kordali *et al.*, 2005). Additionally, essential oils are able to extend shelf-life of foods by lowering lipid oxidation (Oussalah *et al.*, 2004; Zivanovic *et al.*, 2005). Recently, Tongnuanchan *et al*. (2012) incorporated citrus peel essential oils into fish gelatin

based-film and found that it could increase water vapor barrier property. The resulting films also showed antioxidative activity (Tongnuanchan *et al.*, 2012). Essential oils from root are interest of since several roots are abundant in tropical countries including Thailand and they can serve as the potential source of essential oils. Essential oils from roots possess antioxidative activity and are hydrophobic in nature. As a consequence, they could be incorporated into gelatin based-film to improve water vapor barrier property with the concomitant antioxidative activity. Nevertheless, no information regarding the improvement of water barrier property of fish gelatin-based film using root essential oils has been reported. This study aimed to elucidate the impact of different root essential oils on properties and antioxidative activity of fish skin gelatin film.

3.3 Materials and Methods

3.3.1 Fish gelatin and chemicals

Fish gelatin produced from tilapia skin (~240 bloom) was purchased from Lapi Gelatine S.p.A (Empoli, Italy). Essential oils from the roots of ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) and plai *(Zingiber montanum)* were purchased from Botanicessence (Bangkok, Thailand).

2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), glycerol and Tween 20 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol was obtained from Merck (Darmstadt, Germany).

3.3.2 Preparation of film from gelatin incorporated with different essential oils

To prepare film forming solution (FFS), gelatin powder was mixed with distilled water to obtain the protein concentration of 3.5 % (w/v). The mixture was heated at 70 °C for 30 min. Glycerol at 30 % (w/w) of protein content was used as a plasticizer. Different essential oils previously mixed with Tween 20 at 25 % (w/w, based on essential oil) were added into FFS at levels of 25, 50, and 100 %

(w/w, based on protein content). FFS was homogenized at 22,000 rpm for 3 min using a homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The dissolved air in the FFS was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature.

To prepare the film, FFS (4 g) 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). The resulting films were manually peeled off and subjected to analyses. Control films were prepared from FFS without essential oils added.

3.3.3 Determination of film properties

3.3.3.1 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 average thickness determination.

3.3.3.2 Mechanical properties

Prior to testing the mechanical properties, 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.

3.3.3.3 Water vapor permeability (WVP)

WVP was measured using a modified ASTM method (American Society for Testing & Materials, 1989) as described by Shiku *et al.* (2004). The film was sealed on an aluminum permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and a rubber gasket to hold the film 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:

$$WVP\ (gm^{-1}s^{-1}Pa^{-1}) = wlA^{-1}t^{-1}(P_2 - P_1)^{-1}$$

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 (Pa).

3.3.3.4 Color differences

Color differences of film samples were determined using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA). D_{65} (day light) and a measure cell with opening of 30 mm was used. 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 the color parameter of the white standard (L^* = 92.85, a^* = - 1.20, b^* = 0.46).

3.3.3.5 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):

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

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 SDS-Polyacrylamide gel electrophoresis

Protein patterns of gelatin film were analyzed under non-reducing condition using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). Prior to analysis, the film samples were prepared according to the method of Jongjareonrak *et al*. (2006) with some modifications. Film samples (200 mg) were dissolved in 10 mL of 1 % (w/v) SDS. The mixture was stirred continuously at room temperature for 12 h. Supernatants were obtained after centrifuging at 3000 xg for 5 min using Hettich Zentrifuge (MIKRO-20, D-78532, Tuttlingen, Germany). Protein content of the prepared solutions of gelatin film was determined according to the Biuret method (Robinson and Hodgen, 1940). The solutions were then mixed with sample buffer (0.5 M Tris-HCl, pH 6.8 containing 4 % (w/v) SDS, 20 % (v/v) glycerol) at the ratio of 1:1 (v/v). Samples (20 mg protein) were loaded onto the polyacrylamide gel made of 7.5 % running gel and 4 % stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After electrophoresis, gel was stained with 0.05 % (w/v) Coomassie blue R-250 in 15 % (v/v) methanol and 5 % (v/v) acetic acid and destained with 30 % (v/v) methanol and 10 % (v/v) acetic acid. High molecular weight range protein markers were used to estimate the molecular weight of proteins.

3.3.3.7 Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy

Prior to analysis, films were conditioned in a desiccator containing P_2O_5 for 2 weeks at room temperature to obtain the most dehydrated films (Sobral *et al.*, 2001). Films were scanned with a Bruker Model Equinox 55 FTIR spectrometer (Bruker Co., Ettlingen, Germany) equipped with a horizontal ATR Trough plate crystal cell (45º ZnSe; 80 mm long, 10 mm wide and 4 mm thick) (PIKE Technology Inc., Madison, WI, USA) at 25 ºC as described by Nuthong *et al.* (2009). Films were placed onto the crystal cell and the cell was 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 were ratioed against a background spectrum recorded from the clean empty cell at 25 ºC.

3.3.3.8 Thermo-gravimetric analysis (TGA)

Prior to testing, films were conditioned in a desiccator containing P_2O_5 for 2 weeks at room temperature. Conditioned films were scanned using a thermo-gravimetric analyzer (TGA7, PerkinElmer, Norwalk, CT, USA) from 50 to 600 ºC at a rate of 10 ºC/min (Nuthong *et al.*, 2009). Nitrogen was used as the purge gas at a flow rate of 20 ml/min.

3.3.3.9 Scanning electron microscopy (SEM)

Morphology of surface and cross-section of film samples were 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.

3.3.4 Determination of antioxidative activity of fish skin gelatin films incorporated with essential oils

Films were solidified using liquid nitrogen in mortar and ground with a pestle. Ground film (0.25 g) was mixed with 5 ml of methanol and stirred vigorously for 3 h. The mixture was centrifuged at 2700 xg for 10 min using a centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Beckman Coulter, Inc., Palo Alto, CA, USA). The supernatant obtained was analyzed for DPPH radical scavenging activity, ABTS radical scavenging activity and ferric reducing antioxidant power.

3.3.4.1 DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Wu *et al.* (2003) with a slight modification. Sample (1.5 ml) was added with 1.5 ml of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in 95% ethanol. The mixture was mixed vigorously and allowed to stand at room temperature in dark for 30 min. The

absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. Sample blank was prepared in the same manner except that 95% methanol was used instead of DPPH solution. A standard curve was prepared using Trolox in the range of 10 to 60 μM. The activity was calculated after the sample blank substraction and expressed as μmol Trolox equivalents (TE)/g dried film.

3.3.4.2 ABTS radical scavenging activity

ABTS radical scavenging activity was assayed as per the method of Arnao *et al.* (2001) with a slight modification. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS solution with 50 ml of methanol in order to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using spectrophotometer. Fresh ABTS solution was prepared for each assay. Sample (150 μl) was mixed with 2850 μl of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm using a spectrophotometer. Sample blank was prepared in the same manner except that methanol was used instead of ABTS solution. A standard curve of Trolox ranging from 50 to 600 μM was prepared. The activity was calculated after sample blank subtraction and was expressed as μmol Trolox equivalents (TE)/g dried film.

3.3.5 Statistical analysis

All experiments were run in triplicate. 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, 1980). Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL, USA).

3.4 Results and Discussion

3.4.1 Characteristics of gelatin film incorporated with root essential oils

Gelatin films incorporated without and with three essential oils (ginger, turmeric and plai) at different levels (25, 50 and 100 % w/w, based on protein content) were generally flexible and easy to handle. Film had the increased flexibility and extensibility but decreased transparency with increasing essential oil concentration, regardless of types of essential oil used. In general, there was no oil exudates on the film incorporated with low concentration (25%) of essential oil; however, at high concentration of essential oil (100%), some oil exudates were found at the surface of the films. In the presence of root essential oils, fishy odor of gelatin films was masked. The smell intensity of essential oil in films increased with increasing essential oil level. This might limit the application of the film in food when it was incorporated at the high amount. However, smaller amount (25%) of essential oil added did not cause the detrimental effect on smell perception or unacceptability of the film.

3.4.2 Mechanical properties and thickness

Mechanical properties and thickness of fish skin gelatin films incorporated with three essential oils (ginger, turmeric and plai) at different levels (25, 50 and 100 % w/w, based on protein content) are shown in Table 13. Films incorporated with all essential oils at level of 25 % had no changes in thickness, in comparison with control film. However, the increases in film thickness were observed when essential oils at a levels of 50 and 100 % were added ($p < 0.05$), regardless of essential oil types. The interaction of essential oils between peptide chains could reduce the ordered alignment, when the compact network could not be developed.

Among all films, the control film (without essential oil added) showed the highest tensile strength (TS) and the lowest elongation at break (EAB) ($p < 0.05$), compared with others. Films incorporated with essential oils at higher levels showed lower TS, but higher EAB, regardless of types of essential oil, compared with the control ($p < 0.05$). For all films added with essential oils, the lowest TS was found in films added with 100 % essential oil ($p < 0.05$). Generally, when the level of essential

oils concentration increased, EAB of resulting films increased ($p < 0.05$). However, EAB of films incorporated with essential oils from turmeric and plai at 50 and 100 % were similar ($p > 0.05$). In general, no difference in EAB was noticed in film incorporated with different essential oils at same level. Thus, the level of essential oils incorporated had significant impact on the mechanical properties of film.

The addition of lipids, oils or fatty acids with increasing level decreased TS of protein-based films e.g., films from blue shark skin gelatin (Limpisophon *et al.*, 2010), fish muscle protein (Prodpran *et al.*, 2007; Tanaka *et al.*, 2001) and whey protein (Soazo *et al.*, 2011), etc. The incorporation of essential oils into gelatin film could enhance the development of heterogeneous film matrix, leading to discontinuity of film network. Additionally, essential oils which were in miserable in FFS more likely impeded the interaction of gelatin peptide chains. This could lower the bonding between gelatins in film network. Hydrogen bonding and hydrophobic interaction were major bonds stabilizing in fish skin gelatin film (Hoque *et al.*, 2010). Addition of lipids or oils into protein-based films may interrupt the protein network via protein-protein interaction and the lack of cohesive structure integrity of oils or lipids also contributed to poorer mechanical property (Gontard *et al.*, 1995). The reduction in film strength provided the extensibility of film. Thus, an essential oil incorporated exhibited a plasticizing effect on fish gelatin film. Nevertheless, Atarés *et al.* (2010) reported the sodium casinate-based film added with cinnamon and ginger essential oils did not have a significant effect on the tensile strength and elongation at break. This was probably due to the low content of incorporated oil. Thus, the amount of essential oil in gelatin film directly determined mechanical properties of resulting films.

3.4.3 Water vapor permeability (WVP)

WVP of fish skin gelatin films, containing 30 % glycerol, incorporated with ginger, turmeric and plai essential oils at different levels is shown in Table 13. WVP of film incorporated with essential oils decreased ($p < 0.05$), especially with increasing amount of essential oil. WVP of fish skin gelatin film decreased markedly from 3.11 to 1.88, 1.89 and 2.45 × 10^{-11} $gm^{-1}s^{-1}Pa^{-1}$ ($p < 0.05$), when films were incorporated with ginger, turmeric and plai, respectively, at a level of 100 %. The

incorporation of ginger, turmeric and plai essential oils at the highest level (100 % based on protein) reduced WVP of film by 39.54, 39.22 and 21.22 %, respectively. It was noted that ginger and turmeric essential oils were more effective in improving the water vapor barrier property than plai essential oil at all levels tested ($p < 0.05$). The result suggested different hydrophobicity of compounds present in different essential oils used. Monoterpenes are highly hydrophobic substances found in essential oils, in which the content varied with types of essential oils (Turina *et al.*, 2006). Hydrophobic essential oil could increase the hydrophobicity of films, thereby reducing the water vapor migration through the film. Generally, the blend films based on protein have the decreased WVP with increasing content of lipids or hydrocarbons. Their hydrophobicity regulates the water vapor transmission rate through oil blend films (McHugh and Krochta, 1994). This result was in agreement with Pires *et al.* (2011) who reported that thyme essential oil incorporated into hake protein-based film decreased WVP of resulting films, especially with increasing oil amount. Atarés *et al.* (2010) reported that the sodium casinate-based film incorporated with cinnamon and ginger essential oils at protein/oil ratio of 1: 0.025-0.075 had no differences in WVP. Thus, the incorporation of essential oil, particularly those from ginger and turmeric, could enhance water vapor barrier property of fish gelatin-based film.

Table 13. Properties of films from fish skin gelatin incorporated with ginger, turmeric and plai essential oils at different levels.

Essential oils	Level of incorporation (% based on protein content)	TS (MPa)	EAB (%)	WVP ($x10^{-11}$ $gm^{-1}s^{-1}Pa^{-1}$)	Thickness (mm)
Control	Without essential oil	43.62 ± 2.65 ^{a}*	19.59 ± 2.49 ^{c}	3.11 ± 0.05 ^{a}	0.043 ± 0.001 ^{d}
Ginger	25	35.73 ± 1.57 ^{b}	41.70 ± 7.84 ^{b}	2.61 ± 0.09 ^{cd}	0.041 ± 0.002 ^{d}
	50	24.48 ± 2.94 ^{f}	52.44 ± 5.57 ^{b}	2.48 ± 0.15 ^{d}	0.048 ± 0.001 ^{c}
	100	18.58 ± 2.64 ^{g}	72.03 ± 9.58 ^{a}	1.88 ± 0.09 ^{e}	0.057 ± 0.002 ^{a}
Tumeric	25	34.04 ± 1.68 ^{bc}	42.79 ± 13.08 ^{b}	2.48 ± 0.12 ^{d}	0.041 ± 0.001 ^{d}
	50	27.43 ± 1.97 ^{e}	68.79 ± 7.87 ^{a}	2.04 ± 0.15 ^{e}	0.047 ± 0.001 ^{c}
	100	23.34 ± 0.62 ^{f}	72.80 ± 9.17 ^{a}	1.89 ± 0.19 ^{e}	0.053 ± 0.002 ^{b}
Plai	25	32.06 ± 3.00 ^{cd}	44.96 ± 13.64 ^{b}	2.91 ± 0.28 ^{ab}	0.041 ± 0.002 ^{d}
	50	29.68 ± 1.01 ^{de}	65.64 ± 7.69 ^{a}	2.79 ± 0.08 ^{bc}	0.047 ± 0.001 ^{c}
	100	17.20 ± 1.47 ^{g}	74.68 ± 6.23 ^{a}	2.45 ± 0.19 ^{d}	0.055 ± 0.002 ^{b}

*Mean ± SD (n=3).

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

3.4.4 Color

As the levels of essential oils increased, higher b^*- and ΔE^*-values were observed in resulting films with the concomitant lower L^*- and a^*-values ($p < 0.05$) (Table 14). Among all film samples, those incorporated with turmeric essential oil had the highest b^*- and ΔE^*-values ($p < 0.05$) when the same level of essential oil was incorporated. This result suggested that the color of essential oil directly affected the color of resulting film. It was noted that high yellowish color of turmeric oil was correlated with the highest b^*- and ΔE^*-values of resulting film. Plai essential oil also had the slightly yellowish color, which also determined the color of films. The changes in color of resulting films were most likely attributed to the coloring components in essential oils. This result was in agreement with Atarés *et al.* (2010) who reported that increasing amount of cinnamon essential oil markedly increased C^*_{ab} with the concomitant decreases in whiteness index (WI) of sodium casinate-based and soy protein-based film. Nevertheless, color parameters of those films were not affected when ginger essential oil was incorporated. Pranoto *et al.* (2005) also reported that color of alginate based-film added with garlic oil tended to be yellowish and darken. The change in color of biopolymer film was mostly affected by the origin of oil incorporated. However, the addition of thyme oil into hake protein film did not significantly had the impact on color parameter and color attribute, mostly caused by the low content of oil (0 - 0.25 ml oil/g protein) in the film (Pires *et al.*, 2011).

3.4.5 Light transmittance and film transparency

Transmission of UV and visible light in the range of 200 - 800 nm of films incorporated with ginger, turmeric and plai essential oils at different levels is presented in Table 15. The films had the excellent barrier property to the light in UV at 200 nm, regardless of type and level of essential oil incorporated. Higher UV light barrier capacity was reported for gelatin films from bigeye snapper skin (Jongjareonrak *et al.*, 2006) and cuttlefish skin (Hoque *et al.*, 2011). Light transmittance of films at wavelength of 280 nm decreased markedly from 40.73 % (control film) to 0.55, 0.00 and 0.05 % upon the incorporation of ginger, turmeric and plai essential oils at a level of 100 %, respectively. Thus, the incorporation of

essential oils to gelatin film could improve the UV-barrier property, especially for films added with turmeric and plai essential oils. Transmission of visible light range (350-800 nm) of films incorporated with those essential oils also decreased with increasing levels. The decrease in light transmittance was possibly caused by light scattering of lipid droplets distributed throughout the protein network. At the same level of essential oil added, ginger essential oil had the highest efficiency in lowering light transmission in visible range. The difference in light scattering or absorption by different compounds in varying essential oil was also presumed.

Based on transparency value (Table 15), films incorporated with all types of essential oils had the higher transparency value than the control film ($p < 0.05$). The result indicated that films incorporated with all essential oils became less transparent as essential oils at high level were incorporated. At levels of 25 and 50 %, film incorporated with ginger essential oil had higher transparency value than those added with essential oils from turmeric and plai ($p < 0.05$). On the other hand, when essential oils at level of 100 % was incorporated, film added with ginger essential oil showed the highest transparency value, compared with those containing essential oils from turmeric and plai ($p < 0.05$). This result was in agreement with Pires *et al.* (2011) who reported that the addition of thyme essential oil decreased the transparency of hake protein film. Chitosan films had the decreases in transparency when thyme, clove and cinnamon essential oils were added (Hosseini *et al.*, 2009). The result indicated that the addition of those essential oils could increase the opacity of resulting film. The decreases in transparency of gelatin based-films were related with the decrease in light transmittance when essential oil was incorporated. The coloring components in essential oils as well as the decreased ordered film protein network were mostly contributable to the decreased transparency of essential oil incorporated gelatin films. Therefore, the incorporation of essential oil had the impact on appearance and light barrier properties of gelatin film.

Table 14. Color of fish skin gelatin incorporated with ginger, turmeric and plai essential oils at different levels.

Essential oils	Level of incorporation (% based on protein content)	*L**	*a**	*b**	Δ*E**
Control	Without essential oil	90.57 ± 0.10 ^{cd}*	-1.58 ± 0.03 ^{a}	2.15 ± 0.02 ^{i}	2.87 ± 0.08 ^{i}
Ginger	25	90.53 ± 0.28 ^{cd}	-1.88 ± 0.03 ^{b}	4.00 ± 0.33 ^{h}	4.30 ± 0.39 ^{h}
	50	89.84 ± 0.12 ^{f}	-2.15 ± 0.17 ^{d}	6.39 ± 1.20 ^{f}	6.73 ± 1.13 ^{f}
	100	89.86 ± 0.32 ^{f}	-2.83 ± 0.06 ^{f}	12.23 ± 0.52 ^{b}	12.26 ± 0.47 ^{b}
Turmeric root	25	90.92 ± 0.04 ^{ab}	-2.20 ± 0.03 ^{d}	6.34 ± 0.21 ^{f}	6.27 ± 0.19 ^{f}
	50	90.45 ± 0.03 ^{de}	-2.53 ± 0.02 ^{e}	9.73 ± 0.06 ^{d}	9.68 ± 0.06 ^{d}
	100	90.04 ± 0.19 ^{f}	-2.98 ± 0.10 ^{g}	13.43 ± 0.35 ^{a}	13.39 ± 0.31 ^{a}
Plai	25	90.74 ± 0.04 ^{bc}	-1.98 ± 0.03 ^{c}	5.39 ± 0.06 ^{g}	5.42 ± 0.04 ^{g}
	50	91.11 ± 0.06 ^{a}	-2.45 ± 0.05 ^{e}	7.78 ± 0.09 ^{e}	7.63 ± 0.08 ^{e}
	100	90.25 ± 0.08 ^{e}	-3.02 ± 0.03 ^{g}	11.62 ± 0.20 ^{c}	11.60 ± 0.21 ^{c}

*Mean ± SD (n=3).

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

Table 15. Light transmittance and transparency value of fish skin gelatin incorporated with ginger, turmeric and plai essential oils at different levels.

Essential oils	Level of incorporation (% based on protein content)	Light transmittance (%) at different wavelength (nm)								Transparency value
		200	280	350	400	500	600	700	800	
Control	Without essential oil	0.01	40.73	75.45	83.74	87.64	88.91	89.59	90.03	1.20 ± 0.08 ^{g*}
Ginger	25	0.00	17.85	68.99	79.20	84.01	85.55	86.40	86.97	1.60 ± 0.01 ^{d}
	50	0.00	7.75	54.79	69.15	77.03	79.93	81.58	82.71	1.89 ± 0.01 ^{c}
	100	0.00	0.55	26.51	43.93	58.89	66.81	71.86	75.35	3.02 ± 0.01 ^{a}
Turmeric root	25	0.00	0.70	57.36	78.25	83.83	85.49	86.45	87.12	1.45 ± 0.01 ^{f}
	50	0.00	0.00	33.80	72.50	81.27	83.39	84.58	85.43	1.50 ± 0.05 ^{ef}
	100	0.00	0.00	13.44	65.88	78.45	81.25	82.88	84.08	1.63 ± 0.04 ^{d}
Plai	25	0.00	2.39	63.14	78.33	84.49	86.50	87.52	88.13	1.49 ± 0.01 ^{ef}
	50	0.01	0.16	31.61	72.78	82.35	85.64	87.29	88.23	1.52 ± 0.03 ^{e}
	100	0.02	0.05	14.63	49.82	68.51	77.77	82.87	85.75	2.17 ± 0.07 ^{b}

*Mean ± SD (n=3).

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

3.4.6 Protein pattern

Protein patterns of gelatin films incorporated with ginger, turmeric and plai essential oils at different levels are illustrated in Figure 9. All gelatin films contained α1- and α2-chains as major components. α1- and α2-chains constituted as the main components in fish skin gelatin (Benjakul *et al.*, 2009). Band intensities of those two major protein bands in films incorporated with essential oils were similar to those of control film (without essential oil incorporated). For all types of essential oils incorporated, no differences in protein patterns were observed between films containing essential oil at different levels. This result suggested that major bondings in film matrix were weak bonds, which could be interrupted by denaturing agent used for electrophoresis. Additionally, it was suggested that essential oils rarely interacted with gelatin by covalent bonds. It was postulated that essential oils more likely formed hydrophobic interaction with gelatin at hydrophobic domains. Therefore, the essential oils had no impact on protein pattern of fish skin gelatin film.

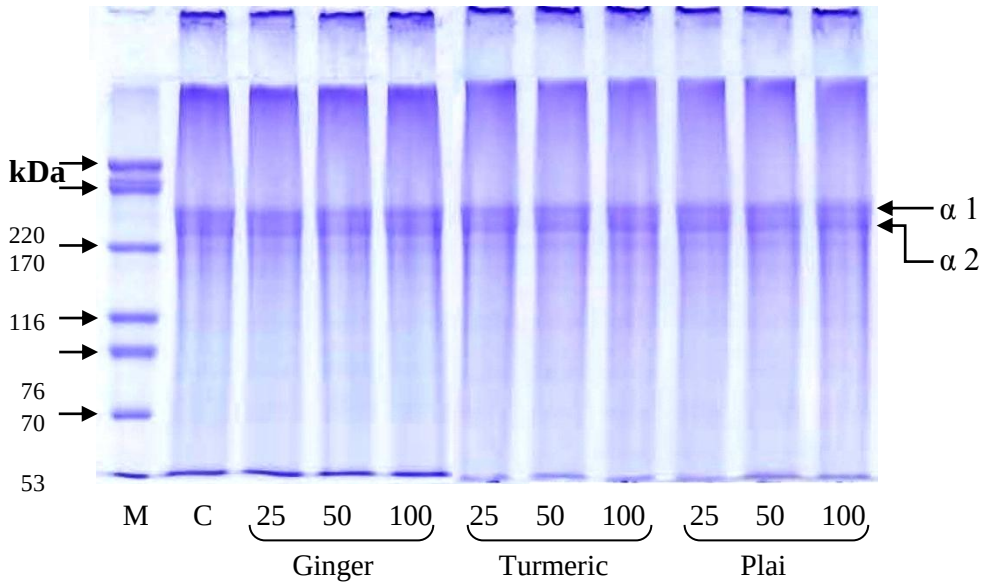


Figure 9. Protein patterns of gelatin films incorporated ginger, turmeric and plai essential oils at different levels. M: protein marker; C: control film (without essential oil incorporated). Numbers denoted the level of incorporation (% based on protein content).

3.4.7 Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra of gelatin films incorporated with three essential oils at different levels are illustrated in Figure 10. In general, FTIR spectra of control film and films containing different essential oils showed the similar major peaks but the amplitudes of peaks varied, depending on the level of essential oils incorporated. The band situated at the wavenumber of 1031-1035 cm^{-1} was found in all 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 this peak decreased with increasing level of essential oils incorporated. This was more likely due to the dilution effect caused by essential oils added. All films had the similar spectra in the range of 1700-700 cm^{-1}, covering amide-I, II and III bands. All films had the major bands at 1630 cm^{-1} (amide-I, illustrating C=O stretching/hydrogen bonding coupled with COO), 1538 cm^{-1} (amide-II, presenting the bending vibrations of N-H groups and stretching vibrations of C-N groups) and 1237 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.*, 2004a). Jongjareonrak *et al.* (2008) also reported the similar spectra for both bigeye snapper and brownstripe red snapper skin gelatin film, where amide-I and II bands were found at the wavenumbers of 1630 and 1545 cm^{-1}, respectively. It was found that the amplitudes of amide I, II and III of control film were higher than those of films incorporated with essential oils, regardless of types, but the amplitudes became lowered with increasing amount of essential oils added. This might be due to the highest protein content in the control film, in comparison with films incorporated with essential oils. Essential oils more likely decreased protein content in film matrix with the concomitant increasing proportion of essential oil in the resulting films.

An amide-A band was observed at the wavenumber of 3273-3306 cm^{-1}, and amide-B band was found at 2922-2933 cm^{-1} for all film samples. The amide-A band represented the NH-stretching coupled with hydrogen bonding and amide-B band represented the CH stretching and $-NH_3^+$ at wavenumber of 2928 cm^{-1} (Muyonga *et al.*, 2004b). Amide-A peak of control film found at wavenumber 3273 cm^{-1} was gradually shifted to higher wavenumbers as the level of essential oil

increased. Wavenumbers of amide-A peak shifted to 3306, 3303 and 3300 cm^{-1} for films incorporated with ginger, turmeric and plai essential oils at a level of 100 %, respectively. Additionally, the amplitude of amide-A band decreased with increasing level of essential oils, especially at a level of 100 %. This confirmed the lower interaction between gelatins, as reflected by the lower TS with the increased EAB of films when essential oils at higher level were incorporated. Moreover, the amplitude and size of amide-B peak of films increased markedly when being incorporated with essential oils at higher levels. The result indicated that the presence of essential oils, which dispersed in the protein film matrix and impeded protein-protein interaction in film matrix.

Peaks with wavenumber of 2869-2877 cm^{-1} and 2922-2933 cm^{-1} were also found in all samples. Peaks at wavenumbers 2853.62 cm^{-1} and 2923.58 cm^{-1} represent the methylene asymmetrical and symmetrical stretching vibration of the aliphatic C-H in CH_2 and CH_3 groups, respectively (Guillén and Cabo, 1997; Guillén and Cabo, 2004; Muik *et al.*, 2007). Both methylene asymmetrical stretching bands at approximately 2853 cm^{-1} and methylene symmetrical stretching band near 2924 cm^{-1} were obviously present in most lipids (Guillén and Cabo, 2004). The higher amplitude of these peaks was obtained in film incorporated with essential oil, indicating the presence of essential oil containing hydrocarbon in film matrix. This was in accordance with the decreases in amplitude of amide-A.

Furthermore, the peak at wavenumber of 1732 cm^{-1} had the increase in amplitude with increasing levels of essential oils. The peak with wavenumber of 1742 cm^{-1} might represent the C=O stretching vibration of aldehyde or ester carbonyl groups (Guillén and Cabo, 1997; Muik *et al.*, 2007). Aldehyde, ketone and ester are the one of main chemical groups in essential oils (Mohamed *et al.*, 2010). Additionally, the increase in hydrophobicity of gelatin film was obtained when essential oils were incorporated, as evidenced by existing peaks at wavenumber of 2876 cm^{-1}, 2929 cm^{-1} and 1732 cm^{-1} (Aewsiri *et al.*, 2011). This was in agreement with the decreases in WVP of film containing essential oil (Table 13).

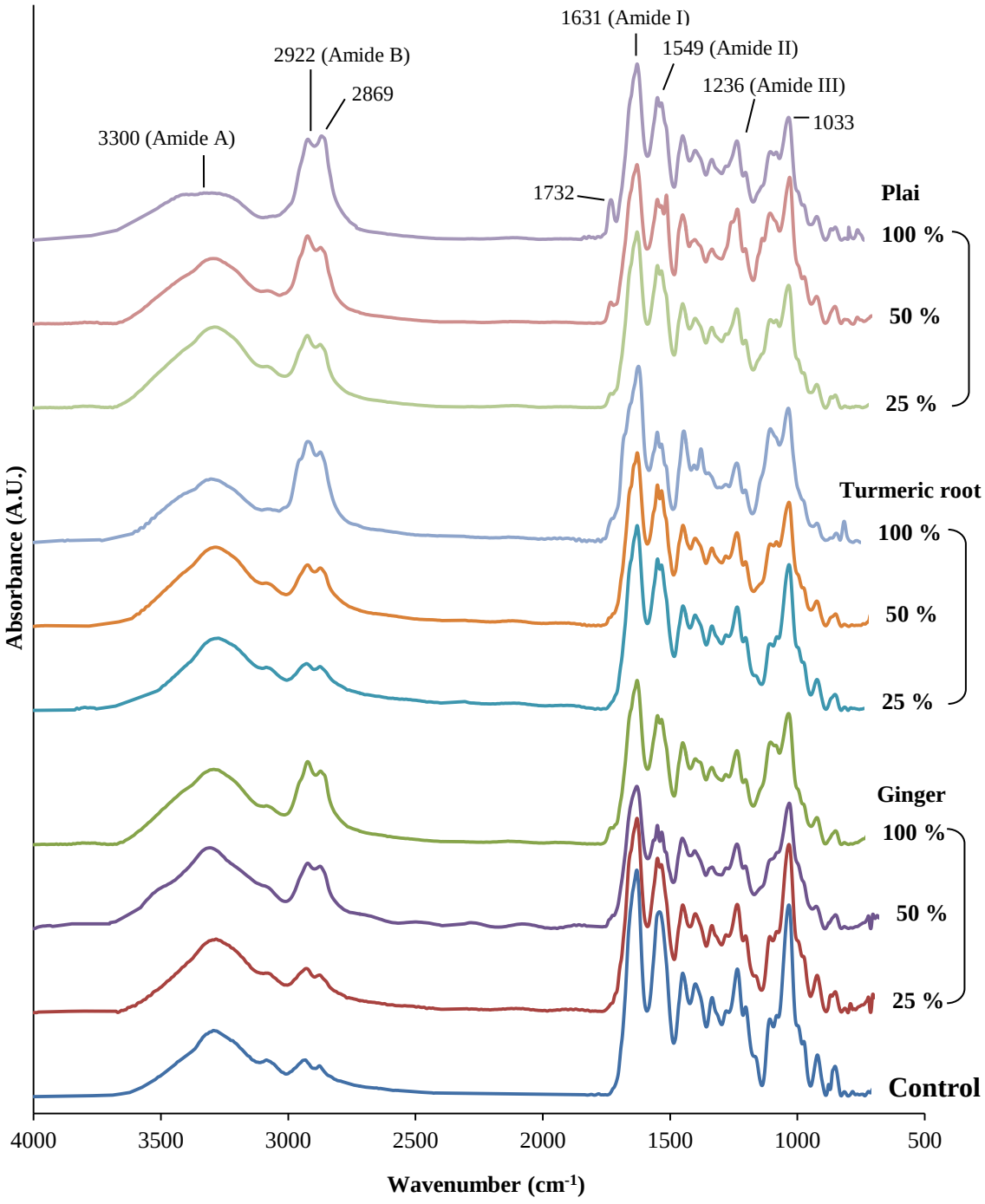


Figure 10. ATR-FTIR spectra of films from fish skin gelatin incorporated with ginger, turmeric and plai essential oils at different levels. Numbers denoted the level of incorporation (% based on protein content).

3.4.8 Thermo-gravimetric analysis (TGA)

TGA thermograms representing thermal degradation behavior of gelatin films incorporated with three essential oils at different levels are presented in Figure 11. The degradation temperatures (T_d), weight loss (Δw) and residue of all film samples are showed in Table 16. Control film and those incorporated with essential oils at a level of 25 % exhibited three main stages of weight loss. However, four main stages of weight loss were observed in films incorporated with all essential oils at levels of 50 % and 100 %, excepted for film incorporated with 50 % turmeric, which had three stages. For all films, the first stage weight loss at 4.15 - 7.34 % was obtained with the temperature (T_{d1}) of 33.01 – 52.94 ºC. The weight loss at this temperature range was plausibly related to the loss of free and absorbed water. The lower weight loss of films added with all essential oils at all levels, in comparison with the control film was possibly due to the lower content of water in the formers associated with their higher hydrophobicity. The similar result was observed in protein-based film from various sources including red tilapia protein isolate (Tongnuanchan *et al.*, 2013) and porcine-plasma protein (Nuthong *et al.*, 2009). The second stage of weight loss (Δw_2 = 18.94 – 26.90 %) was observed at T_{d2} of 214.15 – 264.42 ºC. This stage of weight loss was more likely due to the degradation of glycerol as well as small size protein in the film network. Hoque *et al.* (2011) also reported the degradation temperature in the range of 196.30-216.71ºC for cuttlefish skin gelatin film. For the third stage of weight loss, Δw_3 of 38.79 – 57.88 % and T_{d3} of 306.30 – 326.98 ºC were obtained for all film samples. This was most likely associated with the degradation of the larger size or highly associated protein fraction. For the fourth stage of weight loss, Δw_4 of 14.35 – 25.56 % with T_{d4} of 399.73 – 414.15 ºC were obtained for films incorporated with different essential oils at the level of 50 or 100 %. However, for control film and films incorporated with essential oils at a level of 25 %, the fourth stage of weight loss (Δw_4) was not detectable. This result suggested that the fourth stage of weight loss was possibly related with the loss of high temperature stable components in film containing essential oils. The presence of essential oils in the film network resulted in the higher thermal stability of film matrix.

In general, the T_{d3} for all films incorporated with essential oils was lower than the control film but varied with types and levels of essential oils added. This might be correlated with the discontinuity or interrupted protein-protein interaction of gelatin molecules in film network by essential oils incorporated at different degrees. This result was in agreement with the lowered TS and higher EAB of films incorporated with essential oils, compared with control (Table 13). A reduction in thermal stability can be promoted by changes in the protein structure and provoked by the rupture of low energy intermolecular bonds which maintain the protein conformation (Kaminska and Sionkowska, 1999). Decrease in weight loss on the first, second and third stages of film incorporated with all essential oils, especially at 100 % level, was plausibly associated with the lower protein interaction. The interfering effect of essential oil at a higher level on protein network formation of film matrix was postulated. In general, lower residue from thermal degradation was found in films incorporated with essential oils, compared with the control film, regardless of oil concentration. Thus, both type and level of essential oils had the marked impact on thermal stability of gelatin film.

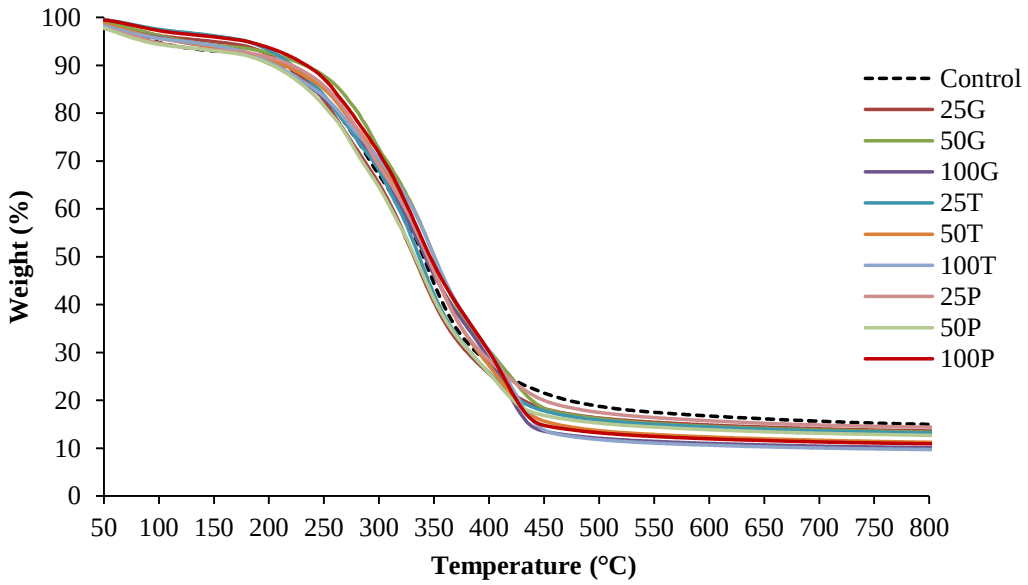


Figure 11. Thermo-gravimetric curves of films from fish skin gelatin incorporated with ginger, turmeric and plai essential oils at different levels. C: control (without essential oil incorporated); G: ginger; T: turmeric; P: plai. Numbers denoted the level of incorporation (% based on protein content).

Table 16. Thermal degradation temperatures (T_d, ºC) and weight loss (Δw, %) of fish skin gelatin film incorporated with ginger, turmeric and plai essential oils at different levels.

Essential oils	Level of incorporation (% based on protein content)	Δ_1		Δ_2		Δ_3		Δ_4		Residue (%)
		$Td_{1,onset}$	Δw_1	$Td_{2,onset}$	Δw_2	$Td_{3,onset}$	Δw_3	$Td_{4,onset}$	Δw_4	
Control	Without essential oil	33.01	7.34	227.21	24.44	326.98	54.37	-	-	13.85
Ginger	25	44.34	5.14	214.15	24.95	306.30	57.23	-	-	12.68
	50	42.68	6.16	252.59	23.54	326.34	43.25	412.08	14.64	12.41
	100	32.74	5.63	221.56	19.74	308.64	38.79	393.49	25.56	10.28
Turmeric root	25	46.60	4.15	229.54	24.71	312.27	57.88	-	-	13.26
	50	34.55	6.72	235.54	24.46	319.59	57.53	-	-	11.29
	100	30.47	4.79	218.33	21.11	324.03	41.09	414.15	23.11	9.90
Plai	25	52.97	6.64	241.05	26.90	323.30	51.72	-	-	14.74
	50	40.07	6.72	264.42	26.50	315.74	39.31	399.73	14.35	13.12
	100	37.88	3.64	230.81	18.94	310.95	41.69	401.28	25.22	10.20

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.

3.4.9 Film morphology

SEM micrographs of the surface and freeze-fractured cross-section of gelatin films incorporated with three essential oils at different levels are presented in Figure 12. The surface of control film (without incorporated essential oil) was smoother than those of films incorporated with essential oils, regardless of type and concentration of oil. However, the rougher surface was found in film incorporated with essential oils for all types and levels. Films incorporated with ginger and plai oils at levels of 25 and 50 % had rough surface. Nevertheless, very small particle was formed when essential oils at a level of 100 % was incorporated. It was postulated that essential oil at high level preferably localized on the upper surface of film after casting and drying. At higher levels, essential oils which were hydrophobic in nature might be segregated from aqueous phase. This was indicated by heterogeneous distribution of essential oils as visualized by crystals formed at the macroscopic level.

Control film showed the smoother cross-section than those incorporated with essential oils. This revealed that control film had more homogeneous structure than other films. Films containing different essential oils showed different cross-section micrographs. Films added with essential oils became rougher, compared with the control film. Oil addition more likely lowered protein-protein interaction in film network, thereby enhancing the discontinuity and roughness of film microstructure. Obviously, films incorporated with 50 % up to 100 % oil concentration showed the phase separation between oil phase (upper) and protein phase (lower), regardless of types of essential oils. As a consequence, the increased content of essential oil droplets could enhance creaming and phase separation. Essential oils at high content and low density were separated and localized at the upper surface of film, thereby forming the bilayer microstructure. It was noted that the bilayer internal structure/morphology of the film was observed only at the microscopic level upon SEM observation. In fact, the bilayer of film was not noticeable at the macroscopic level. Even though, the essential oil seemed to localize at the upper part of film, it was still imbedded and entrapped in the protein matrix of the film. This related with bilayer morphological internal structure. Therefore, there was no oil exudates on the film incorporated with low concentration (25%) of

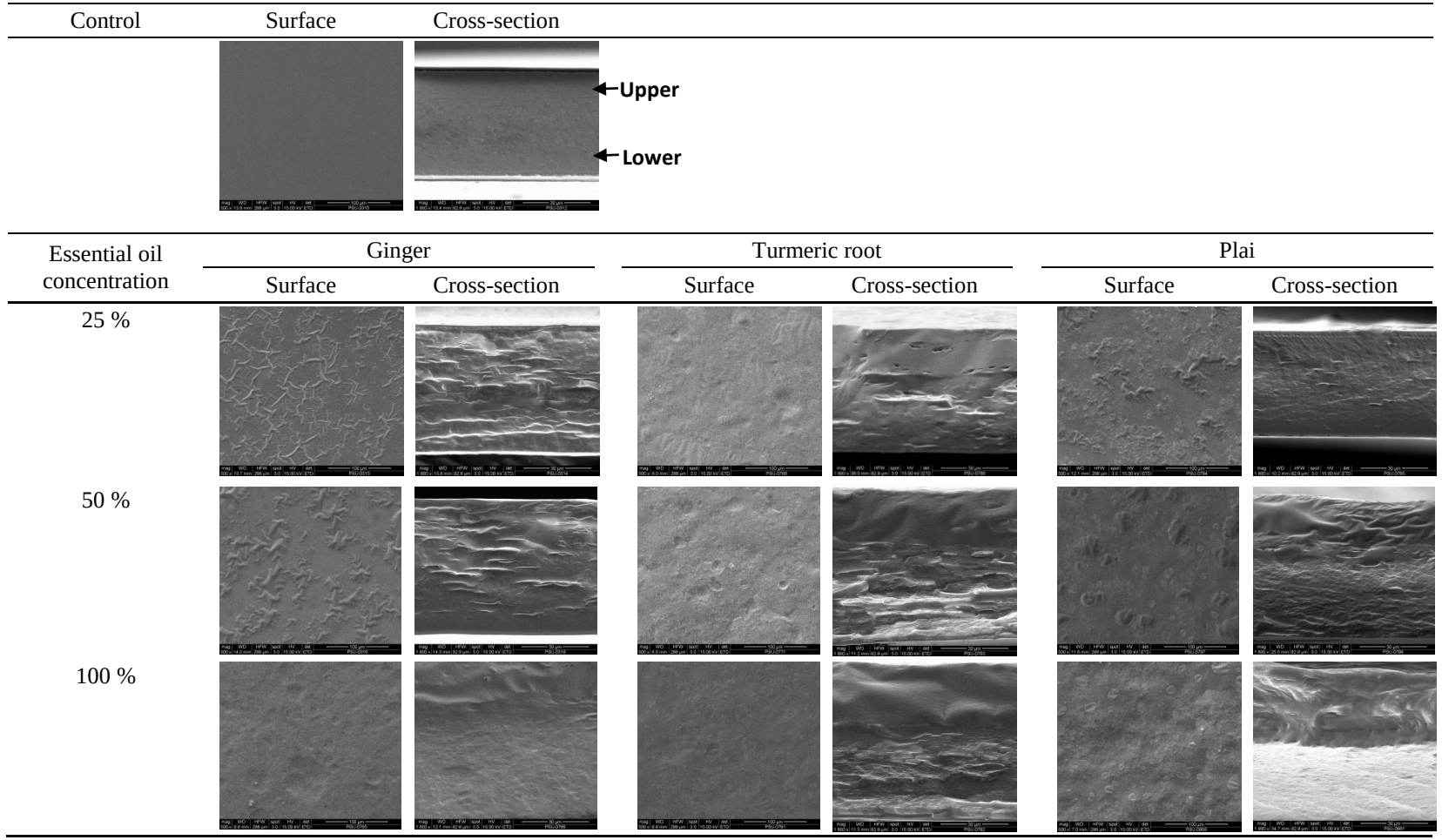


Figure 12. SEM micrographs of surface (5000 x) and cross-section (1800 x) of films from fish skin gelatin incorporated with ginger, turmeric and plai essential oils at different levels. Numbers denoted the level of incorporation (% based on protein content).

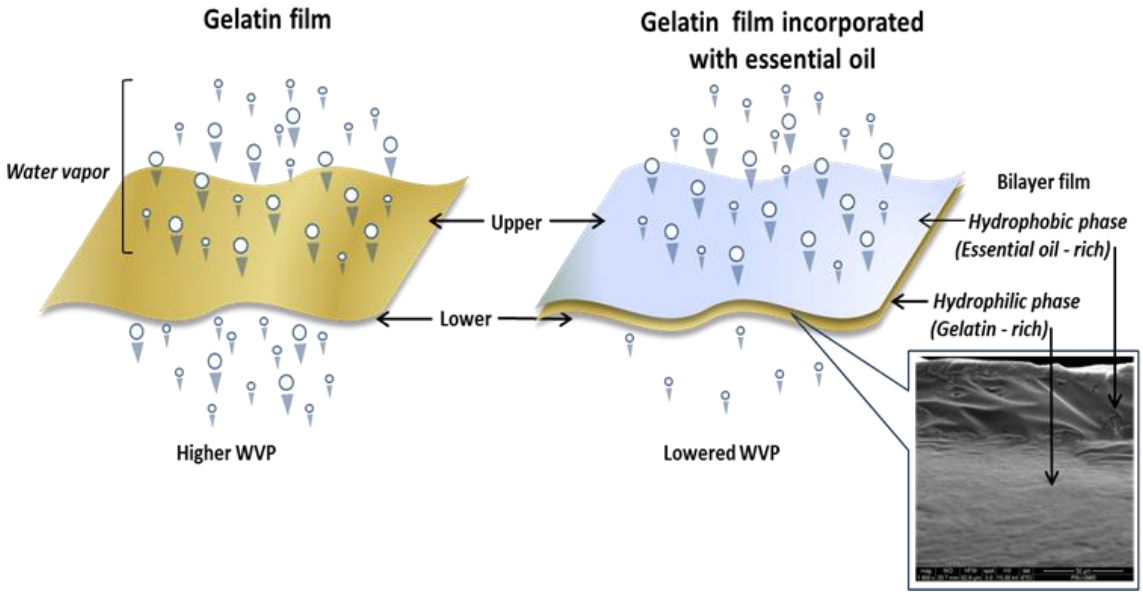


Figure 13. Simplified illustration for the formation of bilayer film from fish skin gelatin incorporated with essential oil.

essential oil; however, some oil exudates were found at the surface of the films as essential oil at high level (100 %) was added. The bilayer-morphological microstructure might contribute to lower WVP of essential oil incorporated gelatin films (Figure 13), compared with the control film, as shown in Table 13. The hydrophobic oil droplet could hinder the water molecules to transfer through the film (Karbowiak *et al.*, 2007).

3.4.10 Antioxidative activity of fish skin gelatin films incorporated with essential oils

Antioxidative activity expressed as DPPH radical scavenging activity and ABTS radical scavenging activity of ginger, turmeric root and plai essential oils are presented in Table 17. DPPH radical-scavenging activity of plai essential oil was highest ($p < 0.05$), followed by plai and ginger essential oil, respectively. The differences in antioxidative activity of different essential oils were mostly due to the differences in types and amounts of antioxidative components presented in the essential oils (Burt, 2004b; Kordali *et al.*, 2005). The major constituents in essential oils are terpenes (monoterpenes and sesquerpenes), aromatic compounds (aldehyde,

alcohol, phenol, methoxy derivative, etc.) and terpenoids (isoprenoids) (Bakkali *et al.*, 2008; Mohamed *et al.*, 2010). From the results, gelatin films incorporated with different essential oils at different levels exhibited different antioxidative activities (Table 18). Control film (without incorporated essential oil) showed DPPH and ABTS radical scavenging activity to some extent. Antioxidant activity of gelatin was due to the peptides containing particular amino acids such as glycine and proline (Bao *et al.*, 2009; Gómez-Estaca *et al.*, 2009; Kim *et al.*, 2001). Gómez-Guillén *et al.* (2007) reported that films prepared from tuna skin gelatin showed antioxidative activity as determined by FRAP and ABTS assays.

Film incorporated with all essential oils showed higher antioxidant activity than the control film ($p < 0.05$). Essential oils have been reported as the excellent source of antioxidant (Wu *et al.*, 1982). In general, both DPPH and ABTS radical scavenging activities of film incorporated with essential oils increased with increasing amount from 25 % to 100 % ($p < 0.05$). However, antioxidant activity varied with types of essential oils. DPPH radical-scavenging activity of film incorporated with plai essential oil was highest ($p < 0.05$), followed by film added with turmeric and ginger essential oil, respectively. For ABTS radical-scavenging activity, film incorporated with turmeric essential oil showed the highest activity ($p < 0.05$), followed by film added with plai and ginger essential oil, respectively. The result indicated that different essential oils contained different antioxidants, which were able to scavenge radicals at varying degrees. Furthermore, various antioxidative compounds could interact with the film matrix in different fashion, in which free antioxidative compounds in essential oils could be released differently. In the present study, antioxidative activities of gelatin films incorporated with root essential oils were lower than those of pure essential oil, regardless of type of essential oil used. This was more likely due to the interaction between gelatin and antioxidative compounds in essential oil, thus lowering the release of those compounds. As a consequence, the lower activity was found in the resulting films. The results suggested that fish gelatin based film incorporated with essential oil from roots could be used as smart films containing antioxidant for further uses.

Table 17. Antioxidant activity of ginger, turmeric and plai essential oils.

Essential oils	Antioxidant activity (μmol Trolox equivalents (TE)/ml essential oil)	
	DPPH	ABTS
Ginger	0.75 ± 0.22 [a]*	5.23 ± 0.97 [a]
Turmeric root	5.55 ± 0.14 [b]	107.28 ± 5.45 [b]
Plai	8.71 ± 0.36 [c]	54.75 ± 0.99 [c]

*Mean ± SD (n=3).

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

Table 18. Antioxidant activity of fish skin gelatin incorporated with ginger, turmeric and plai essential oils at different levels.

Essential oils	Level of incorporation (% based on protein content)	Antioxidant activity (μmol Trolox equivalents (TE)/g dried film)	
		DPPH	ABTS
Control	Without essential oil	0.07 ± 0.08 [f]*	0.49 ± 0.31 [i]
Ginger	25	0.26 ± 0.13 [f]	3.47 ± 0.03 [h]
	50	0.66 ± 0.12 [e]	6.97 ± 0.20 [g]
	100	1.30 ± 0.13 [c]	13.43 ± 0.22 [d]
Turmeric root	25	0.72 ± 0.09 [de]	8.96 ± 0.11 [f]
	50	1.41 ± 0.13 [c]	16.52 ± 0.25 [c]
	100	1.97 ± 0.10 [b]	20.26 ± 0.14 [a]
Plai	25	0.95 ± 0.12 [d]	8.68 ± 0.17 [f]
	50	1.90 ± 0.12 [b]	12.34 ± 0.31 [e]
	100	2.39 ± 0.02 [a]	19.51 ± 0.14 [b]

*Mean ± SD (n=3).

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

3.5 Conclusion

Incorporation of root essential oils from ginger, turmeric and plai markedly decreased TS with the concomitant increase in EAB of fish skin gelatin films, particularly with increasing levels. Essential oils could act as plasticizer, in which the protein interaction in film network was impeded. 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 excessive level (100 %) resulted in the presence of some oil exudates on the film surface. Film containing essential oils showed antioxidative activity but the activity was governed by types and amount of essential oils. Nevertheless, root essential oil incorporated also affected the smell of the gelatin film, especially when high concentration was used. This might limit its application in foods. Thus, use of appropriate root essential oils at proper amount could not only enhance water vapor barrier property of fish skin gelatin film, but also provide antioxidative activity for resulting films.

3.6 References

Aewsiri, T., Benjakul, S. and Visessanguan, W. 2009. Functional properties of gelatin from cuttlefish (*Sepia pharaonis*) skin as affected by bleaching using hydrogen peroxide. Food Chem. 115: 243-249.

Aewsiri, T., Benjakul, S., Visessanguan, W., Wierenga, P.A. and Gruppen, H. 2011. Improvement of foaming properties of cuttlefish skin gelatin by modification with *N*-hydroxysuccinimide esters of fatty acid. Food Hydrocolloid. 25: 1277-1284.

Arnao, M.B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73: 239-244.

Arvanitoyannis, I.S. 2002. Formation and properties of collagen and gelatin films and coatings. *In* Protein-based films and coatings. (Gennadios, A., ed.). p. 275-304. CRC Press. Boca Raton.

ASTM. 1989. Standard test methods for water vapor transmission of materials. Standard designation E96-E80. *In* Annual book of ASTM standards. p. 730-739. ASTM. Philadelphia.

Atarés, L., Bonilla, J. and Chiralt, A. 2010. Characterization of sodium caseinate-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 100: 678-687.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils - A review. Food Chem. Toxicol. 46: 446-475.

Bao, S., Xu, S. and Wang, Z. 2009. Antioxidant activity and properties of gelatin films incorporated with tea polyphenol-loaded chitosan nanoparticles. J. Sci. Food Agr. 89: 2692-2700.

Benjakul, S., Oungbho, K., Visessanguan, W., Thiansilakul, Y. and Roytrakul, S. 2009. Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. Food Chem. 116: 445-451.

Bergo, P. and Sobral, P.J.A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. Food Hydrocolloid. 21: 1285-1289.

Burt, S. 2004a. Essential oils: their antibacterial properties and potential applications in foods-a review. Int. J. Food Microbiol. 94: 223-253.

Burt, S. 2004b. Essential oils: Their antibacterial properties and potential applications in foods - A review. Int. J. Food Microbiol. 94: 223-253.

Cuq, B., Gontard, N. and Guilbert, S. 1997. Thermal properties of fish myofibrillar protein-based films as affected by moisture content. Polymer. 38: 2399-2405.

Gennadios, A., McHugh, T.H., Weller, C.L. and Krochta, J.M. 1994. Edible coatings and films based on proteins. *In* Edible coatings and films to improve food quality. (Krochta, J. M. *et al.*, eds.). p. 201-277. Technomic Publishing Company, Inc. Lancaster, PA.

Gennadios, A., Ghorpade, V.M., Weller, C.L. and Hanna, M.A. 1996. Heat curing of soy protein films. T. ASABE. 39: 575-579.

Gómez-Estaca, J., Bravo, L., Gómez-Guillén, M.C., Alemán, A. and Montero, P. 2009. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chem. 112: 18-25.

Gomez-Guillen, M.C., Perez-Mateos, M., Gomez-Estaca, J., Lopez-Caballero, E., Gimenez, B. and Montero, P. 2009. Fish gelatin: a renewable material for developing active biodegradable films. Trends Food Sci. Technol. 20: 3-16.

Gómez-Guillén, M.C., Ihl, M., Bifani, V., Silva, A. and Montero, P. 2007. Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae Turcz*). Food Hydrocolloid. 21: 1133-1143.

Gontard, N., Marchesseau, S., Cuq, J.L. and Guilbert, S. 1995. Water vapour permeability of edible bilayer films of wheat gluten and lipids. Int. J. Food Sci. Technol. 30: 49-56.

Guillén, M.D. and Cabo, N. 1997. Infrared spectroscopy in the study of edible oils and fats. J. Sci. Food Agr. 75: 1-11.

Guillén, M.D. and Cabo, N. 2004. Study of the effects of smoke flavourings on the oxidative stability of the lipids of pork adipose tissue by means of Fourier transform infrared spectroscopy. Meat Sci. 66: 647-657.

Han, J.H. and Floros, J.D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. J. Plast. Film Sheet. 13: 287-298.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2010. Effect of heat treatment of film-forming solution on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. J. Food Eng. 96: 66-73.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011. Properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin incorporated with cinnamon, clove and star anise extracts. Food Hydrocolloid. 25: 1085-1097.

Hosseini, M.H., Razavi, S.H. and Mousavi, M.A. 2009. Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. J. Food Process. Pres. 33: 727-743.

Huber, K.C., Embuscado, M.E., Pavlath, A.E. and Orts, W. 2009. Edible Films and Coatings: Why, What, and How? *In* Edible Films and Coatings for Food Applications. p. 1-23. Springer New York.

Iwata, K., Ishizaki, S., Handa, A. and Tanaka, M. 2000. Preparation and characterization of edible films from fish water-soluble proteins. Fisheries Sci. 66: 372-378.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid. 20: 492-501.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2008. Antioxidative activity and properties of fish skin gelatin films incorporated with BHT and α-tocopherol. Food Hydrocolloid. 22: 449-458.

Kaminska, A. and Sionkowska, A. 1999. The effect of UV radiation on the values of thermal parameters of collagen containing β-carotene. Polym. Degrad. Stabil. 65: 87-90.

Karbowiak, T., Debeaufort, F. and Voilley, A. 2007. Influence of thermal process on structure and functional properties of emulsion-based edible films. Food Hydrocolloid. 21: 879-888.

Kester, J.J. and Fennema, O.R. 1986. Edible films and coatings: a review. Food Technol. 40: 47-59.

Kim, S.K., Kim, Y.T., Byun, H.G., Nam, K.S., Joo, D.S. and Shahidi, F. 2001. Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska pollack skin. J. Agr. Food Chem. 49: 1984-1989.

Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A. and Yildirim, A. 2005. Determination of the chemical composition and antioxidant activity of the essential oil of artemisia dracunculus and of the antifungal and antibacterial activities of turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. J. Agr. Food Chem. 53: 9452-9458.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-685.

Limpisophon, K., Tanaka, M. and Osako, K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionace glauca*) skin gelatin. Food Chem. 122: 1095-1101.

McHugh, T.H. and Krochta, J.M. 1994. Water vapor permeability properties of edible whey protein-lipid emulsion films. J. Am. Soc. 71: 307-312.

Mohamed, A.A., El-Emary, G.A. and Ali, H.F. 2010. Influence of some citrus essential oils on cell viability, glutathione-s-transferase and lipid peroxidation in *Ehrlich ascites* Carcinoma Cells. J. Am. Sci. 6: 820-826.

Moorhous, S. and Grudon, V. 1994. Encapsulating: A new concept for the food industry. Nutr. Food Sci. 94: 17-19.

Muik, B., Lendl, B., Molina-Diaz, A., Valcarcel, M. and Ayora-Cañada, M.J. 2007. Two-dimensional correlation spectroscopy and multivariate curve resolution for the study of lipid oxidation in edible oils monitored by FTIR and FT-Raman spectroscopy. Anal. Chim. Acta. 593: 54-67.

Muyonga, J.H., Cole, C.G.B. and Duodu, K.G. 2004a. Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chem. 85: 81-89.

Muyonga, J.H., Cole, C.G.B. and Duodu, K.G. 2004b. Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (*Lates niloticus*). Food Chem. 86: 325-332.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009. Characterization of porcine plasma protein-based films as affected by pretreatment and cross-linking agents. Int. J. Biol. Macromol. 44: 143-148.

Oussalah, M., Caillet, S., Salmieri, S., Saucier, L. and Lacroix, M. 2004. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. J. Agr. Food Chem. 52: 5598-5605.

Park, H.J. and Chinnan, M.S. 1995. Gas and water vapor barrier properties of edible films from protein and cellulosic materials. J. Food Eng. 25: 497-507.

Pires, C., Ramos, C., Teixeira, G., Batista, I., Mendes, R., Nunes, L. and Marques, A. 2011. Characterization of biodegradable films prepared with hake proteins and thyme oil. J. Food Eng. 105: 422-428.

Pranoto, Y., Salokhe, V.M. and Rakshit, S.K. 2005. Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. Food Res. Int. 38: 267-272.

Prodpran, T., Benjakul, S. and Artharn, A. 2007. Properties and microstructure of protein-based film from round scad (*Decapterus maruadsi*) muscle as affected by palm oil and chitosan incorporation. Int. J. Biol. Macromol. 41: 605-614.

Robinson, H.W. and Hodgen, C.G. 1940. The biuret reaction in the determination of serum protein. I. A study of condition necessary for the production of the stable color which bears a quantitative relationship to the protein concentration. J. Biol. Chem. 135: 707-725.

Shiku, Y., Hamaguchi, P.Y., Benjakul, S., Visessanguan, W. and Tanaka, M. 2004. Effect of surimi quality on properties of edible films based on Alaska pollack. Food Chem. 86: 493-499.

Soazo, M., Rubiolo, A.C. and Verdini, R.A. 2011. Effect of drying temperature and beeswax content on physical properties of whey protein emulsion films. Food Hydrocolloid. 25: 1251-1255.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics. McGraw-Hill Book. New York.

Tanaka, M., Ishizaki, S., Suzuki, T. and Takai, R. 2001. Water vapor permeability of edible films prepared from fish water soluble proteins as affected by lipid type. J. Tokyo Univ. Fisheries. 87: 31-37.

Tharanathan, R.N. 2003. Biodegradable films and composite coatings: Past, present and future. Trends Food Sci. Technol. 14: 71-78.

Tongnuanchan, P., Benjakul, S., Prodpran, T. and Songtipya, P. 2013. Properties and stability of protein-based films from red tilapia (*Oreochromis niloticus*) protein isolate incorporated with antioxidant during storage. Food Bioprocess Tech. 6: 1113-1126.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2012. Properties and antioxidant activity of fish skin gelatin film incorporated with citrus essential oils. Food Chem. 134: 1571-1579.

Turina, A.V., Nolan, M.V., Zygadlo, J.A. and Perillo, M.A. 2006. Natural terpenes: self-assembly and membrane partitioning. Biophys. Chem. 122: 101-113.

Wu, H.C., Chen, H.M. and Shiau, C.Y. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res. Int. 36: 949-957.

Wu, J., Lee, M.-H., Ho, C.-T. and Chang, S. 1982. Elucidation of the chemical structures of natural antioxidants isolated from rosemary. J. Am. Soc. 59: 339-345.

Zivanovic, S., Chi, S. and Draughon, A.F. 2005. Antimicrobial activity of chitosan films enriched with essential oils. J. Food Sci. 70: 45-51.

CHAPTER 4

CHARACTERISTICS AND ANTIOXIDANT ACTIVITY OF LEAF ESSENTIAL OIL-INCORPORATED FISH GELATIN FILMS AS AFFECTED BY SURFACTANTS

4.1 Abstract

Characteristics of film from fish skin gelatin incorporated with four leaf essential oils (lemongrass, basil, citronella and kaffir lime), as influenced by different surfactants (Tween-20, Tween-80 and soy lecithin) were investigated. Films incorporated with all essential oils had lower tensile strength (TS) with higher elongation at break (EAB) and thickness, compared with the control film ($p < 0.05$). Films added with Tween-20 showed higher TS, compared with those containing other surfactants ($p < 0.05$). Water vapor permeability (WVP) of films incorporated with all types of essential oils markedly decreased in comparison with control, particularly when soy lecithin was used as surfactant ($p < 0.05$). Films generally became darker and more yellowness, when incorporated with essential oils. Film containing basil essential oil had the highest DPPH and ABTS radical scavenging activity, compared with those added with essential oils from lemongrass, citronella and kaffir lime leaf. Higher antioxidative activity was obtained in films incorporated with essential oils when soy lecithin was used as surfactant, probably due to the combined effect of both constituents.

4.2 Introduction

Biodegradable and smart packaging has gained increasing attention as an alternative for plastic packaging due to its versatile uses and environment protection. Proteins from diverse sources have been used as material for biodegradable film because of their relative abundance and good film-forming ability (Hamaguchi *et al.*, 2007b; Krochta, 1997). Among proteins from several sources, gelatin from either mammalian or fish has been used as the material for biodegradable and active film such as antimicrobial film (Emiroğlu *et al.*, 2010; Seydim and Sarikus, 2006) or antioxidative film (Jongjareonrak *et al.*, 2008; Tongnuanchan *et al.*, 2012).

Owing to the hydrophilic character, these films have poor water vapor barrier property, thereby restricting their use as packing materials (Hoque *et al.*, 2010; Jongjareonrak *et al.*, 2008). To improve the barrier property, 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 (Prodpran *et al.*, 2007; Tongnuanchan *et al.*, 2012) have been implemented. Tongnuanchan *et al*. (2012) reported that the addition of citrus essential oils into fish gelatin based film could improve the water vapor barrier property. Thyme essential oil was able to lower water vapor permeability of hake protein based film (Pires *et al.*, 2011). Several essential oils have been produced commercially from leaves, which are abundant and have low market value. Essential oils are hydrophobic in nature (Ikeda *et al.*, 1962; Mohamed *et al.*, 2010) and possess antioxidative activity (Bakkali *et al.*, 2008). Thus, the incorporation of essential oil not only improves the water vapor barrier property, but also increases antioxidant activity of resulting gelatin films. As a consequence, the film can serve as active packaging material for food protection and preservation. To produce emulsion film, the effective surfactants are required to stabilize emulsion during preparation of film forming solution and film casting (Hsu and Nacu, 2003). Those surfactants have been reported to affect the properties of film from biomaterials based films (Chen *et al.*, 2009; Villalobos *et al.*, 2006). However, little information about the properties of fish gelatin film incorporated with leaf essential oils, especially those from tropical plants. Thus, the objectives of this study were to characterize the properties of fish gelatin film containing essential oils from the leaves of lemongrass, basil, citronella and kaffir lime and to investigate the effect of lecithin, Tween-20 and tween-80, used as the surfactants, on the properties of films added with essential oils.

4.3 Materials and Methods

4.3.1 Chemicals

2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picryl hydrazyl (DPPH), glycerol, Tween-20, Tween-80 and soy lecithin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*,*p*′-disulfonic acid monosodium salt hydrate (Ferrozine) and iron (II) chloride were obtained from Merck (Darmstadt, Germany). All chemicals were of analytical grade.

4.3.2 Fish gelatin and essential oils

Fish gelatin produced from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Essential oils from the leaves of lemongrass (*Cymbopogon citratus*), basil (*Ocimum basilicum*), citronella grass (*Cymbopogon nardus*) and kaffir lime (*Citrus hystrix*) were purchased from *Botanicessence* (Bangkok, Thailand).

4.3.3 Preparation of film from fish gelatin incorporated with different essential oils and surfactants

To prepare film forming solution (FFS), gelatin powder was mixed with distilled water to obtain the protein concentration of 3.5 % (w/v). The mixture was heated at 70 °C for 30 min. Glycerol at 30 % (w/w) of protein content was used as a plasticizer. Prior to addition into solution, essential oils were mixed with various surfactants (Tween-20, Tween-80, soy lecithin) at 25 % (w/w, based on essential oil). Thereafter, the prepared essential oils were added into the gelatin solution at gelatin/essential oil ratio of 1:1 (w/w). The obtain suspension was homogenized at 22,000 rpm for 3 min using a homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The dissolved air in the FFS was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature.

For film preparation, FFS (4 g) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown for 12 h at room temperature. The films were further dried 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 were prepared from FFS without essential oils and surfactants.

4.3.4 Determination of film properties

4.3.4.1 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 Mechanical properties

Prior to testing the mechanical properties, 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.

4.3.4.3 Water vapor permeability (WVP)

WVP was measured using a modified ASTM method (American Society for Testing & Materials, 1989) as described by Shiku *et al.* (2004). The film was sealed on an aluminum permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and a rubber gasket to hold the film 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:

$$WVP\ (gm^{-1}s^{-1}Pa^{-1}) = wlA^{-1}t^{-1}(P_2 - P_1)^{-1}$$

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 (Pa).

4.3.4.4 Color differences

Color differences of film samples were determined using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA). D_{65} (day light) and a measure cell with opening of 30 mm was used. 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 the color parameters of the white standard (L^* = 92.85, a^* = - 1.20, b^* = 0.46).

4.3.4.5 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):

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

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.

4.3.5 Determination of antioxidative activity of fish skin gelatin films incorporated with essential oils

Films were solidified using liquid nitrogen in a mortar and ground with a pestle. Ground film (0.25 g) was mixed with 5 ml of methanol and stirred

vigorously for 3 h. The mixture was centrifuged at 2700 xg for 10 min using a centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Beckman Coulter, Inc., Palo Alto, CA, USA). The supernatant obtained was determined for antioxidative activities.

4.3.5.1 DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Binsan *et al.* (2008) with a slight modification. Sample (1.5 ml) was added with 1.5 ml of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in 95 % ethanol. The mixture was mixed vigorously using a mixer (Vertex-Genie 2, Model G-560E, Scientific Industries, inc., Bohemia, New York, USA) and allowed to stand at room temperature in dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. Sample blank was prepared in the same manner except that 95 % methanol was used instead of DPPH solution. A standard curve was prepared using Trolox in the range of 10 - 60 μM. The activity was calculated after the sample blank substraction and expressed as μmol Trolox equivalents (TE)/g dried film.

4.3.5.2 ABTS radical scavenging activity

ABTS radical scavenging activity was assayed as per the method of Arnao *et al.* (2001) with a slight modification. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml of ABTS solution with 50 ml of methanol in order to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using a spectrophotometer. ABTS solution was prepared freshly prior to assay. Sample (150 μl) was mixed with 2850 μl of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm using a spectrophotometer. Sample blank was prepared in the same manner except that methanol was used instead of ABTS solution. A standard curve of Trolox ranging from 50 to 600 μM was prepared. The activity was calculated

after sample blank subtraction and was expressed as μmol Trolox equivalents (TE)/g dried film.

4.3.5.3 Ferrous ion chelating activity

Ferrous ion chelating activity was measured by the method of Thiansilakul *et al.* (2007). Diluted sample (4.7 ml) was mixed with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine. The reaction mixture was allowed to stand for 20 min at room temperature. The absorbance was then read at 562 nm. EDTA with the concentration range of 0–50 μM was used as the standard. Ferrous ion chelating activity was expressed as μmol EDTA equivalents (EE)/g dried film.

4.3.6 Statistical analysis

All experiments were run in triplicate. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test (Steel and Torrie, 1980). Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL, USA).

4.4 Results and Discussion

4.4.1 Effect of different leaf essential oils and surfactants on mechanical and physical properties of fish gelatin films

4.4.1.1 Thickness

All films incorporated with essential oils had higher thickness than the control film, regardless of surfactants ($p < 0.05$) (Table 19). Interaction between essential oils and peptide chains might reduce the ordered alignment. As a result, the compact network could not be developed and the protruded network was formed as shown by the increased thickness. Moreover, it was noted that films prepared using different surfactants had varying thickness ($p < 0.05$). The use of soy lecithin as surfactant resulted in the highest increase in thickness, compared with Tween-20 and Tween-80. Lecithin with a larger and bulky structure more likely impeded the alignment of gelatin in film network to a higher extent. Additionally, the droplet of essential oils were dispersed and localized inside the film network, thereby lowering

the interaction between gelatin chains. It was noted that the difference in bulk densities between materials used in FFS, e.g. essential oil and surfactants could be another factor governing the thickness of resulting films. Both essential oils and surfactants could therefore affect the film matrix differently.

4.4.1.2 Mechanical properties

Mechanical properties expressed as tensile strength (TS) and elongation at break (EAB) of gelatin films incorporated with various essential oils in the presence of different surfactants are shown in Table 19. Films incorporated with essential oils exhibited the lower TS but higher EAB than the control film (without essential oil and surfactant), regardless of surfactants ($p < 0.05$). It was found that surfactants affected TS and EAB of resulting films differently. The highest TS was obtained in films when incorporated with lemongrass, basil, and citronella when Tween-20 was used as surfactant ($p < 0.05$). Nevertheless, film added with kaffir lime essential oil had no difference in TS between those containing Tween-20 and soy lecithin ($p > 0.05$). No difference in TS was observed between films containing lemongrass, basil and citronella essential oils when Tween-80 and soy lecithin were present ($p > 0.05$). Essential oils and surfactants also affected EAB of films to different degrees. Tween-80 rendered the films with the lowest EAB ($p < 0.05$). Similar EAB of films containing Tween-20 and soy lecithin as surfactant was observed when added with lemongrass, basil and citronella essential oils ($p > 0.05$). Tween-20 with higher hydrophobic-lipophilic balance (HLB) might be more effective in stabilizing emulsion than Tween-80 (Lee *et al.*, 2005). As a consequence, oil droplets were distributed more uniformly in the film containing Tween-20, in comparison with that having Tween-80 as the surfactant. As a result, interaction or aggregation between protein chains could be lower in film containing Tween-20 more potentially as indicated by the higher EAB. No difference in EAB was observed in films incorporated with all essential oils when Tween-80 were used as a surfactant ($p > 0.05$). Thus, the type of surfactants used had the marked impact on the mechanical properties of film. Different surfactants might have varying activity in lowering surface tension. As a result, size and dispersion of oil droplets stabilized by surfactant

could be varied. These might affect the uniformity of film matrix as evidenced by different mechanical properties.

When the same surfactant was used, TS and EAB of films also varied with different types of essential oils incorporated. The addition of hydrophobic substances such as fatty acids, oil or wax decreased TS of films from whey protein (Chick and Hernandez, 2002; Soazo *et al.*, 2011), sodium caseinate (Fabra *et al.*, 2009), fish skin gelatin (Limpisophon *et al.*, 2010; Tongnuanchan *et al.*, 2012), etc. The incorporation of essential oil decreased TS and increased EAB of gelatin based films. The results suggested that essential oils functioned as plasticizer in resulting film. Plasticizers are molecules, which penetrate into biopolymer matrix, thereby increasing the volume of empty spaces between the chains of molecules and causing a decrease in the strength of intermolecular forces along the matrix (Krochta, 1997). Essential oils more likely interfered or interrupted the protein–protein interaction of gelatin chains in film network. The major bonds stabilizing gelatin-based film were hydrogen bond and hydrophobic interaction (Hoque *et al.*, 2010; Jongjareonrak *et al.*, 2006a). The lack of cohesive structure integrity led to discontinuity of film network. Due to the differences in reactivity in binding or interacting with protein molecules in film network, different essential oils affected the mechanical properties of fish gelatin films differently. Essential oils are very complex natural mixtures containing 20 – 60 compounds at different concentrations such as aldehydes, ketones and phenol, etc (Bakkali *et al.*, 2008). Aldehydes or ketones (De Carvalho and Grosso, 2004; Hernández-Muñoz *et al.*, 2004) and phenols or phenolic compounds (Hoque *et al.*, 2011; Nuthong *et al.*, 2009) have been reported to interact with proteins, determining the property of resulting films. Polyphenol containing hydrophobic groups could interact with gelatin by hydrophobic interaction, while hydroxyl group of polyphenol was able to interact with gelatin via hydrogen bonds. Moreover, the monoterpenes hydrocarbon could interact with the hydrophobic region of gelatin chain via hydrophobic interaction. As a result, the incorporation of essential oil directly affected TS and EAB, but the value varied with types of essential oils incorporated.

4.4.1.3 Water vapor permeability (WVP)

WVP of gelatin films incorporated with different essential oils and surfactants is shown in Table 19. Gelatin film is known to have poor water barrier properties, due to its hydrophilic nature (Denavi *et al.*, 2009; Jongjareonrak *et al.*, 2006a). WVP of films decreased when all essential oils were incorporated ($p < 0.05$), for all surfactants used. For the film added with the same essential oil, those containing soy lecithin as surfactant had the lowest WVP, in comparison with those having Tween-20 and Tween-80 ($p < 0.05$). Films incorporated with lemongrass, basil, citronella and kaffir lime essential oils and used soy lecithin as surfactant had the decreases in WVP by 49.64, 57.14, 61.78 and 63.21 %, respectively, in comparison with control film (without essential oils and surfactants added) ($p < 0.05$). Among all films, that incorporated with kaffir lime essential oil and soy lecithin had the lowest WVP ($p < 0.05$). However, it had the similar WVP to those containing citronella essential oil and soy lecithin ($p > 0.05$). It was suggested that the incorporation of non-polar or hydrophobic materials including essential oil increased the hydrophobicity of films, thereby lowering the adsorptivity as well as diffusivity of water vapor through the film as indicated by lower WVP. WVP of films varied with hydrophobicity of compounds in essential oils. Monoterpenes are highly hydrophobic compounds in essential oils (Turina *et al.*, 2006). The monoterpenes are formed from coupling of two isoprene units (C10) and constitute 90 % of essential oils (Bakkali *et al.*, 2008).

Surfactant was found to influence WVP of films. Soy lecithin or phospholipids yielded the films with the lowest WVP. Soy lecithin might facilitate oil droplets to distribute uniformly in film matrix, thereby lowering the diffusion of water molecules through the film (Dickinson, 2003; Fang and Dalgleish, 1993). Soy lecithin was able to prevent creaming or oil phase separation more effectively than other surfactants (data not shown). Furthermore, hydrophobic domain of phospholipid or lecithin might assist in lowering the transfer of water vapor as indicated by lower WVP. Thus, the incorporation of essential oil could enhance water vapor barrier property of fish gelatin film effectively, particularly when soy lecithin was used as surfactant.

Table 19. Mechanical properties, water vapor permeability and thickness of films from fish skin gelatin containing different leaf essential oils in the presence of different surfactants.

Essential oils	Surfactants	TS (MPa)	EAB (%)	WVP ($x10^{-11}$ $gm^{-1}s^{-1}Pa^{-1}$)	Thickness (mm)
Control	-	36.83 ± 1.51 ^{a}*	31.07 ± 5.40 ^{f}	2.80 ± 0.16 ^{a}	0.043 ± 0.002 ^{h}
Lemongrass	Tween-20	25.13 ± 0.46 ^{b}	77.25 ± 6.11 ^{cd}	1.79 ± 0.04 ^{c}	0.056 ± 0.002 ^{g}
	Tween-80	18.04 ± 0.88 ^{de}	52.81 ± 1.54 ^{e}	1.59 ± 0.06 ^{d}	0.070 ± 0.001 ^{ef}
	Soy lecithin	18.42 ± 1.54 ^{de}	72.85 ± 8.54 ^{d}	1.41 ± 0.17 ^{e}	0.073 ± 0.002 ^{de}
Basil	Tween-20	21.37 ± 0.21 ^{c}	85.06 ± 7.50 ^{bc}	2.11 ± 0.13 ^{b}	0.054 ± 0.001 ^{g}
	Tween-80	17.49 ± 0.90 ^{de}	46.53 ± 4.47 ^{e}	1.84 ± 0.07 ^{c}	0.066 ± 0.002 ^{f}
	Soy lecithin	18.70 ± 0.67 ^{d}	83.59 ± 4.71 ^{bc}	1.20 ± 0.05 ^{f}	0.084 ± 0.002 ^{a}
Citronella	Tween-20	21.85 ± 0.79 ^{c}	97.29 ± 8.48 ^{a}	1.42 ± 0.06 ^{e}	0.068 ± 0.002 ^{bc}
	Tween-80	17.10 ± 0.57 ^{e}	44.63 ± 2.87 ^{e}	1.43 ± 0.04 ^{e}	0.060 ± 0.001 ^{f}
	Soy lecithin	17.39 ± 1.37 ^{de}	92.40 ± 9.84 ^{ab}	1.07 ± 0.04 ^{fg}	0.080 ± 0.002 ^{b}
Kaffir lime	Tween-20	26.21 ± 0.72 ^{b}	95.08 ± 7.56 ^{a}	1.59 ± 0.04 ^{d}	0.066 ± 0.002 ^{f}
	Tween-80	15.21 ± 1.89 ^{f}	43.95 ± 4.07 ^{e}	1.17 ± 0.02 ^{f}	0.076 ± 0.002 ^{cd}
	Soy lecithin	25.07 ± 0.44 ^{b}	79.60 ± 9.71 ^{cd}	1.03 ± 0.10 ^{g}	0.081 ± 0.002 ^{b}

* Mean ± SD (n=3).

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

Control film was prepared from FFS without addition of essential oils and surfactants.

4.4.1.4 Color

The color expressed as L^*, a^*, b^* and ΔE^*-values of gelatin films incorporated with different essential oils and surfactants is shown in Table 20. Films incorporated with all essential oils had lower L^*-value and higher b^*- and ΔE^*-values than the control film ($p < 0.05$). Among all types of essential oils, lemongrass essential oil yielded the films with the highest b^*- and ΔE^*-values ($p < 0.05$). Essential oil from lemongrass had higher yellowish color than others, due to the presence of coloring pigment or compounds. Thus, color of essential oil directly had the impact on the color of resulting films. Surfactants were also found to impact the color of resulting films. Films with soy lecithin as surfactant showed the lowest L^*-value with the concomitant highest b^*- and ΔE^*-values ($p < 0.05$) when the same type of essential oil was added. This result indicated the higher yellowness in film prepared using soy lecithin as surfactant in comparison with others. It was noted that brown color of soy lecithin mainly correlated with the higher b^*-value and lower L^*-value of resulting film. Moreover, films containing soy lecithin showed the higher a^*-value than those having Tween-20 and Tween-80 as surfactants, when lemongrass, citronella and kaffir lime essential oils were added ($p < 0.05$). The incorporation of thyme oil at low content (0 - 0.25 ml oil/g protein) in the film had no influence on color of film from hake protein (Pires *et al.*, 2011). Thus, the changes in color of films were more likely due to coloring compounds in essential oils and surfactants incorporated.

4.4.1.5 Light transmittance and transparency

All films incorporated with essential oils had the excellent barrier property against UV light at 200 and 280 nm, regardless of surfactants (Table 21). Similar result was obtained for the control film. 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.*, 2007a). This result was in accordance with those of gelatin films from tilapia skin (Tongnuanchan *et al.*, 2012), bigeye snapper and brownstripe red snapper (Jongjareonrak *et al.*, 2006b). The lower transmission of visible light in the range of 350-800 nm was observed in films incorporated with

essential oils, compared with control films. Droplets of essential oils might prevent the transmission of light through the films. Among all essential oils incorporated, kaffir lime essential oil showed the highest efficiency in lowering the light transmittance in visible range of films. The barrier property became more pronounced when soy lecithin was used as surfactant, in which light transmittance of 5.24 - 55.34 % at wavelengths of 350 - 800 nm was observed. The difference in light scattering or absorption by different compounds in varying essential oils was presumed. Additionally, light transmittance of films was also varied with types of surfactants.

The transparency values of all film samples are presented in Table 21. The control film had the lowest transparency value, compared with those films added with all essential oils ($p < 0.05$). The lower transparency value indicated that the film was more transparent. Films generally became more turbid upon the addition of essential oils. This result suggested that the incorporation of essential oils yielded less transparent films. Among all films, those incorporated with kaffir lime essential oil had the highest transparency value ($p < 0.05$), followed by those added with citronella essential oil. However, no difference in transparency value was observed between films added with lemongrass and basil essential oils when the same surfactant was used ($p > 0.05$). This result was in agreement with Tongnuanchan *et al.* (2012) who reported that the addition of citrus essential oils could lower the transparency value of fish skin gelatin film. For films incorporated with basil or citronella essential oils, those containing soy lecithin had lower transparency value ($p < 0.05$). On the other hand, higher transparency value was found for films incorporated with kaffir lime essential oil when soy lecithin was used as surfactant. The opaqueness of film was more likely associated with light scattering effect of oil droplet in films. Moreover, transparency value of film also varied with the thickness of film. The higher transparency value was in agreement with higher L^*-value of films. Thus, the incorporation of essential oils and surfactants had the pronounced impacts on light transmittance and transparency of films.

Table 20. Color of films from fish skin gelatin containing different leaf essential oils in the presence of different surfactants.

Essential oils	Surfactants	*L**	*a**	*b**	Δ*E**
Control	-	91.42 ± 0.08 [a]*	-1.12 ± 0.03 [d]	1.54 ± 0.10 [j]	1.14 ± 0.08 [j]
Lemongrass	Tween-20	88.92 ± 0.08 [h]	-2.87 ± 0.03 [h]	14.19 ± 0.50 [d]	14.07 ± 0.51 [d]
	Tween-80	89.15 ± 0.02 [f]	-2.81 ± 0.04 [h]	14.09 ± 0.06 [d]	13.92 ± 0.07 [d]
	Soy lecithin	84.30 ± 0.09 [j]	-0.65 ± 0.04 [c]	32.09 ± 0.67 [a]	32.47 ± 0.67 [a]
Basil	Tween-20	90.87 ± 0.01 [d]	-1.67 ± 0.04 [g]	4.61 ± 0.03 [f]	4.23 ± 0.03 [f]
	Tween-80	91.29 ± 0.01 [c]	-1.53 ± 0.02 [f]	4.67 ± 0.02 [f]	4.23 ± 0.02 [f]
	Soy lecithin	89.07 ± 0.02 [g]	-1.73 ± 0.04 [g]	14.70 ± 0.05 [c]	14.47 ± 0.05 [c]
Citronella	Tween-20	91.73 ± 0.02 [a]	-1.09 ± 0.02 [d]	2.24 ± 0.06 [i]	1.81 ± 0.05 [i]
	Tween-80	91.40 ± 0.02 [b]	-1.40 ± 0.04 [e]	3.48 ± 0.08 [g]	3.03 ± 0.08 [g]
	Soy lecithin	88.40 ± 0.04 [i]	0.84 ± 0.12 [a]	17.05 ± 0.06 [b]	17.05 ± 0.04 [b]
Kaffir lime	Tween-20	91.29 ± 0.01 [c]	-1.39 ± 0.04 [e]	3.15 ± 0.05 [h]	2.71 ± 0.05 [h]
	Tween-80	91.78 ± 0.02 [a]	-1.71 ± 0.04 [g]	4.66 ± 0.02 [f]	4.21 ± 0.02 [f]
	Soy lecithin	90.65 ± 0.03 [e]	-0.30 ± 0.09 [b]	8.83 ± 0.05 [e]	8.50 ± 0.04 [e]

* Mean ± SD (n=3).

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

Control film was prepared from FFS without addition of essential oils and surfactants.

Table 21. Light transmittance and transparency value of films from fish skin gelatin containing different leaf essential oils in the presence of different surfactants.

Essential oils	Surfactants	Light transmittance (%) at different wavelength (nm)								Transparency value
		200	280	350	400	500	600	700	800	
Control	-	0.03	43.72	78.47	82.58	85.64	87.18	88.18	88.86	1.39 ± 0.05 ^{i}*
Lemongrass	Tween-20	0.01	0.00	7.19	44.16	64.07	69.56	72.51	74.60	2.65 ± 0.12 ^{g}
	Tween-80	0.01	0.01	5.25	37.33	56.88	62.72	66.01	68.52	3.24 ± 0.16 ^{f}
	Soy lecithin	0.01	0.00	0.12	22.24	58.14	68.77	73.81	77.07	2.48 ± 0.11 ^{gh}
Basil	Tween-20	0.01	0.02	36.24	58.74	68.44	72.11	74.45	76.28	2.56 ± 0.32 ^{g}
	Tween-80	0.01	0.03	33.71	49.14	57.72	62.00	65.03	67.57	3.26 ± 0.19 ^{f}
	Soy lecithin	0.01	0.00	28.75	43.85	59.40	68.27	73.70	77.60	2.18 ± 0.05 ^{h}
Citronella	Tween-20	0.01	0.27	19.59	29.40	41.84	51.28	58.66	64.43	3.92 ± 0.12 ^{cd}
	Tween-80	0.00	0.57	25.07	36.51	45.31	50.99	55.41	59.07	4.41 ± 0.14 ^{c}
	Soy lecithin	0.01	0.01	9.19	21.33	37.33	49.89	58.70	65.32	3.67 ± 0.20 ^{e}
Kaffir lime	Tween-20	0.02	1.17	24.80	37.19	46.64	52.57	57.04	60.63	4.25 ± 0.69 ^{cd}
	Tween-80	0.02	3.35	26.47	34.01	41.36	46.45	50.56	54.14	5.64 ± 0.03 ^{b}
	Soy lecithin	0.02	0.00	5.24	12.72	26.09	38.18	48.04	55.94	6.08 ± 0.03 ^{a}

* Mean ± SD (n=3).

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

Control film was prepared from FFS without addition of essential oils and surfactants.

4.4.2 Effect of different leaf essential oils and surfactants on antioxidative activities of fish gelatin films

Antioxidative activities expressed as DPPH radical scavenging activity, ABTS radical scavenging activity and chelating activity of gelatin films incorporated with different essential oils and surfactants are depicted in Figure 14, 15 and 16, respectively. Very low antioxidative activities were observed in the control film (without incorporated essential oil and surfactant). When films were incorporated with essential oils, the marked increases in antioxidant activities were obtained ($p < 0.05$). With the same surfactant, films incorporated with basil essential oil had the highest DPPH and ABTS radical scavenging activities ($p < 0.05$), followed by those added with citronella, lemongrass and kaffir lime essential oils, respectively. It has been reported that essential oils contained various antioxidant compounds (Wu *et al.*, 1982). The main constituents in essential oil are terpenes (monoterpenes and sesquerpitenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, etc.) and terpenoides (isoprenoids) (Bakkali *et al.*, 2008). Hydroxyl group of tertenes or aromatic compounds in essential oils generally acts as hydrogen donor (Bakkali *et al.*, 2008). As a result, the different essential oils containing various antioxidants were able to scavenge radicals at varying degrees. It was suggested that the antioxidant compounds in essential oils might interact with protein molecules in film network via mainly hydrogen bonding, upon film casting and drying. This might result in the lowered antioxidative activity of films in comparison with free essential oil. It was noted that films having soy lecithin as surfactant had higher DPPH and ABTS radical-scavenging activities than those with Tween-20 and Tween-80 ($p < 0.05$). It was suggested that lecithin might act as antioxidant. Lecithin is an efficient antioxidant with metal chelating activities (Judde *et al.*, 2003).

For chelating activity, films incorporated with lemongrass essential oil using Tween-20 and Tween-80 as surfactants had the highest activities ($p < 0.05$), followed by those incorporated with kaffir lime, citronella and basil essential oils, respectively. For films incorporated with kaffir lime essential oil, the highest chelating activity was observed when soy lecithin was used ($p < 0.05$). Higher chelating activity was generally found when soy lecithin was used as surfactant.

Lecithin containing phospholipid polar head with capacity of chelation might be able to sequester Fe ions. Apart from serving as surfactant, lecithin simultaneously act as antioxidant. It was noted that films containing basil essential oil showed different ABTS and DPPH radical scavenging activities when Tween-20 and Tween-80 were used. Distribution of oil droplets and their interaction with protein determined antioxidant activity. Thus, the incorporation of leaf essential oils into gelatin-based film and the use of soy lecithin as surfactant could augment antioxidative activity.

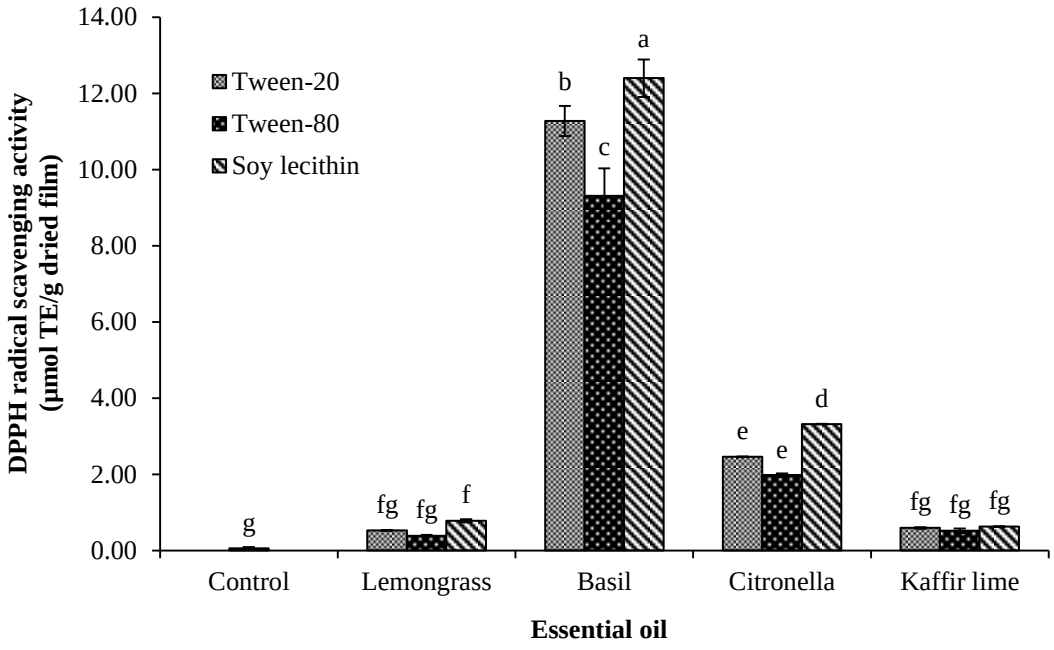


Figure 14. DPPH radical scavenging activity of films from fish skin gelatin containing different leaf essential oils in the presence of different surfactants. Bars represent the standard deviation (n=3). Different letters on the bars for the same assay indicate the significant differences ($p < 0.05$).

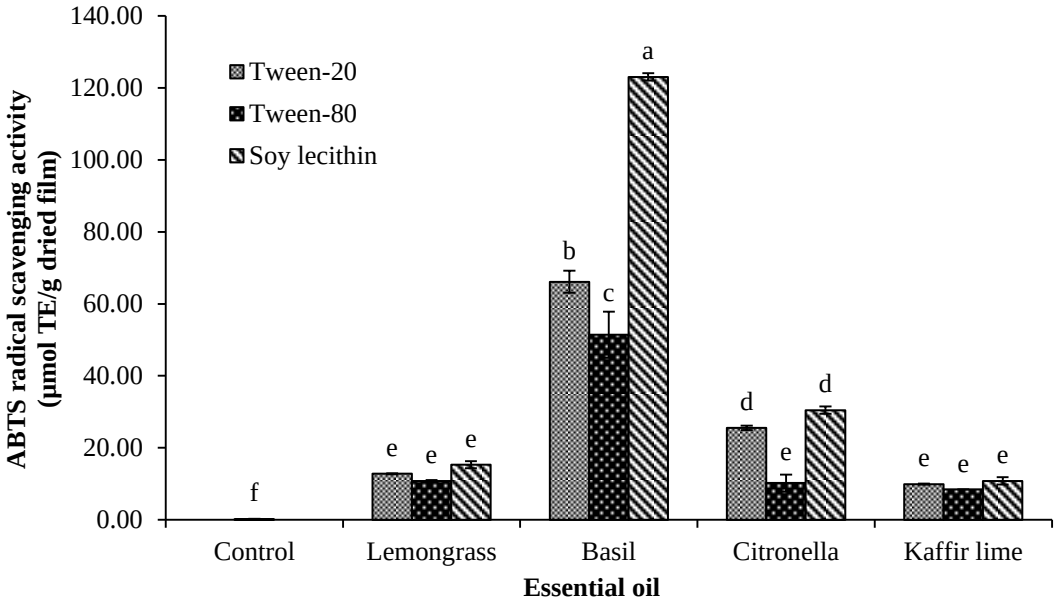


Figure 15. ABTS radical scavenging activity of films from fish skin gelatin containing different leaf essential oils in the presence of different surfactants. Bars represent the standard deviation (n=3). Different letters on the bars for the same assay indicate the significant differences ($p < 0.05$).

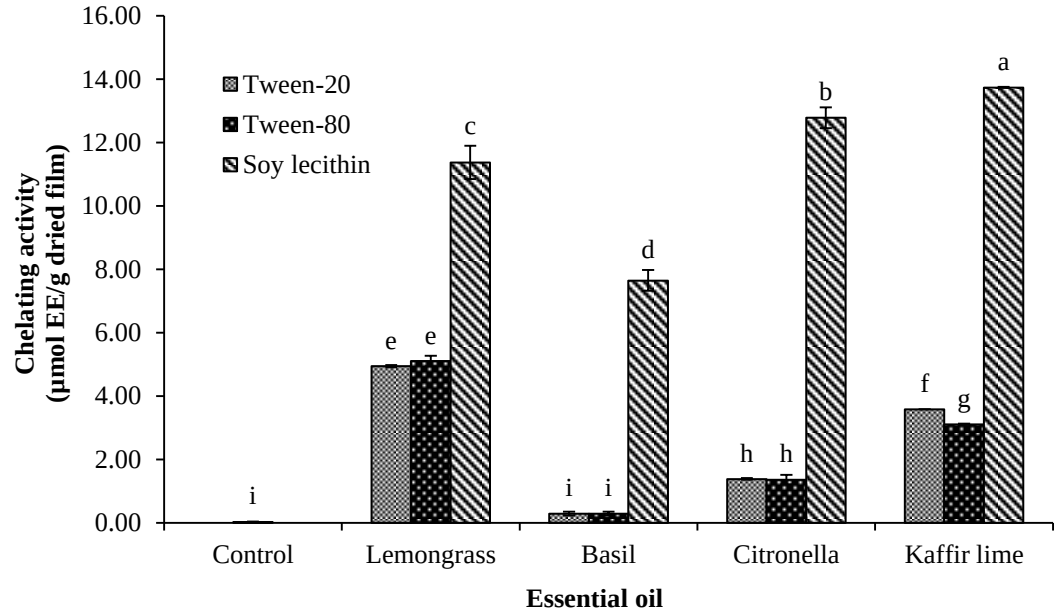


Figure 16. Chelating activity of films from fish skin gelatin containing different leaf essential oils in the presence of different surfactants. Bars represent the standard deviation (n=3). Different letters on the bars for the same assay indicate the significant differences ($p < 0.05$).

4.5 Conclusion

Mechanical properties, WVP and antioxidative activities of fish skin gelatin film were generally affected by the incorporation of leaf essential oils and surfactants. Essential oils exhibited plasticizing effect and lowered WVP of films. Among surfactants used, soy lecithin was the most appropriate surfactant, which yielded the film with improved WVP and antioxidative activity. Therefore, gelatin film incorporated with leaf essential oils using soy lecithin as surfactant can be used as active packaging due to their enhanced antioxidative activity. The antioxidative activity of gelatin film containing only surfactant should be further elucidated.

4.6 References

Arnao, M.B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73: 239-244.

ASTM. 1989. Standard test methods for water vapor transmission of materials. Standard designation E96-E80. *In* Annual book of ASTM standards. p. 730-739. ASTM. Philadelphia.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils - A review. Food Chem. Toxicol. 46: 446-475.

Binsan, W., Benjakul, S., Visessanguan, W., Roytrakul, S., Tanaka, M. and Kishimura, H. 2008. Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). Food Chem. 106: 185-193.

Chen, C.-H., Kuo, W.-S. and Lai, L.-S. 2009. Effect of surfactants on water barrier and physical properties of tapioca starch/decolorized hsian-tsao leaf gum films. Food Hydrocolloid. 23: 714-721.

Chick, J. and Hernandez, R.J. 2002. Physical, thermal, and barrier characterization of casein-wax-based edible films. J. Food Sci. 67: 1073-1079.

De Carvalho, R.A. and Grosso, C.R.F. 2004. Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. Food Hydrocolloid. 18: 717-726.

Denavi, G.A., Pérez-Mateos, M., Añón, M.C., Montero, P., Mauri, A.N. and Gómez-Guillén, M.C. 2009. Structural and functional properties of soy protein isolate and cod gelatin blend films. Food Hydrocolloid. 23: 2094-2101.

Dickinson, E. 2003. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocolloid. 17: 25-39.

Emiroğlu, Z.K., Yemiş□, G.P., Coşkun, B.K. and Candoğan, K. 2010. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Sci. 86: 283-288.

Fabra, M.J., Talens, P. and Chiralt, A. 2009. Microstructure and optical properties of sodium caseinate films containing oleic acid-beeswax mixtures. Food Hydrocolloid. 23: 676-683.

Fang, Y. and Dalgleish, D.G. 1993. Casein adsorption on the surfaces of oil-in-water emulsions modified by lecithin. Colloids Surfaces B: Biointerfaces. 1: 357-364.

Gennadios, A., Weller, C.L., Hanna, M.A. and Froning, G.W. 1996. Mechanical and barrier properties of egg albumen films. J. Food Sci. 61: 585-589.

Hamaguchi, P.Y., Weng, W., Kobayashi, T., Runglertkreingkrai, J. and Tanaka, M. 2007a. Effect of fish meat quality on the properties of biodegradable protein films. Food Sci. Technol. Res. 13: 200-204.

Hamaguchi, P.Y., WuYin, W. and Tanaka, M. 2007b. Effect of pH on the formation of edible films made from the muscle proteins of blue marlin (*Makaira mazara*). Food Chem. 100: 914-920.

Han, J.H. and Floros, J.D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. J. Plast. Film Sheet. 13: 287-298.

Hernández-Muñoz, P., Villalobos, R. and Chiralt, A. 2004. Effect of cross-linking using aldehydes on properties of glutenin-rich films. Food Hydrocolloid. 18: 403-411.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2010. Effect of heat treatment of film-forming solution on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. J. Food Eng. 96: 66-73.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011. Properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin incorporated with cinnamon, clove and star anise extracts. Food Hydrocolloid. 25: 1085-1097.

Hsu, J.-P. and Nacu, A. 2003. Behavior of soybean oil-in-water emulsion stabilized by nonionic surfactant. J. Colloid Interf. Sci. 259: 374-381.

Ikeda, R.M., Stanley, W.L., Vannier, S.H. and Spitler, E.M. 1962. The Monoterpene Hydrocarbon Composition of Some Essential Oils. J. Food Sci. 27: 455-458.

Iwata, K., Ishizaki, S., Handa, A. and Tanaka, M. 2000. Preparation and characterization of edible films from fish water-soluble proteins. Fisheries Sci. 66: 372-378.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006a. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid. 20: 492-501.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006b. Effects of plasticizers on the properties of edible films from skin gelatin of bigeye snapper and brownstripe red snapper. Eur. Food Res. Technol. 222: 229-235.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2008. Antioxidative activity and properties of fish skin gelatin films incorporated with BHT and α-tocopherol. Food Hydrocolloid. 22: 449-458.

Judde, A., Villeneuve, P., Rossignol-Castera, A. and Guillou, A. 2003. Antioxidant effect of soy lecithins on vegetable oil stability and their synergism with tocopherols. J. Am. Soc. 80: 1209-1215.

Krochta, J.M. 1997. Edible protein films and coatings. *In* Food proteins and their applications. (Damodaran, S. and Paraf, A., eds.). p. 529-549. Marcel Dekker. New York.

Lee, D.-H., Kim, E.-S. and Chang, H.-W. 2005. Effect of Tween surfactant components for remediation of toluene-contaminated groundwater. Geosci. J. 9: 261-267.

Limpisophon, K., Tanaka, M. and Osako, K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionace glauca*) skin gelatin. Food Chem. 122: 1095-1101.

Mohamed, A.A., El-Emary, G.A. and Ali, H.F. 2010. Influence of some citrus essential oils on cell viability, glutathione-s-transferase and lipid peroxidation in *Ehrlich ascites* Carcinoma Cells. J. Am. Sci. 6: 820-826.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009. Effect of phenolic compounds on the properties of porcine plasma protein-based film. Food Hydrocolloid. 23: 736-741.

Pires, C., Ramos, C., Teixeira, G., Batista, I., Mendes, R., Nunes, L. and Marques, A. 2011. Characterization of biodegradable films prepared with hake proteins and thyme oil. J. Food Eng. 105: 422-428.

Prodpran, T., Benjakul, S. and Artharn, A. 2007. Properties and microstructure of protein-based film from round scad (*Decapterus maruadsi*) muscle as affected by palm oil and chitosan incorporation. Int. J. Biol. Macromol. 41: 605-614.

Seydim, A.C. and Sarikus, G. 2006. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food Res. Int. 39: 639-644.

Shiku, Y., Hamaguchi, P.Y., Benjakul, S., Visessanguan, W. and Tanaka, M. 2004. Effect of surimi quality on properties of edible films based on Alaska pollack. Food Chem. 86: 493-499.

Soazo, M., Rubiolo, A.C. and Verdini, R.A. 2011. Effect of drying temperature and beeswax content on physical properties of whey protein emulsion films. Food Hydrocolloid. 25: 1251-1255.

Staroszczyk, H., Pielichowska, J., Sztuka, K., Stangret, J. and Kołodziejska, I. 2012. Molecular and structural characteristics of cod gelatin films modified with EDC and TGase. Food Chem. 130: 335-343.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics. McGraw-Hill Book. New York.

Thiansilakul, Y., Benjakul, S. and Shahidi, F. 2007. Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). Food Chem. 103: 1385-1394.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2012. Properties and antioxidant activity of fish skin gelatin film incorporated with citrus essential oils. Food Chem. 134: 1571-1579.

Turina, A.V., Nolan, M.V., Zygadlo, J.A. and Perillo, M.A. 2006. Natural terpenes: self-assembly and membrane partitioning. Biophys. Chem. 122: 101-113.

Vanin, F.M., Sobral, P.J.A., Menegalli, F.C., Carvalho, R.A. and Habitante, A.M.Q.B. 2005. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. Food Hydrocolloid. 19: 899-907.

Villalobos, R., Hernández-Muñoz, P. and Chiralt, A. 2006. Effect of surfactants on water sorption and barrier properties of hydroxypropyl methylcellulose films. Food Hydrocolloid. 20: 502-509.

Wu, J., Lee, M.-H., Ho, C.-T. and Chang, S. 1982. Elucidation of the chemical structures of natural antioxidants isolated from rosemary. J. Am. Soc. 59: 339-345.

CHAPTER 5

STRUCTURAL, MORPHOLOGICAL AND THERMAL BEHAVIOR CHARACTERIZATIONS OF FISH GELATIN FILM INCORPORATED WITH BASIL AND CITRONELLA ESSENTIAL OILS AS AFFECTED BY SURFACTANTS

5.1 Abstract

Structural, morphological and thermal properties of fish skin gelatin films incorporated with basil and citronella essential oils at a ratio of 1:1 (w/w), as influenced by different surfactants (Tween-20, Tween-80 and soy lecithin at 25% based on essential oil), were characterized. Smoother surface and more homogeneous oil distribution was observed via SEM in films containing both basil and citronella essential oils when soy lecithin was used as surfactant. Essential oil containing gelatin films exhibited bi-layer morphology when Tween-80 was used as surfactant. FTIR results suggested the decreased protein-protein interaction in the matrix of gelatin film when essential oils were incorporated, depending on type of surfactants used. Films added with both essential oils had lower glass-transition and degradation temperatures than the control films, indicating a poorer protein-protein interaction in film network. Therefore, both essential oils and surfactants had the impact on molecular structure and thermal properties of resulting films.

5.2 Introduction

Proteins are biopolymers capable of forming the film and their properties can be varied with many factors, e.g. level of plasticizer, amount and type of protein (Krochta, 1997). Gelatins from several sources have been widely used as film forming materials (Arvanitoyannis, 2002; Hoque *et al.*, 2011a; Jongjareonrak *et al.*, 2008; Tongnuanchan *et al.*, 2012). Gelatin film properties were determined by gelatin source and film making process (Limpisophon *et al.*, 2010). Gelatin films have excellent barrier properties against gas, volatile compounds and UV light (Hoque *et al.*, 2011a; Jongjareonrak *et al.*, 2006). However, water vapor barrier property of gelatin films was poorer than that of other biopolymer films, due to the hydrophillic

nature of gelatin and hydrophilic plasticizer required for film preparation (Jongjareonrak *et al.*, 2006; Limpisophon *et al.*, 2010; Tongnuanchan *et al.*, 2012). Among plasticizers used, glycerol is a plasticizer of choice widely used to incorporate into gelatin film because it is well miscible with the gelatin molecule, providing excellent plasticizing effect on gelatin film (Arvanitoyannis, 2002; Bergo and Sobral, 2007; Krochta, 1997). Moreover, glycerol is a major by-product, which generated by the production of biodiesel. Using glycerol as plasticizer for biodegradable film production is a potential way for increasing value of this low-grade by-product (Arrieta *et al.*, 2013; Ye *et al.*, 2012).

The incorporation of non-polar or hydrophobic substances, such as oils, fats and fatty acids, are commonly used to improve the water vapor barrier property of hydrophilic biopolymer films (Limpisophon *et al.*, 2010; Prodpran *et al.*, 2007). Fat and lipids of different types have been successfully incorporated into protein-based films by different means including coating, lamination or multilayer and dispersion or emulsion to form composite films (Atarés *et al.*, 2010a; Bahram *et al.*, 2014; Guerrero *et al.*, 2011; Krochta, 2002).

Essential oils are natural volatile complex compounds formed as the secondary metabolites in plants (Bakkali *et al.*, 2008). Essential oils have been largely employed because of their antibacterial, antifungal, antiviral and antioxidant properties (Burt, 2004; Kordali *et al.*, 2005). Therefore, the incorporation of essential oils into the films could provide antioxidant activity for resulting films. Atarés *et al.* (2010b) studied the mechanical properties of soy protein isolate incorporated with cinnamon and ginger essential oil at different concentration (protein to oil mass ratios: 1:0.025, 1:0.050, 1:0.075 and 1:0.100). A slight decreasing trend of elastic modulus was observed as the oil content increased. Protein-based film incorporated with essential oil showed the increased water vapour barrier properties (Pires *et al.*, 2011; Tongnuanchan *et al.*, 2012) and possessed antioxidant and antimicrobial activities (Emiroğlu *et al.*, 2010; Oussalah *et al.*, 2004; Tongnuanchan *et al.*, 2012). Seydim and Sarikus (2006) evaluated antimicrobial activity of whey protein isolate-based edible films incorporated with oregano essential oil and found that oregano essential oil added films exhibited larger inhibitory zone on *S. aureus* with increasing levels of essential oil added. Recently, Tongnuanchan, Benjakul, and Prodpran (2013b)

reported that gelatin film containing basil essential oil exhibited the highest DPPH radical- and ABTS radical-scavenging activities, followed by film containing citronella essential oil, while no differences in those activities were observed between films added with lemongrass and kaffir lime essential oils. Therefore, leaf essential oils such as basil and citronella essential oils can serve as hydrophobic substances with antioxidant activity and to be used to improve the property of biopolymer film as an active packaging. Furthermore, the amount of essential oils incorporated in the film directly affected properties and antioxidative activity of film. The lowest water vapor permeability and the highest radical scavenging activity were observed in gelatin film when root essential oils were incorporated at a gelatin/essential oil ratio of 1:1 (w/w) (Tongnuanchan *et al.*, 2013b). However, the disruption of protein-protein interaction in the film matrix arose from droplets of essential oils, thereby causing roughness or discontinuity of film matrix and decreasing strength of film (Atarés *et al.*, 2010a; Tongnuanchan *et al.*, 2012). The increased content of essential oil droplets could enhance creaming and phase separation. Essential oils with low density, especially at high content were separated and localized at the upper surface of film (Tongnuanchan *et al.*, 2013a).

Therefore, the preparation of emulsion films generally requires proper surfactant in order to provoke the stable state of two-phase emulsion system with homogeneity of oil droplet distribution. Different surfactants incorporated would contribute to film structure or morphology (oil droplet distribution) and film properties differently. Prodpran *et al.* (2007) prepared fish muscle protein-based film containing palm oil, in which Tween-20 was used as surfactant. Peng and Li (2014) also used Tween-20 to prepared chitosan-based film incorporated with lemon, thyme and cinnamon essential oils. Tween-80 has been reported to prepared soluble soybean polysaccharide film added with essential oils (Salarbashi *et al.*, 2013), whey protein concentrate film containing cinnamon essential oil (Bahram *et al.*, 2014), cassava starch blend film (Brandelero *et al.*, 2010). Tanaka *et al.* (2001) prepared fish protein-lipid emulsion films using lecithin as surfactant. Furthermore, Limpisophon *et al.* (2010) reported the use of sucrose stearate as surfactant for preparation of gelatin film incorporated with stearic and oleic acids. More recently, the physico-chemical properties of gelatin/essential oil films as affected by different surfactants used were

reported (Tongnuanchan *et al.*, 2012). However, little information regarding the effect of different surfactants on fish gelatin-essential oil emulsion film, particularly on its microstructures, molecular characteristics and thermal properties has been reported. Thus, the present study was undertaken to investigate morphological, molecular and thermal properties of emulsion films based on fish gelatin incorporated with basil and citronella essential oils in the presence of different surfactants used.

5.3 Materials and Methods

5.3.1 Chemicals

Glycerol and soy lecithin (HLB = 4.0) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tween-20 (HLB = 16.7) and Tween-80 (HLB = 15.0) were obtained from Merck (Darmstadt, Germany). All chemicals are of analytical grade.

5.3.2 Fish gelatin and essential oils

Fish gelatin produced from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Essential oils from the leaves of basil (*Ocimum basilicum*) and citronella (*Cymbopogon nardus*) were purchased from *Botanicessence* (Bangkok, Thailand).

5.3.3 Preparation of film from fish gelatin incorporated with different essential oils and surfactants

Gelatin powder was mixed with distilled water to obtain the protein concentration of 3.5 % (w/v). The mixture was heated at 70 °C for 30 min. Glycerol at 30 % (w/w) of protein content was used as a plasticizer. Prior to the addition into solution, essential oils were mixed with various surfactants (Tween-20, Tween-80, soy lecithin) at 25 % (w/w, based on essential oil). Subsequently, the prepared essential oils were added into the solution at gelatin/essential oil ratio of 1:1 (w/w). The suspension was homogenized at 22,000 rpm for 3 min using a homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The dissolved air in the suspension

was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature.

To prepare the films, the suspension (4 g) was cast onto a rimmed silicone resin plate (50×50 mm^2) and air-blown for 12 h at room condition (27±2 °C and 75±10 % relative humidity (RH)). The films were further dried 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 were prepared from solution containing gelatin and glycerol without essential oils and surfactants.

5.3.4 Characterization of fish gelatin-essential oils emulsion film

Prior to analyses, films were conditioned in desiccators containing dried silica gel for 2 weeks and 1 week in desiccators containing P_2O_5 at room temperature (25-30 °C) to obtain the most dehydrated films.

5.3.4.1 Scanning electron microscopy (SEM)

Morphology of surface and cross-section of film samples were 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.

5.3.4.2 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 -40 to 150 °C, with a heating rate of 5 °C/min. Dry ice was used as cooling medium and the system was equilibrated at -40 °C for 5 min prior to the scan. An empty aluminium pan was used as the reference. The maximum transition temperature was estimated from the endothermic peak of the

DSC thermogram and transition enthalpy was determined from the area under the endothermic peak. A second scan was also performed in the same manner, followed by quench-cooling of the sample after completing the first scan. The T_g of basil and citronella essential oils as well as glycerol was also evaluated over the temperature range of -170 to 150 °C using liquid nitrogen as cooling medium, with a heating rate of 5 °C/min.

5.3.4.3 Thermo-gravimetric analysis (TGA)

Films were scanned using a thermo-gravimetric analyzer (TGA7, PerkinElmer, Norwalk, CT, USA) from 25 to 1000 ºC at a rate of 10 ºC/min (Nuthong *et al.*, 2009). Nitrogen was used as the purge gas at a flow rate of 20 ml/min.

5.3.4.4 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 horizontal ATR Trough plate crystal cell (45º ZnSe; 80 mm long, 10 mm wide and 4 mm thick) (PIKE Technology Inc., Madison, WI, USA) at 25 ºC as described by Nuthong *et al.* (2009). Films were placed onto the crystal cell and the cell was 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 were ratioed against a background spectrum recorded from the clean empty cell at 25 ºC.

5.4 Results and Discussion

5.4.1 Film appearance and morphology

Photographs of gelatin films incorporated without and with basil and citronella essential oils using different surfactants are shown in Figure 17. Films containing both essential oils had the higher opaqueness than the control film, regardless of surfactant types used. Transparency and color of films varied with essential oils and surfactants used.

Surface and freeze-fractured cross-sectional images of gelatin films incorporated with basil and citronella essential oils in combination with different surfactants are shown in Figure 18. The control film (without incorporated essential oils and surfactants) had the smooth and continuous surface. This might be owing to the presence of ordered-phase and homogenous network structure. The surface of film containing both basil and citronella essential oils with Tween-80 as surfactant and film added with basil oil using Tween-20 as surfactant had rougher surface, compared with other films. The SEM images of those films indicated the collapsed oil droplets localized toward the upper surface of film, but those oil droplets were still remained imbedding in the film matrix. This result suggested that the essential oil droplets were unstable in emulsion systems stabilized by those surfactants (Tween-80 and Tween-20). Essential oils which were hydrophobic in nature might be separated from aqueous phase (Atarés *et al.*, 2010a). This was indicated by heterogeneous distribution of essential oils as visualized by crystals formed. However, the smooth and continuous surface was observed in films prepared using soy lecithin as a surfactant when both basil and citronella essential oils were incorporated. This might be due to the uniform distribution of oil droplets in the film network. This suggested that soy lecithin could maintain the oil droplet and lowered the collapse of droplets to creaming of essential oil-gelatin suspension. The result indicated the profound role of surfactant in morphology of resulting film incorporated with essential oils.

For cross-section, the control film had the smoother and more compact structure than those incorporated with essential oils. It indicated that the control film had more homogeneous structure than other films. Films containing different essential oils exhibited different cross-section morphologies. Protein-protein interaction in the

film network could be disrupted when oil droplets were inserted, thereby providing the roughness in film cross-section. Among all films incorporated with both essential oils, those prepared using soy lecithin as surfactant had the smoother cross-section than having Tween-20 and Tween-80 as surfactants. Those oil droplets were more likely localized inside the film network, where as no oil droplet on the surface of film was noticeable. Nevertheless, films prepared with Tween-80 exhibited the most roughness or discontinuity of cross-section, compared with those using other surfactants. Obviously, films prepared using Tween-80 for both essential oils incorporated and those containing basil essential oil and Tween-20 showed the phase separation between oil phase (upper) and protein phase (lower). Films prepared using Tween-20 and Tween-80 could not maintain the dispersion of oil droplets, which underwent creaming and phase separation, particularly during casting. Furthermore, essential oils with low density were able to separate and localize at the upper surface of film, thereby forming the bi-layer film (Tongnuanchan *et al.*, 2013a). This result was in agreement with Atarés *et al*. (2010a) who reported that sodium caseinate film containing ginger essential oil had more discontinuity of cross-section and underwent phase separation with increasing level of essential oils incorporated, while this phenomenon was not occurred when cinnamon essential oil was used. As a consequence, the different types of surfactants exhibited the varying ability to stabilize the oil droplets in emulsion system. Soy lecithin was therefore the appropriate surfactant to produce protein-essential oil emulsion film, in which smooth or homogeneous surface and cross-section of film were obtained.

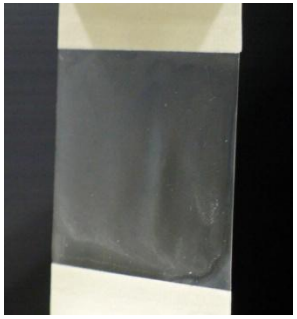
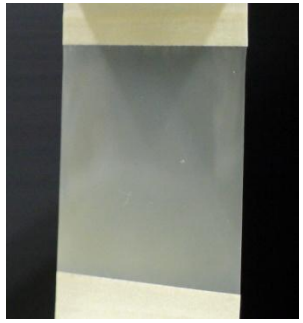
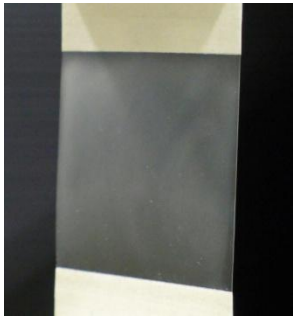
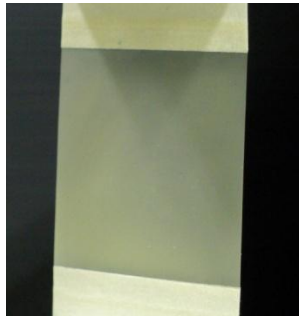
Control	Type of essential oil	Type of surfactant		
		Tween 20	Tween 80	Soy Lecithin
	Basil			
	Citronella			

Figure 17. Photographs of films from fish skin gelatin containing basil and citronella essential oils in the presence of different surfactants. Black background was used.

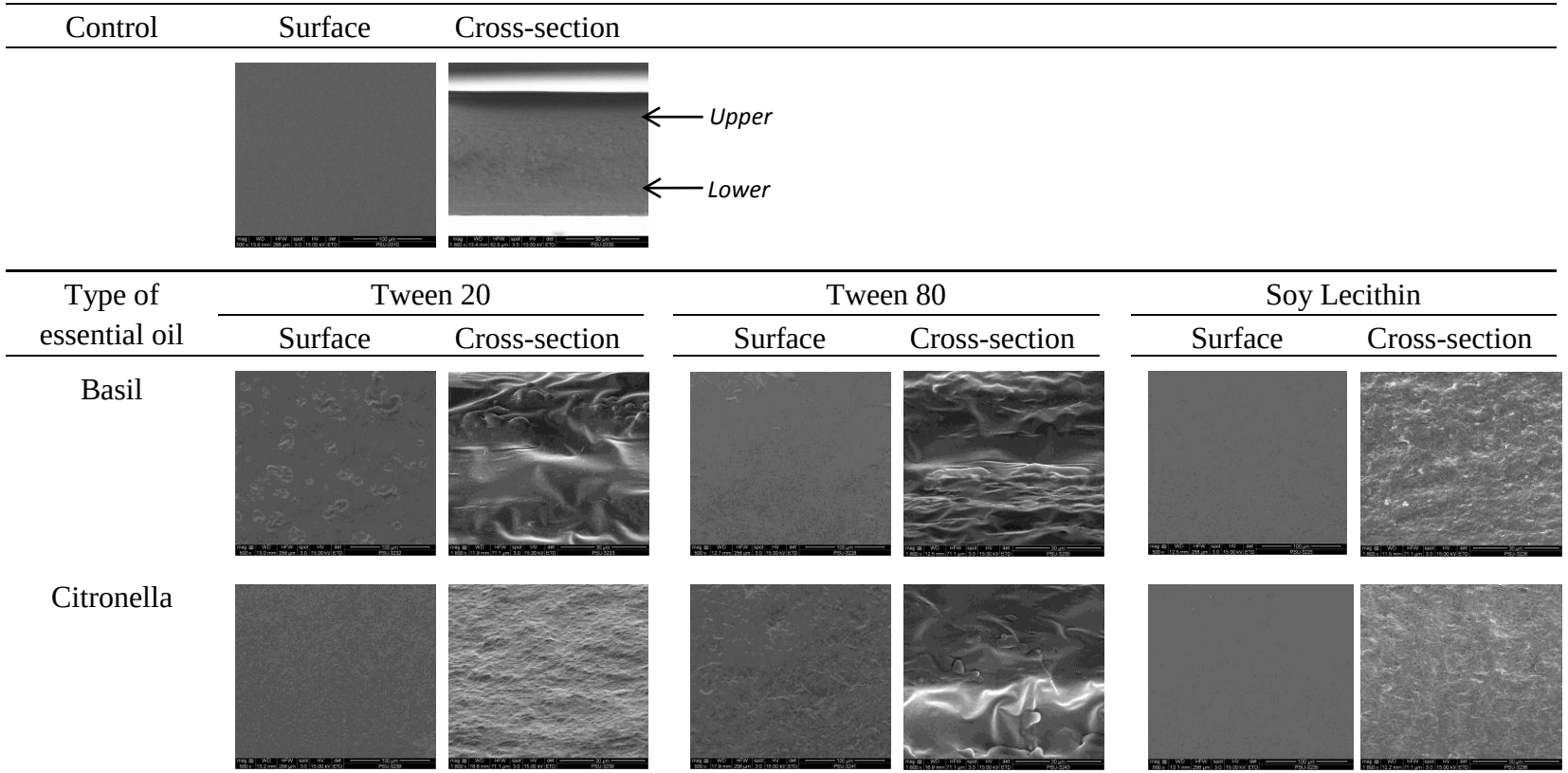


Figure 18. SEM micrographs of surface exposed to air upon drying (5000 x) and cross-section (1800 x) of films from fish skin gelatin containing basil and citronella essential oils in the presence of different surfactants. In cross-sectional images, the upper surface represents the film surface exposed to the air during film casting.

5.4.2 Differential scanning calorimetry (DSC)

DSC thermograms of the 1st and 2nd-heating scans of gelatin films incorporated without and with basil and citronella essential oils in the presence of various surfactants are illustrated in Figure 19 and 20, respectively. Glass transition temperature (T_g), melting transition temperature (T_{max}) and enthalpy (ΔH) of all film samples in comparison to those of anhydrous glycerol and essential oils are summarized in Table 22. DSC was used to determine the transitions of films when the sample was subjected to thermal change.

From thermogram of the 1st heating scan (from -40 to 120 ºC), the control film (without essential oils and surfactants) exhibited two step-like transitions, which represented the glass transition temperatures (T_{g1} and T_{g2}), followed by an endothermic melting transition (T_{max}). The glass transition temperature (T_g) of polymers is an important parameter in many applications, which affects the thermo-rheological behavior (Backfolk *et al.*, 2007). T_g is generally related with molecular segmental motion of disordered (amorphous) structure, while T_{max} of the protein film indicated the temperature causing a destruction of ordered or aggregated structure, which was stabilized by various protein inter-actions during film formation (Jongjareonrak *et al.*, 2006; Tang *et al.*, 2009). T_g is a very important physical parameter, which depends on molecular structure and interaction as well as chain stiffness. It thus serves to explain the physical and chemical behavior of material system (Perdomo *et al.*, 2009). From the result, for the control film, T_{g1} found at low temperature (-26.67 ºC) was likely associated with T_g of glycerol- rich phase, which was higher than that of pure glycerol at around -93 ºC (Pol *et al.*, 2002). T_{g2} observed at higher temperature of (41.30 ºC) was associated with T_g of plasticized gelatin-rich phase. Similar observation of multiple T_g relaxations was reported for various plasticized protein-based films such as glycerol-plasticized wheat gluten film (Song and Zheng, 2008), sorbitol-plasticized sodium caseinate film (Kristo *et al.*, 2008) and fructose-containing casein and sodium caseinate films (Kalichevsky *et al.*, 1993). It was reported that the T_g of beta–lactoglobulin film varied with types and their characteristic of plasticizer used. Similar T_g values were observed in films plasticized with lower molecular weight (MW) and liquid-state plasticizers (propylene glycol,

glycerol and PEG 200), while those films containing higher MW and solid-state plasticizers (sorbitol and sucrose) exhibited higher T_g values (Sothornvit and Krochta, 2000). Therefore, MW and state of plasticizer directly influenced the T_g of film.

Gelatin films containing basil and citronella essential oils, irrespective of surfactants used, had lower T_g than did the control film. The result suggested that the incorporation of essential oils could interrupt protein-protein interaction, thus increasing chain mobility of gelatin. In general, T_g of protein-based films increases with increasing chain stiffness mediated by inter/intramolecular attractive forces. Thus, the weaker structure of film was developed as essential oils were incorporated. From the result, the decrease in T_g could explain in part a decrease in resistance and rigidity of essential oil containing gelatin films as evidenced by the decreased strength of films as reported in the previous investigation (Tongnuanchan *et al.*, 2013b). It is worth to note that the loss of mechanical resistance of gelatin films containing essential oil was mainly due to the lack of structural integrity with disconnected network. It was noted that T_g, corresponded to immiscible essential oil phase, could not be detected in the thermogram in temperature range tested of -40-150 ºC. This was due to the fact that basil and citronella essential oils had T_g of -93.98 and -105.60 ºC (Table 1), respectively, which were lower than the temperature range used for film characterization.

For films incorporated with basil essential oil, those prepared using Tween-80 as a surfactant showed the highest T_g, followed by those containing Tween-20 and soy lecithin, respectively. However, films added with citronella essential oil showed the highest T_g when Tween-20 was used and had the lowest T_g when soy lecithin was present. It was noted that gelatin film added with basil or citronella essential oils showed the lowest T_g when soy lecithin was used as surfactant. Different surfactants more likely affected the dispersion of oil droplets and the alignment of gelatin molecule in film network in different fashions. More uniform dispersion and stable distribution of oil droplets regulated by soy lecithin could interfere inter/intra-molecular interaction of protein network. This result was in agreement with the FTIR results and the lowest tensile strength (TS) of films prepared with soy lecithin in comparison with those films prepared with Tween-20 and control film as reported by Tongnuanchan *et al.* (2013b).

For endothermic/melting transition, the control film exhibited clearly endothermic peak at temperature (T_{max}) of 76.92 ºC. This endothermic transition appeared after the glass transition was most likely involved in helix-coil transition (Sobral *et al.*, 2001; Vanin *et al.*, 2005) and was plausibly associated with the disruption of other kinds of ordered molecular structure. When essential oils were incorporated into gelatin film, the melting/ordered-phase transition peak became broader and decreased in intensity, especially when soy lecithin was used as a surfactant. Films incorporated with both essential oils had slightly higher $T_{max,peak}$ than the control film, regardless of surfactants used. For film added with citronella essential oil, $T_{max,peak}$ were higher when Tween-80 and soy lecithin were used as surfactants, compared with Tween-20. The result suggested that some particular surfactants in film network could enhance the strong protein-protein interaction rather than surfactant-protein interaction. For Tween with high HLB, the interaction with proteins could be more favored. As a result, the migration of Tween to oil interface could be lowered. This might be associated with the lowered ability in stabilizing the oil droplets in the system. For lecithin with higher HLB, this compound has both hydrophobic and hydrophobic domains, in which hydrophobic portion can be exposed and localized at oil interface. In this system, lecithin showed the better emulsifying property. Thus, the mechanism of both compounds might be different, in which the stability of emulsion could be varied. The control film showed the highest ΔH, compared with others. Films incorporated with both essential oils required a lower enthalpy for disruption of the inter-chain interaction. The lower thermal stability was observed in weaker film network, which required a lower enthalpy for precluding the inter-molecular interactions. ΔH or the area under endothermic melting peak also correlated with the amount of order-phase fraction in the film. The result suggested that the control films contained higher fraction of order-phase structure, compared to those added with essential oils. Gelatin molecules, which formed the film network, could undergo partial renaturation during film formation process. The essential oils, glycerol and water retained more likely yielded amorphous phase (non-ordered structure) in the film. Apart from plasticizing effects, high amount of essential oils incorporated might increase the amorphous phase with the concomitant decrease in the ordered phase as indicated by lower ΔH and thus increasing molecular mobility.

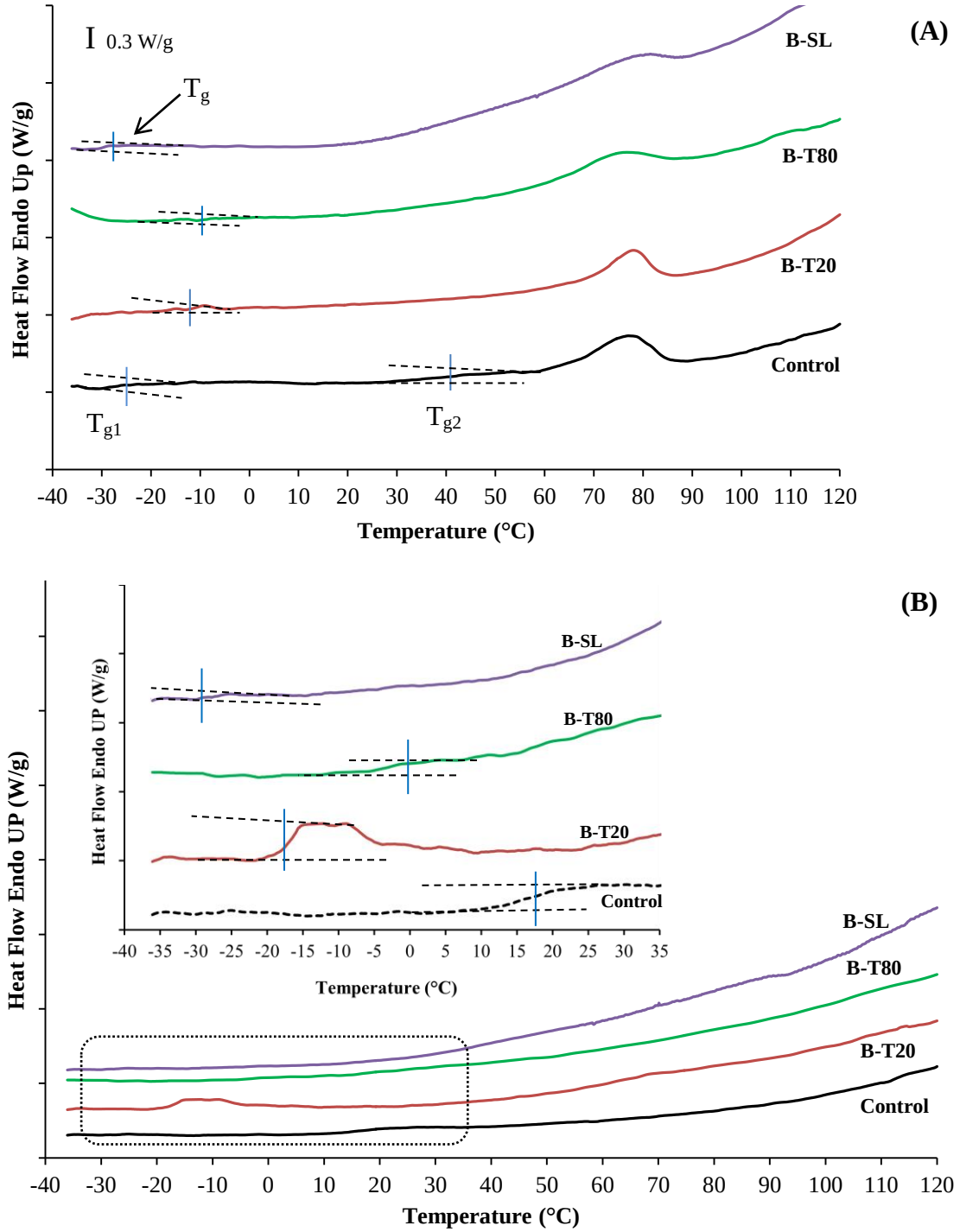


Figure 19. DSC thermograms of 1st-heating scan (A) and 2nd-heating scan (B) of films from fish skin gelatin containing basil essential oil in the presence of different surfactants. Control: without addition of essential oils and surfactants. T20, T80, SL: Tween-20, Tween-80 and soy lecithin, respectively.

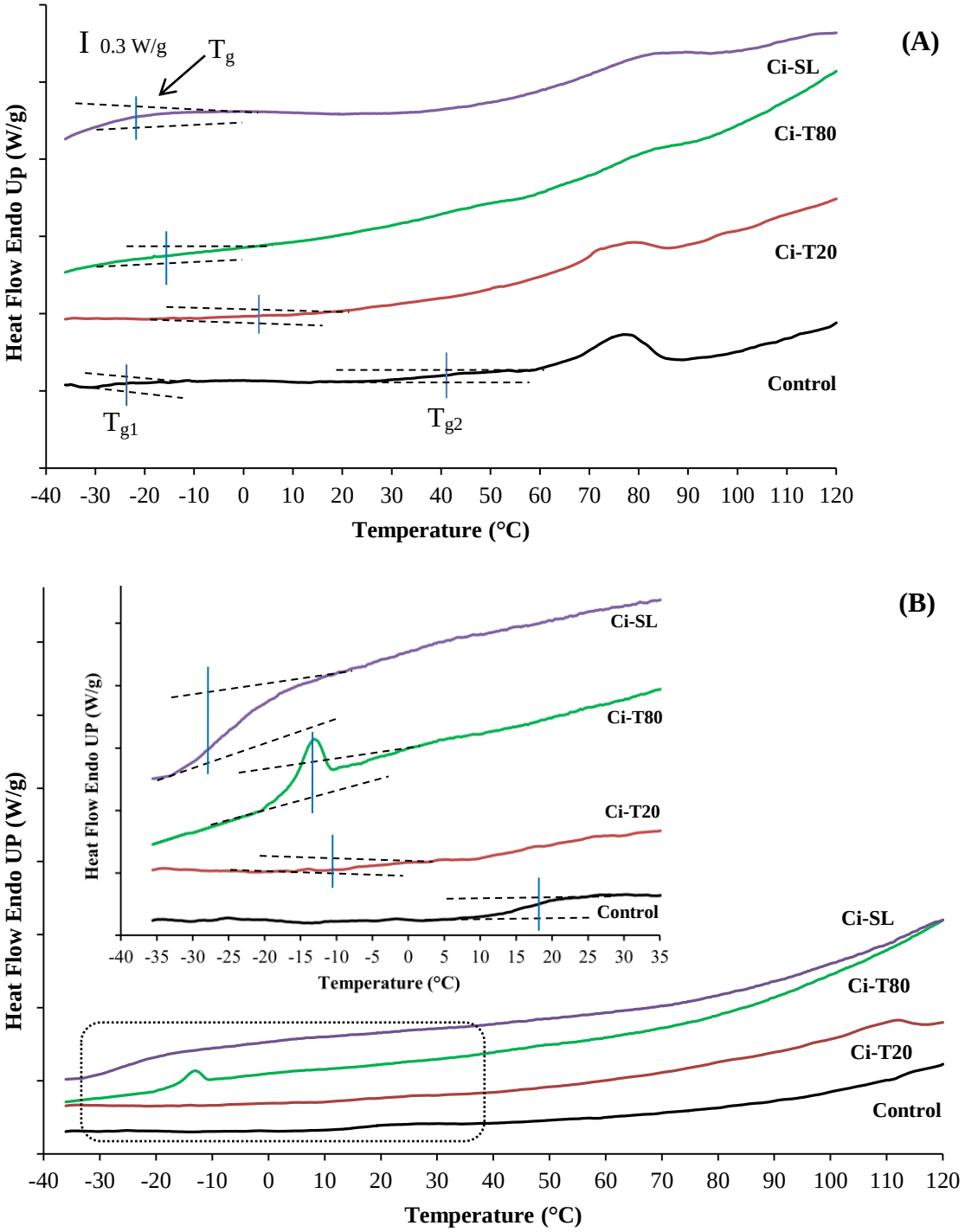


Figure 20. DSC thermograms of 1st-heating scan (A) and 2nd-heating scan (B) of films from fish skin gelatin containing citronella essential oil in the presence of different surfactants. Control: without addition of essential oils and surfactants. T20, T80, SL: Tween-20, Tween-80 and soy lecithin, respectively.

Table 22. Glass transition temperature (T_g), melting/order-phase transition temperature (T_{max}) and enthalpy (ΔH) of films from fish skin gelatin containing basil and citronella essential oils in the presence of different types of surfactants.

Essential oils	Surfactants	1st - Heating					2nd - Heating
		Glass transition	Melting/order-phase transition				
		T_g (°C)	T_{onset} (°C)	$T_{peak, max}$ (°C)	T_{end} (°C)	ΔH (J/g)	T_g (°C)
Control	-	-26.67 (T_{g1}), 41.30 (T_{g2})	65.07	76.92	84.89	13.56	16.12
Basil	Tween-20	-11.78	69.83	77.92	83.52	9.19	-17.18
	Tween-80	-8.42	62.97	74.83 (broad)	86.89	10.59	-1.25
	Soy lecithin	-29.58	62.63	77.83 (very broad)	85.86	5.11	-28.42
Citronella	Tween-20	2.35	67.99	77.58 (broad)	85.32	7.45	-13.95
	Tween-80	-15.30	61.25	83.08 (very broad)	95.38	3.48	-14.73
	Soy lecithin	-26.54	67.78	83.08 (very broad)	96.42	9.43	-28.09
Basil essential oil		-93.98	-	-	-	-	-89.38
Citronella essential oil		-105.60	-	-	-	-	-111.09
Glycerol		-85.04	-	-	-	-	-81.13

Control: without addition of essential oils and surfactants.

From the thermograms of the 2nd-heating scan (Figure 19B and 20B), the endothermic peak was not observed for all film samples. The result suggested that upon fast (quenched) cooling before starting the 2nd-heating scan, gelatin was not able to rearrange themselves into the order structure. The lower order phase could provoke the increased mobility of gelatin molecules in the amorphous phase; as a consequence, the shift of T_g to lower value was observed in the 2nd-heating scan, compared to the 1st-heating scan for all film samples, excepted for those containing both essential oils in the presence of Tween-80 (Table 1). The similar trend of T_g was observed for all films, compared to that obtained from the 1st-heating scan. Among all film samples, the control film had the highest T_g, compared with others. For films incorporated with the same essential oil, those having soy lecithin as a surfactant had the lowest T_g, in comparison with those using other surfactants. Soy lecithin might enhance the uniform distribution of essential oil droplets throughout the gelatin network of film. Therefore, lower interaction between protein chains was postulated.

5.4.3 Thermo-gravimetric analysis (TGA)

TGA thermograms presenting thermal degradation behavior of gelatin films incorporated with basil and citronella essential oils in the presence of different surfactants are illustrated in Figure 21A and 21B, respectively. The degradation temperatures (T_d), weight loss (Δw) and residue of all film samples are shown in Table 23. Control film generally exhibited three main stages of weight loss. Four main stages of weight loss were observed in films incorporated with both essential oils when Tween-20 and Tween-80 were used as surfactants. However, those films prepared using soy lecithin as surfactant had three main stages of weight loss, regardless of essential oil types. For all films, the first stage weight loss (Δw_1 = 3.28-5.27 %) occurred approximately at temperature (T_{d1}) of 30.25-52.94 ºC. The weight loss at this temperature range could be associated with the evaporation of low molecular weight components in the films probably including glycerol and water in glycerol as well as aroma compounds from essential oil. It was noted that films incorporated with both essential oils and all surfactants had lower weight loss than the control film. This might be associated with the lower amount of water in films incorporated with essential oils and also ascribed to less amount of hydrophilic gelatin

component in the films caused by the presence of hydrophobic essential oils (Tongnuanchan *et al.*, 2013a). The similar result was observed in protein-based film from various sources including cuttlefish skin gelatin (Hoque *et al.*, 2011b), tilapia skin gelatin (Tongnuanchan *et al.*, 2012), porcine-plasma protein (Nuthong *et al.*, 2009) and red tilapia protein isolate (Tongnuanchan *et al.*, 2013). In addition, the effect of essential oil incorporation on the observed weight loss, due to the evaporation of water and other volatile substances, could also be caused by the water diffusion reduction owing to the presence of hydrophobic layer of essential oil close to the film surface as evidenced from the SEM images (Figure 18). Indeed, in many films containing oil droplet, there is creaming that concentrate the oil phase toward the surface and then limit water diffusion and evaporation. Among all samples, films using soy lecithin as a surfactant showed the lower Δw_1 than others. Lecithin might be able to bind water to some extent via their polar head, leading to the lowered loss of that water.

The second stage of weight loss (Δw_2 = 15.65-32.27 %) was observed at T_{d2} of 181.75-253.20 ºC. This stage of weight loss was more likely due to the degradation of glycerol compounds (plasticizer) as well as lower molecular weight protein fractions or structurally bound water in the film network (Hoque *et al.*, 2011b). The loss of low molecular weight compounds in essential oils was also presumed. Hoque *et al.* (2011b) and Tongnuanchan *et al.* (2013) also reported the T_{d2} in the range of 202.76-217.40 ºC and 171.61-184.96 ºC for cuttlefish skin gelatin film and fish protein isolate film, respectively. For the third stage of weight loss, Δw_3 of 34.89 - 70.36 % and T_{d3} of 301.29-334.48 ºC were observed for all film samples, possibly associated with the degradation of the larger size or highly associated protein fraction. Guerrero *et al.* (2011) observed the three main stages of weight loss for soy protein film prepared with acid and oils, where the first, second and third stages of weight loss were associated with the loss of water, the evaporation of glycerol and protein degradation, respectively. Lower thermal degradation temperature of second and third stages was observed in films incorporated with both essential oils, compared with the control film. Nevertheless, the behavior varied with surfactants used. The lower thermal stability of those films added with essential oils was plausibly associated with the lower inter/intra-molecular protein interaction in film network

than the control film. Plasticizing effect of essential oils was evidenced in film network, as shown by lower tensile strength (TS) and higher elongation at break (EAB), reported in the previous study (Tongnuanchan *et al.*, 2012). For films incorporated with both basil and citronella essential oils, those prepared using Tween-20 as surfactant had the highest thermal degradation temperature, T_{d2} and T_{d3}, followed by films added with Tween-80 and soy lecithin, respectively. The results indicated that film prepared using Tween-20 showed higher heat resistance than did those using other surfactants. Films prepared using soy lecithin as a surfactant had the lowest T_{d2} and weight loss, compared with others. However, it had the highest weight loss for Δw_3, whilst showed the lowest T_{d3}. The uniform distribution of oil droplet might contribute to higher stability of film network. The high Δw_3 of film containing soy lecithin might be a result of loss in volatile compounds at high temperature. A reduction in thermal stability can be promoted by changes in the protein structure and provoked by the rupture of low energy intermolecular bonds which maintain the protein conformation (Kaminska and Sionkowska, 1999).

For the fourth stage of weight loss, Δw_4 of 22.35-36.38 % with T_{d4} of 413.10-427.63 ºC were obtained for films incorporated with both basil and citronella essential oils in the presence of Tween-20 and Tween-80. However, the fourth stage of weight loss (Δw_4) was undetectable for the control film and films containing both essential oils in the presence of soy lecithin. The fourth stage of weight loss observed in film stabilized with Tween-20 and Tween-80 was possibly associated with the loss of highly stable substances in film containing essential oils. Moreover, Tween-20 and Tween-80 might also possess higher heat resistance than soy lecithin.

Generally, films incorporated with both basil and citronella essential oils had lower residual mass (representing char content) than control film, regardless of surfactant types used. The lower thermal stability of film added with essential oils was in agreement with the weaker film network, as indicated by the lowered TS (Tongnuanchan *et al.*, 2012). Among all films containing essential oil, those prepared using soy lecithin had the highest residual, compared with others. Thus, both essential oils and surfactants had the marked impact on thermal stability of gelatin film.

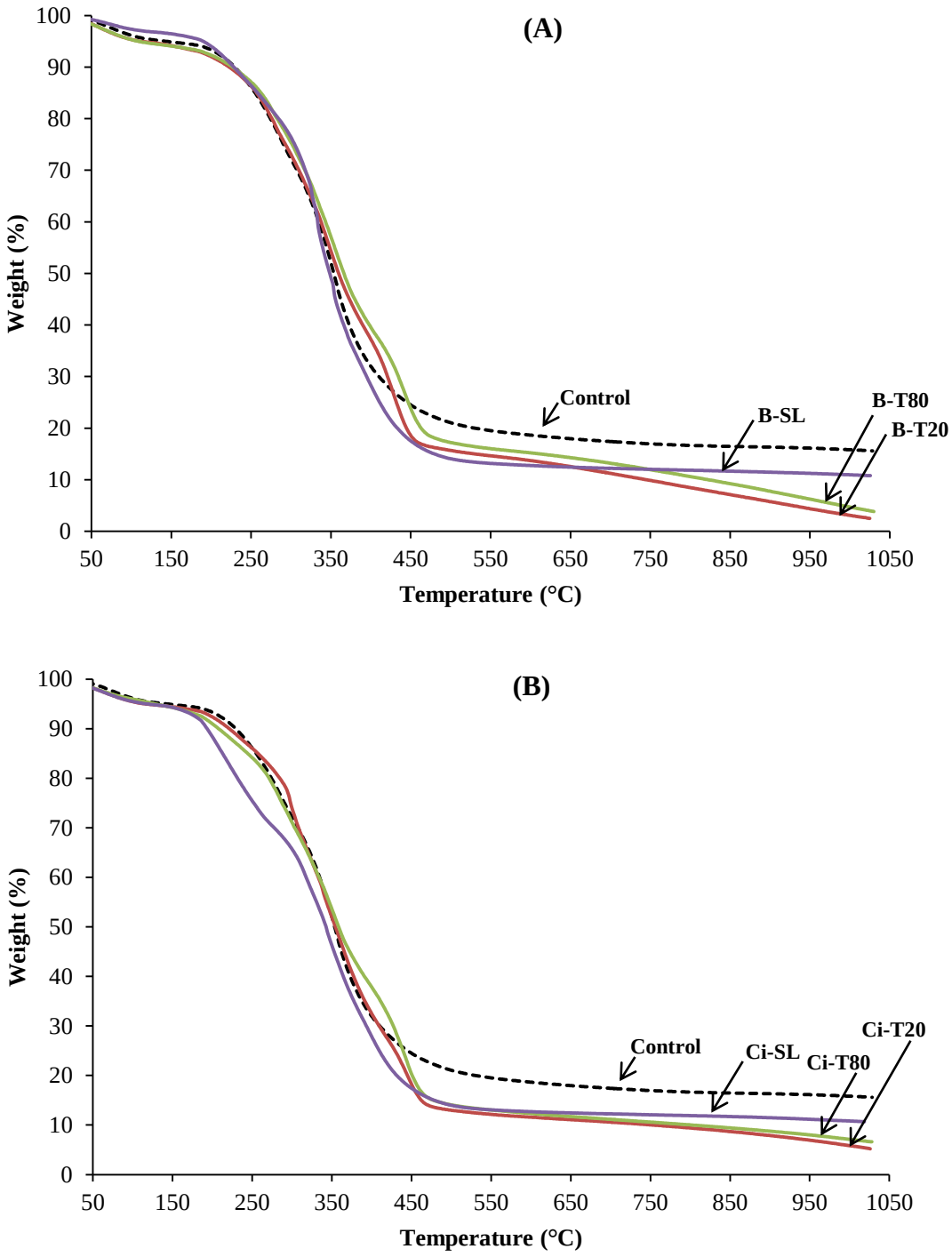


Figure 21. Thermo-gravimetric curves of films from fish skin gelatin containing basil and citronella essential oils in the presence of different surfactants. Control: without addition of essential oils and surfactants. B, Ci: basil and citronella essential oil, respectively. T20, T80, SL: Tween-20, Tween-80 and soy lecithin, respectively.

Table 23. Thermal degradation temperatures (T_d, ºC) and weight loss (Δw, %) of films from fish skin gelatin containing basil and citronella essential oils in the presence of different types of surfactants.

Essential oils	Surfactants	Δ_1		Δ_2		Δ_3		Δ_4		Residue (%)
		$Td_{1,onset}$	Δw_1	$Td_{2,onset}$	Δw_2	$Td_{3,onset}$	Δw_3	$Td_{4,onset}$	Δw_4	
Control	-	34.88	5.27	233.20	23.52	334.48	52.30	-	-	18.91
Basil	Tween-20	39.96	5.19	243.16	20.15	319.75	35.79	413.10	36.38	2.49
	Tween-80	49.99	5.75	237.88	15.75	319.12	40.46	424.80	34.17	3.87
	Soy lecithin	55.31	3.28	192.17	15.65	321.29	70.56	-	-	10.51
Citronella	Tween-20	31.88	5.18	195.19	32.27	337.46	34.89	427.63	22.35	5.31
	Tween-80	28.25	4.76	185.73	27.83	323.95	28.16	420.34	32.47	6.78
	Soy lecithin	42.20	4.95	181.75	25.25	311.65	58.99	-	-	10.81

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.

Control: without addition of essential oils and surfactants.

5.4.4 Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra of gelatin films incorporated with basil and citronella essential oils using different surfactants are illustrated in Figure 22. Generally, the control film and those containing basil and citronella essential oils with different surfactants showed the similar major peaks but the amplitudes of peaks varied, depending on essential oils and surfactants used. The band situated at the wavenumber of 1034-1038 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 1700-700 cm^{-1}, covering amide-I, II and III bands. All films had the major bands at 1630 cm^{-1} (amide-I, illustrating C=O stretching/hydrogen bonding coupled with COO), 1538 cm^{-1} (amide-II, presenting the bending vibrations of N-H groups and stretching vibrations of C-N groups) and 1237 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). Staroszczyk *et al.* (2012) also reported the similar spectra for cod skin gelatin film, where amide-I, II and III bands were found at the wavenumbers of 1633, 1538 and 1238 cm^{-1}, respectively. It was noted that the amplitudes of amide I, II and III of control film were slightly higher than those of films incorporated with essential oils, regardless of surfactants used. This was mainly due to the dilution effect found in essential oil incorporated-gelatin films.

An amide-A band was observed at the wavenumber of 3288-3295 cm^{-1}, and amide-B band was found at 3081-3085 cm^{-1} for all film samples. The amide-A band represents the NH-stretching coupled with hydrogen bonding and amide-B band illustrated NH-stretching vibration and asymmetric CH-stretching vibration at wavenumber of ~3300 and ~3100 cm^{-1}, respectively (Elliott and Ambrose, 1950; Kong and Yu, 2007; Krimm and Bandekar, 1986). Amide-A peak of control film found at wavenumber of 3290 cm^{-1} was slightly shifted to higher wavenumbers when basil and citronella essential oils were incorporated. Wavenumbers of amide-A peak shifted to 3288, 3291 and 3292 cm^{-1} for films incorporated with basil essential oil, and to 3294, 3292 and 3295 cm^{-1} for films incorporated with citronella essential oil, when Tween-20, Tween-80 and soy lecithin were used as surfactants, respectively. Bahram

et al. (2014) reported that the difference in wavenumber and their amplitude peak might be associated with the converting of the functional groups of protein with the functional groups of essential oil due to the interaction between them. Typically, the presence of amide-A at lower wavenumber indicated the existing of hydrogen bonding between protein molecules (Xie *et al.*, 2006). Films added with soy lecithin exhibited the highest wavenumber of amide-A, suggesting the greatest decrease in protein-protein interaction. This was more likely attributable to the more homogeneous distribution of oil droplets in the matrix of films stabilized by soy lecithin, as compared to those stabilized by Tween-20 and Tween-80. The spectra of films incorporated with both essential oils showed the lower amplitude of amide-A band, regardless of surfactants used. The result suggested that the incorporation of essential oils into gelatin film might disrupt the protein-protein interaction in the film network. As a result, the reactive group could undergo interaction to the lower extent, thereby causing the more availability of those groups. This was confirmed by the decreased strength and increased extension of film in comparison with the control film (Tongnuanchan *et al.*, 2012).

Additionally, peaks at wavemunbers of 2937 and 2878 cm^{-1} for the control film were shifted to lower wavenumbers of 2924-2926 cm^{-1} and 2855-2872 for film added with basil essential oil, and at wavenumbers of 2923-2926 cm^{-1} and 2855-2876 cm^{-1} for film added with citronella essential oil. The amplitude of both peaks was much higher in films added with essential oils, in comparison with the control film. Peaks at wavenumbers around 2853 cm^{-1} and 2924 cm^{-1} represent the methylene asymmetrical and symmetrical stretching vibration of the aliphatic C-H in CH_2 and CH_3 groups, respectively (Guillén and Cabo, 1997; Guillén and Cabo, 2004; Muik *et al.*, 2007). Those vibration stretching bands are obviously present in most lipids and hydrophobic substances (Guillén and Cabo, 2004). The result could be an indirect suggestion of an increase in hydrophobicity of gelatin films added with essential oils as compared to the control gelatin film. This was evidenced by existing peaks having wavenumbers of 2876 cm^{-1}, 2929 cm^{-1} and 1732 cm^{-1} (Aewsiri *et al.*, 2011). It was noted that films using soy lecithin as a surfactant had the lowest shift of peak. The result suggested that various surfactants determined the changes in molecular organization and interaction in the film matrix of resulting films differently.

Furthermore, C=O stretching vibration of aldehyde or ester carbonyl groups occurred at wavenumber of 1742 cm^{-1} (Guillén and Cabo, 1997; Muik *et al.*, 2007). Aldehyde, ketone and ester are the one of main chemical groups in essential oils (Mohamed *et al.*, 2010). For films incorporated with both essential oils in the presence of all surfactants used, the carbonyl absorption of aldehyde or ester carbonyl groups was observed at wavenumber of 1733-1743 cm^{-1} (Figure 22). As expected, no peak at wavenumber of 1733-1743 cm^{-1} was found in the control film. Guerrero *et al.* (2011) also reported the similar peak for soy protein incorporated with epoxydized soybean oil and olive oil, where carbonyl group (C=O) was found at wavenumber 1749 cm^{-1} and 1747 cm^{-1}, respectively. The difference in wavenumbers depended on the varying compounds in different essential oils. It should be noted also that the difference in absorbance intensity of ATR-FTIR spectra of the samples depends in part on several factors such as the moisture content, sample surface topology and diffraction index of the samples which contribute to the penetration depth and angle of IR beam. Therefore, the result suggested that the presence of essential oils and surfactants directly influenced the molecular interaction of protein chains in film matrix.

Based on all supporting data revealing the obtained films' microscopic morphology and molecular phase structure, a simplified illustration of the matrix of gelatin-based films incorporated without and with essential oils in the presence of different surfactants is depicted in Figure 23. It was postulated that gelatin film without essential oil and surfactant exhibited the homogenous network with the presence of higher ordered phase due to partial renaturation (Figure 23A). A non-uniform and bilayer-like oil distribution with lower amount of ordered phase was postulated for essential oil containing gelatin film added with less effective surfactant (i.e., Tween-80 and Tween-20) (Figure 23B). For gelatin-based film containing essential oil and more effective surfactant (i.e., soy lecithin), a uniform oil distribution with less ordered phase structure within the film matrix was obtained (Figure 23C).

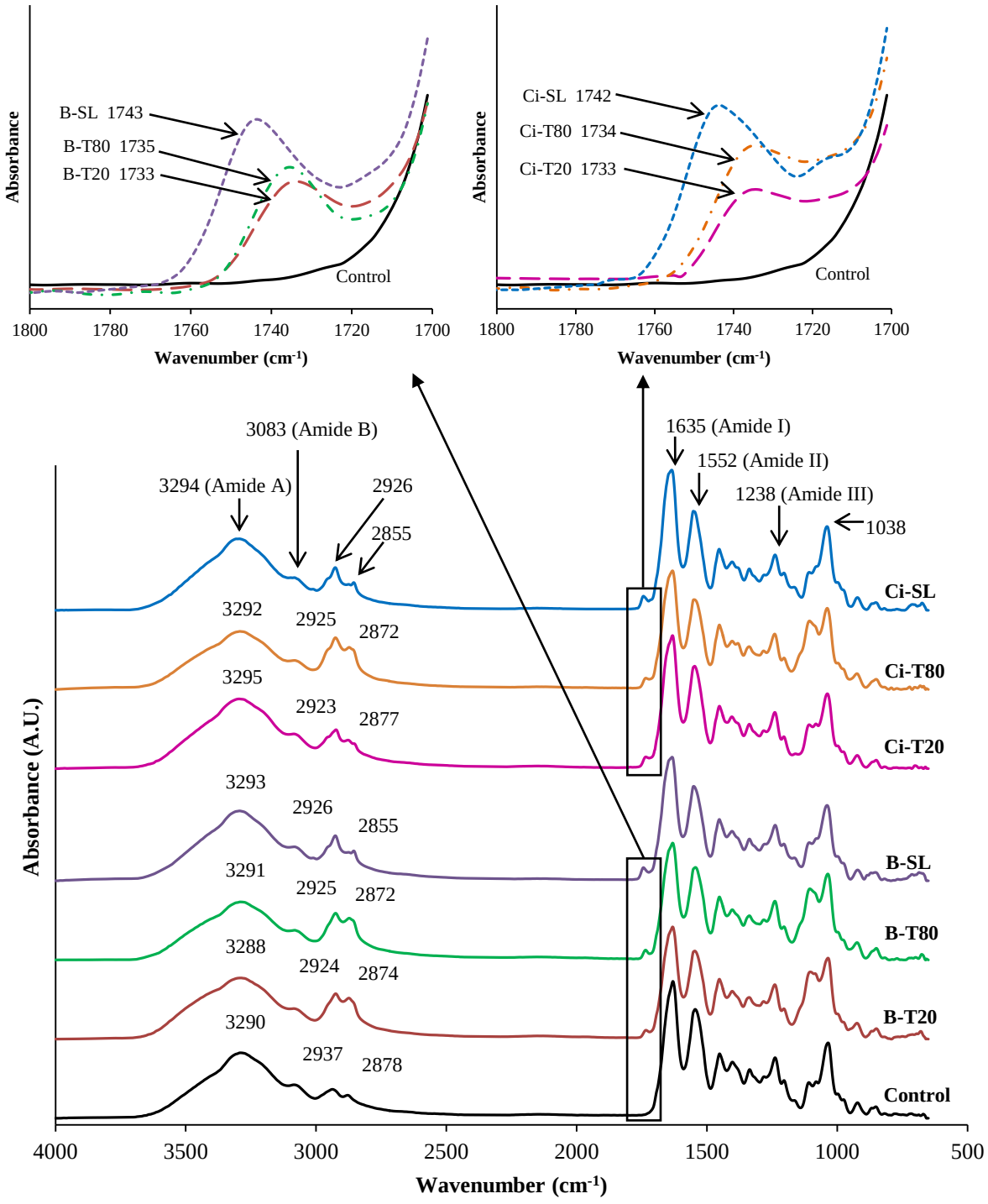


Figure 22. ATR-FTIR of films from fish skin gelatin containing basil and citronella essential oils in the presence of different surfactants. Control: without addition of essential oils and surfactants. B, Ci: basil and citronella essential oil, respectively. T20, T80, SL: Tween-20, Tween-80 and soy lecithin, respectively.

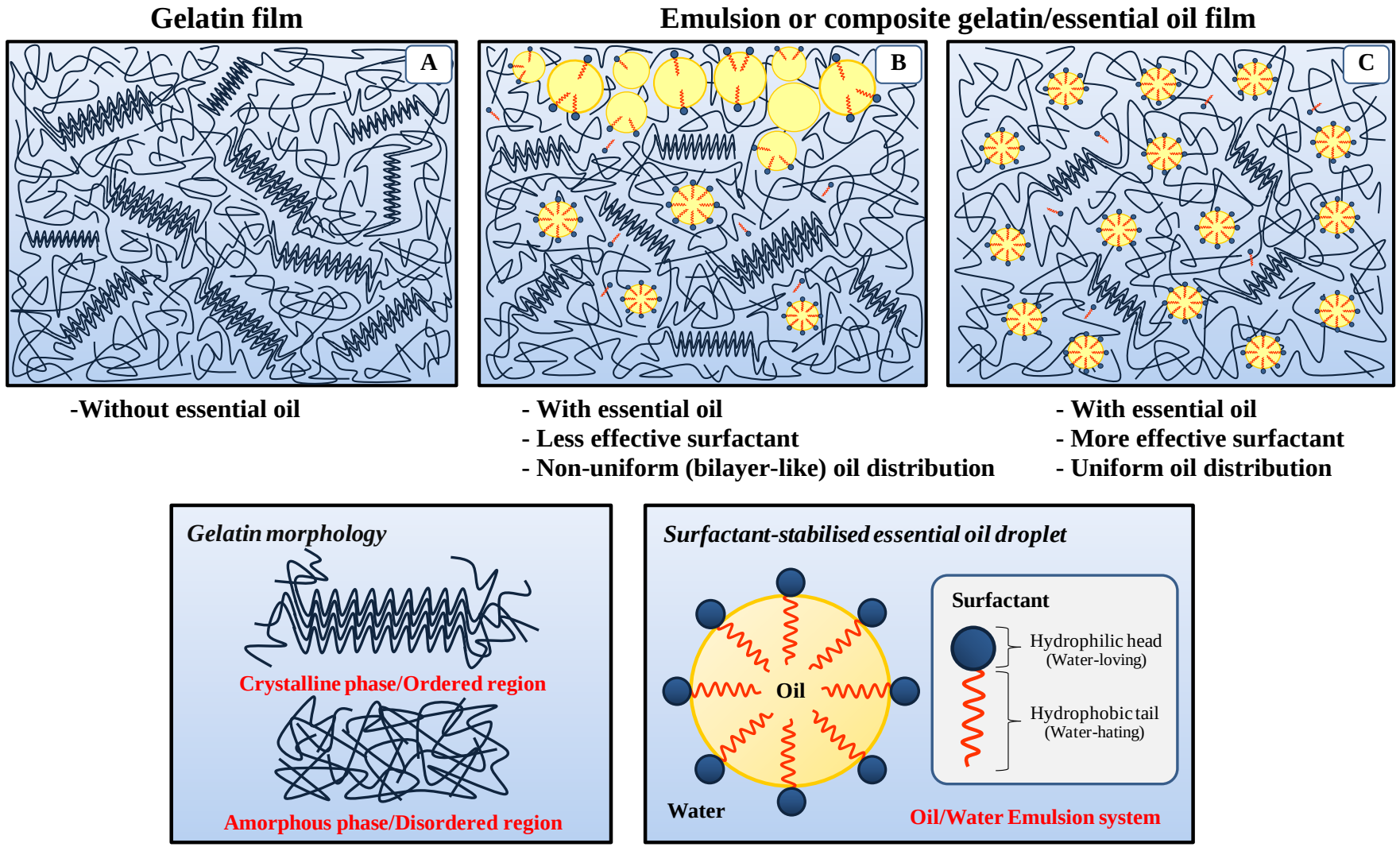


Figure 23. Simplified illustration of gelatin film matrix without and with essential oils in the presence of different surfactants.

5.5 Conclusion

Both basil and citronella essential oils as well as surfactants had the impact on protein interaction in the matrix of gelatin-based film. Type of surfactant used had a strong influence on microscopic morphological structure and molecular phase structure of gelatin-essential oil composite films. Thermal property of film was also affected by both essential oils and surfactant used. In general, soy lecithin was the appropriate surfactant, which potentially enhanced stability of emulsion providing homogenous oil distribution in the matrix of gelatin-essential oil composite films. The obtained gelatin-based emulsion films containing appropriate essential oil and surfactant, which have antioxidant activity, could be potentially used as active packaging such as soluble pouch for ingredients or condiments for some types of food, e.g. instant noodle, etc.

5.6 References

Aewsiri, T., Benjakul, S. and Visessanguan, W. 2009. Functional properties of gelatin from cuttlefish (*Sepia pharaonis*) skin as affected by bleaching using hydrogen peroxide. Food Chem. 115: 243-249.

Aewsiri, T., Benjakul, S., Visessanguan, W., Wierenga, P.A. and Gruppen, H. 2011. Improvement of foaming properties of cuttlefish skin gelatin by modification with *N*-hydroxysuccinimide esters of fatty acid. Food Hydrocolloid. 25: 1277-1284.

Arrieta, M.P., Peltzer, M.A., Garrigós, M.d.C. and Jiménez, A. 2013. Structure and mechanical properties of sodium and calcium caseinate edible active films with carvacrol. J. Food Eng. 114: 486-494.

Arvanitoyannis, I.S. 2002. Formation and properties of collagen and gelatin films and coatings. *In* Protein-based films and coatings. (Gennadios, A., ed.). p. 275-304. CRC Press. Boca Raton.

Atarés, L., Bonilla, J. and Chiralt, A. 2010a. Characterization of sodium caseinate-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 100: 678-687.

Atarés, L., De Jesús, C., Talens, P. and Chiralt, A. 2010b. Characterization of SPI-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 99: 384-391.

Backfolk, K., Holmes, R., Ihalainen, P., Sirviö, P., Triantafillopoulos, N. and Peltonen, J. 2007. Determination of the glass transition temperature of latex films: Comparison of various methods. Polym. Test. 26: 1031-1040.

Bahram, S., Rezaei, M., Soltani, M., Kamali, A., Ojagh, S.M. and Abdollahi, M. 2014. Whey protein concentrate edible film activated with cinnamon essential oil. J. Food Process. Pres. 38: 1251-1258.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils - A review. Food Chem. Toxicol. 46: 446-475.

Bergo, P. and Sobral, P.J.A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. Food Hydrocolloid. 21: 1285-1289.

Brandelero, R.P.H., Yamashita, F. and Grossmann, M.V.E. 2010. The effect of surfactant Tween 80 on the hydrophilicity, water vapor permeation, and the mechanical properties of cassava starch and poly(butylene adipate-co-terephthalate) (PBAT) blend films. Carbohyd. Polym. 82: 1102-1109.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. Int. J. Food Microbiol. 94: 223-253.

Elliott, A. and Ambrose, E.J. 1950. Structure of synthetic polypeptides. Nature. 165: 921−922.

Emiroğlu, Z.K., Yemiş□, G.P., Coşkun, B.K. and Candoğan, K. 2010. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Sci. 86: 283-288.

Guerrero, P., Nur Hanani, Z.A., Kerry, J.P. and de la Caba, K. 2011. Characterization of soy protein-based films prepared with acids and oils by compression. J. Food Eng. 107: 41-49.

Guillén, M.D. and Cabo, N. 1997. Infrared spectroscopy in the study of edible oils and fats. J. Sci. Food Agr. 75: 1-11.

Guillén, M.D. and Cabo, N. 2004. Study of the effects of smoke flavourings on the oxidative stability of the lipids of pork adipose tissue by means of Fourier transform infrared spectroscopy. Meat Sci. 66: 647-657.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011a. Properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin incorporated with cinnamon, clove and star anise extracts. Food Hydrocolloid. 25: 1085-1097.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011b. Effects of partial hydrolysis and plasticizer content on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. Food Hydrocolloid. 25: 82-90.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid. 20: 492-501.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2008. Antioxidative activity and properties of fish skin gelatin films incorporated with BHT and α-tocopherol. Food Hydrocolloid. 22: 449-458.

Kalichevsky, M.T., Blanshard, J.M.V. and Tokargzuk, P.F. 1993. Effect of water content and sugars on the glass transition of casein and sodium caseinate. Int. J. Food Sci. Technol. 28: 139-151.

Kaminska, A. and Sionkowska, A. 1999. The effect of UV radiation on the values of thermal parameters of collagen containing β-carotene. Polym. Degrad. Stabil. 65: 87-90.

Kong, J. and Yu, S. 2007. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Bioch. Bioph. Sin. 39: 549-559.

Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A. and Yildirim, A. 2005. Determination of the chemical composition and antioxidant activity of the essential oil of artemisia dracunculus and of the antifungal and antibacterial activities of turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. J. Agr. Food Chem. 53: 9452-9458.

Krimm, S. and Bandekar, J. 1986. Vibrational spectroscopy and conformation of peptides, polypeptides, and proteins. Adv. Protein Chem. 38: 181−364.

Kristo, E., Koutsoumanis, K.P. and Biliaderis, C.G. 2008. Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on Listeria monocytogenes. Food Hydrocolloid. 22: 373-386.

Krochta, J.M. 1997. Edible protein films and coatings. *In* Food proteins and their applications. (Damodaran, S. and Paraf, A., eds.). p. 529-549. Marcel Dekker. New York.

Krochta, J.M. 2002. Protein as raw materials for films and coatings: definitions, current status, and opportunities. *In* Protein-Based Films and Coating (Gennadios, A., ed.). p. 1-39. CRC Press. New York.

Limpisophon, K., Tanaka, M. and Osako, K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionace glauca*) skin gelatin. Food Chem. 122: 1095-1101.

Mohamed, A.A., El-Emary, G.A. and Ali, H.F. 2010. Influence of some citrus essential oils on cell viability, glutathione-s-transferase and lipid peroxidation in ehrlich ascites carcinoma cells. J. Am. Sci. 6: 820-826.

Muik, B., Lendl, B., Molina-Diaz, A., Valcarcel, M. and Ayora-Cañada, M.J. 2007. Two-dimensional correlation spectroscopy and multivariate curve resolution for the study of lipid oxidation in edible oils monitored by FTIR and FT-Raman spectroscopy. Anal. Chim. Acta. 593: 54-67.

Muyonga, J.H., Cole, C.G.B. and Duodu, K.G. 2004. Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chem. 85: 81-89.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009. Characterization of porcine plasma protein-based films as affected by pretreatment and cross-linking agents. Int. J. Biol. Macromol. 44: 143-148.

Oussalah, M., Caillet, S., Salmieri, S., Saucier, L. and Lacroix, M. 2004. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. J. Agr. Food Chem. 52: 5598-5605.

Peng, Y. and Li, Y. 2014. Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films. Food Hydrocolloid. 36: 287-293.

Perdomo, J., Cova, A., Sandoval, A.J., García, L., Laredo, E. and Müller , A.J. 2009. Glass transition temperatures and water sorption isotherms of cassava starch. Carbohyd. Polym. 76: 305-313.

Pires, C., Ramos, C., Teixeira, G., Batista, I., Mendes, R., Nunes, L. and Marques, A. 2011. Characterization of biodegradable films prepared with hake proteins and thyme oil. J. Food Eng. 105: 422-428.

Pol, H., Dawson, P., Acton, J. and Ogale, A. 2002. Soy protein isolate/corn-zein laminated films: transport and mechanical properties. J. Food Sci. 67: 212-217.

Prodpran, T., Benjakul, S. and Artharn, A. 2007. Properties and microstructure of protein-based film from round scad (*Decapterus maruadsi*) muscle as affected by palm oil and chitosan incorporation. Int. J. Biol. Macromol. 41: 605-614.

Salarbashi, D., Tajik, S., Ghasemlou, M., Shojaee-Aliabadi, S., Shahidi Noghabi, M. and Khaksar, R. 2013. Characterization of soluble soybean polysaccharide film incorporated essential oil intended for food packaging. Carbohyd. Polym. 98: 1127-1136.

Seydim, A.C. and Sarikus, G. 2006. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food Res. Int. 39: 639-644.

Sobral, P.J.A., Menegalli, F.C., Hubinger, M.D. and Roques, M.A. 2001. Mechanical, water vapor barrier and thermal properties of gelatin based edible films. Food Hydrocolloid. 15: 423-432.

Song, Y. and Zheng, Q. 2008. Improved tensile strength of glycerol-plasticized gluten bioplastic containing hydrophobic liquids. Bioresource Technol. 99: 7665-7671.

Sothornvit, R. and Krochta, J.M. 2000. Plasticizer effect on oxygen permeability of β-lactoglobulin films. J. Agr. Food Chem. 48: 6298-6302.

Staroszczyk, H., Pielichowska, J., Sztuka, K., Stangret, J. and Kołodziejska, I. 2012. Molecular and structural characteristics of cod gelatin films modified with EDC and TGase. Food Chem. 130: 335-343.

Tanaka, M., Ishizaki, S., Suzuki, T. and Takai, R. 2001. Water vapor permeability of edible films prepared from fish water soluble proteins as affected by lipid type. J. Tokyo Univ. Fisheries. 87: 31-37.

Tang, C.-H., Xiao, M.-L., Chen, Z., Yang, X.-Q. and Yin, S.-W. 2009. Properties of cast films of vicilin-rich protein isolates from *Phaseolus* legumes: Influence of heat curing. LWT - Food Sci. Technol. 42: 1659-1666.

Tongnuanchan, P., Benjakul, S., Prodpran, T. and Songtipya, P. 2013. Properties and stability of protein-based films from red tilapia (*Oreochromis niloticus*) protein isolate incorporated with antioxidant during storage. Food Bioprocess Tech. 6: 1113-1126.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2012. Properties and antioxidant activity of fish skin gelatin film incorporated with citrus essential oils. Food Chem. 134: 1571-1579.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2013a. Physico-chemical properties, morphology and antioxidant activity of film from fish skin gelatin incorporated with root essential oils. J. Food Eng. 117: 350-360.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2013b. Characteristics and antioxidant activity of leaf essential oil–incorporated fish gelatin films as affected by surfactants. Int. J. Food Sci. Technol. 48: 2143–2149.

Vanin, F.M., Sobral, P.J.A., Menegalli, F.C., Carvalho, R.A. and Habitante, A.M.Q.B. 2005. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. Food Hydrocolloid. 19: 899-907.

Xie, Y.-L., Zhou, H.-M. and Qian, H.-F. 2006. Effect of addition of peach gum on physicochemical properties of gelatin-based microcapsule. J. Food Biochem. 30: 302-312.

Ye, Z., Xiu, S., Shahbazi, A. and Zhu, S. 2012. Co-liquefaction of swine manure and crude glycerol to bio-oil: Model compound studies and reaction pathways. Bioresource Technol. 104: 783-787.

CHAPTER 6

COMPARATIVE STUDIES ON PROPERTIES AND ANTIOXIDATIVE ACTIVITY OF FISH SKIN GELATIN FILMS INCORPORATED WITH ESSENTIAL OILS FROM VARIOUS SOURCES

6.1 Abstract

Properties of fish skin gelatin films incorporated with three essential oils from various sources (basil, plai and lemon) with a gelatin/essential oil ratio of 1:1 (w/w) were determined. Films containing different essential oils had lower tensile strength (TS) and elastic modulus (EM) but higher elongation at break (EAB) and thickness, compared with the control film (without essential oils) ($p < 0.05$). Lower water vapor permeability (WVP) was observed when essential oils were incorporated, in comparison with the control, particularly when basil and lemon essential oils were used ($p < 0.05$). Films with essential oils had varying L^*-, a^*-, b^*- and ΔE^*-values (total color difference) and WI (whiteness). Among all films, that incorporated with plai essential oil showed the highest b^*- and ΔE^*-values but lowest WI ($p < 0.05$). Lower light transmittance and higher transparency value, indicating more opaqueness, were observed when films were incorporated with essential oils ($p < 0.05$), especially for film added with lemon essential oil. Film containing basil essential oil showed the highest DPPH and ABTS radical scavenging activities ($p < 0.05$), whilst those incorporated with lemon essential oil had the highest chelating activity ($p < 0.05$). Thus, the incorporation of various essential oils from various sources determined properties and antioxidative activity of fish skin gelatin film differently.

6.2 Introduction

Potential processing and management are very important for fish industry, where great economic, nutritional and environmental values can be obtained by the better uses of byproducts. Fish processing generates solid wastes that can be as high as 50–80% of the original raw material (Shahidi *et al.*, 1994). About 30% of the wastes consists of skin and bone with high collagen content (Wasswa *et al.*, 2007). Gelatin can be obtained by partial hydrolysis or thermal denaturation of collagen in

skin and bone. Moreover, gelatins have been widely used as film forming material, particularly for preparation of biodegradable or edible films (Gómez-Estaca *et al.*, 2009). 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 protect foods against the external factors e.g. water, oxygen, carbon dioxide and lipids (Krochta, 1997). Although gelatins yielded transparent, colorless and highly extensible films, they have poor water resistance. This could be a drawback when they are applied to food products with high moisture content, because the films may swell, partially dissolve or disintegrate upon the contact with wet surface (Núñez-Flores *et al.*, 2012). The use of essential oils from plants can be another approach to improve water vapor barrier property of gelatin films. Due to antioxidant and antimicrobial activities of essential oils (Burt, 2004), they may make the film become active. As a consequence, smart films with varying property can be produced, especially for shelf-life extension of foods. In our previous study, gelatin-based films incorporated with essential oils containing 30% glycerol exhibited the higher antioxidative activity than those with 20% glycerol, owing to their more loosen structure of film matrix (Tongnuanchan *et al.*, 2012). Moreover, essential oil as high level of 100% (w/w based on protein) could be incorporated into gelatin film and yielded the bilayer film with the lowest water vapor permeability with the highest antioxidative activity (Tongnuanchan *et al.*, 2013a). Recently, Tongnuanchan *et al.* (2013b) reported that soy lecithin could be potentially used as a surfactant for gelatin film added with leaf essential oils, where homogeneous internal film network was formed.

Essential oils from different parts of plants or different plants showed varying antioxidative activity. Among essential oils from leaf, root and citrus peel, those from basil, plai and lemon showed the higher antioxidative activity, respectively (Tongnuanchan *et al.*, 2012; Tongnuanchan *et al.*, 2013a; Tongnuanchan *et al.*, 2013b). However, there is no information on the effect of essential oils from different parts of plants (leaf, root and citrus peel) on the properties of fish gelatin film. Thus, the objectives of this study were to comparatively study the effects of three different essential oils (basil, plai and lemon) on the properties and antioxidative activity of film from fish skin gelatin.

6.3 Materials and Methods

6.3.1 Chemicals

2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picryl hydrazyl (DPPH), glycerol, and soy lecithin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,p'*-disulfonic acid monosodium salt hydrate (Ferrozine) and iron (II) chloride were obtained from Merck (Darmstadt, Germany). All chemicals were of analytical grade.

6.3.2 Fish gelatin and essential oils

Fish gelatin produced from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Essential oils from basil (*Ocimum basilicum*), plai (*Zingiber montanum*) and lemon (*Citrus limonum*) were purchased from *Botanicessence* (Bangkok, Thailand).

6.3.3 Preparation of film from fish gelatin incorporated with different essential oils

To prepare film forming solution (FFS), gelatin powder was mixed with distilled water to obtain the protein concentration of 3.5 % (w/v). The mixture was heated at 70 °C for 30 min. Glycerol at 30 % (w/w) of protein content was used as a plasticizer. Prior to addition into solution, essential oils were mixed with soy lecithin at 25 % (w/w, based on essential oil) as surfactant. Thereafter, the prepared essential oils were added into the gelatin solution at gelatin/essential oil ratio of 1:1 (w/w). The obtain suspension was homogenized at 22,000 rpm for 3 min using a homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The dissolved air in the FFS was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature.

For film preparation, FFS (4 g) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown for 12 h at room temperature. The films were further dried 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 were prepared from FFS without essential oils and surfactants.

6.3.4 Determination of film properties

Prior to testing, films were conditioned for 48 h at 50 ± 5% relative humidity (RH), at 25 ± 0.5 °C.

6.3.4.1 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.

6.3.4.2 Mechanical properties

Tensile strength (TS), elastic modulus (EM) 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.

6.3.4.3 Water vapor permeability (WVP)

WVP was measured using a modified ASTM method (ASTM, 1989) as described by Shiku *et al*. (2004). The film was sealed on an aluminum permeation cup containing dried silica gel (0 % RH) with silicone vacuum grease and a rubber gasket to hold the film 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:

$$WVP\ (gm^{-1}s^{-1}Pa^{-1}) = wlA^{-1}t^{-1}(P_2 - P_1)^{-1}$$

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 (Pa).

6.3.4.4 Color

Film samples were subjected to color measurement using a CIE colorimeter (Hunter associates laboratory, Inc., 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), b^*-value (yellowness/blueness), total difference of color (ΔE^*) and whiteness (WI) were calculated as follows (Gennadios *et al.*, 1996; Ghanbarzadeh *et al.*, 2010):

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

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences between the color parameter of the samples and the color parameters of the white standard (L^* = 92.82, a^* = - 1.21, b^* = 0.45).

6.3.4.5 Light transmittance and transparency value

The light transmittance of films was measured at the ultraviolet and visible ranges (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):

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

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.

6.3.5 Determination of antioxidative activity of fish skin gelatin films incorporated with essential oils

Films were solidified using liquid nitrogen in a mortar and ground with a pestle. Ground film (0.25 g) was mixed with 5 ml of methanol and stirred vigorously for 3 h. The mixture was centrifuged at 2700 xg for 10 min using a centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Beckman Coulter, Inc., Palo Alto, CA, USA). The supernatant obtained was determined for antioxidative activities.

6.3.5.1 DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Binsan *et al.* (2008) with a slight modification. Sample (1.5 ml) was added with 1.5 ml of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in 95 % ethanol. The mixture was mixed vigorously using a mixer (Vertex-Genie 2, Model G-560E, Scientific Industries, inc., Bohemia, New York, USA) and allowed to stand at room temperature in dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. Sample blank was prepared in the same manner except that 95 % methanol was used instead of DPPH solution. A standard curve was prepared using Trolox in the range of 10 - 60 μM. The activity was calculated after the sample blank substraction and expressed as μmol Trolox equivalents (TE)/g dried film.

6.3.5.2 ABTS radical scavenging activity

ABTS radical scavenging activity was assayed as per the method of Arnao *et al.* (2001) with a slight modification. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities. The mixed solutions were allowed to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml of ABTS solution with 50 ml of methanol in order to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using a spectrophotometer. ABTS solution was prepared freshly prior to assay. Sample (150 μl) was mixed with 2850 μl

of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm using a spectrophotometer. Sample blank was prepared in the same manner except that methanol was used instead of ABTS solution. A standard curve of Trolox ranging from 50 to 600 μM was prepared. The activity was calculated after sample blank subtraction and was expressed as μmol Trolox equivalents (TE)/g dried film.

6.3.5.3 Ferrous ion chelating activity

Ferrous ion chelating activity was measured by the method of Thiansilakul *et al.* (2007). Diluted sample (4.7 ml) was mixed with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine. The reaction mixture was allowed to stand for 20 min at room temperature. The absorbance was then read at 562 nm. EDTA with the concentration range of 0–50 μM was used as the standard. Ferrous ion chelating activity was expressed as μmol EDTA equivalents (EE)/g dried film.

6.3.6 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, SPSS Inc., Chicago, IL, USA).

6.4 Results and Discussion

6.4.1 Thickness, mechanical and physical properties

6.4.1.1 Thickness

Thickness of fish skin gelatin films incorporated with different essential oils (basil, plai and lemon) and control film (without essential oils) is shown in Table 24. The thickness of all films containing essential oils was higher than that of the control film ($p < 0.05$). Essential oil droplets might insert and localize themselves in the film network. As a result, the interaction between gelatin chains could be impeded. The loss of compact network and the decrease in ordered alignment of gelatin chains might bring about the protruded structure as suggested by the increased

thickness. Moreover, it was noted that films prepared using different types of essential oils had varying thickness ($p < 0.05$). Among all essential oils used, lemon essential oil yielded the film with the highest thickness ($p < 0.05$), followed by those added with basil and plai essential oils, respectively. This might be governed by the differences in surface tension or size of oil droplets. Additionally, the essential oils might have different compositions, which could interact with gelatin chain in the film matrix differently. As a result, the alignment of gelatin molecules in the film matrix might be different, leading to the differences in film thickness.

6.4.1.2 Mechanical properties

Mechanical properties expressed as tensile strength (TS), elastic modulus (EM) and elongation at break (EAB) of gelatin films incorporated with different essential oils are shown in Table 24. Fish skin gelatin films with and without essential oils were flexible and visually homogeneous. Oil exudation was not found on the surface of films containing essential oils in spite of high amount of oil added. Addition of all types of essential oils led to the change in mechanical properties of films. Films with essential oils incorporated had lower TS and EM, but higher EAB, as compared with the control film ($p < 0.05$). TS of films were decreased by 29.5, 40.8 and 53.9% when basil, plai and lemon essential oils were incorporated, respectively, compared with that of control film. EM of films also decreased by 59.7, 67.7 and 69.5%, when basil, plai and lemon essential oils were added, respectively. Among all essential oils incorporated, basil essential oil yielded the film with the highest TS and EM ($p < 0.05$), followed by those containing plai and lemon essential oils, respectively. However, EAB of films incorporated with basil, plai and lemon essential oil were increased by 149.6, 175.5 and 122.6%, respectively. The highest EAB was obtained in film added with plai essential oil, followed by basil and lemon essential oils ($p < 0.05$). It was found that the incorporation of essential oil affected TS, EM and EAB of resulting films and the value varied with types of essential oils incorporated.

Several parameters such as the characteristics of the lipid and their capability to interaction with protein molecules in film network affected properties of resulting emulsion films (Gontard *et al.*, 1994). In general, the incorporation of lipid

decreased TS and puncture strength of protein-based film from gelatin (Limpisophon *et al.*, 2010), whey protein (Soazo *et al.*, 2013) and soy protein isolate (Guerrero *et al.*, 2011). This result was in agreement with Zinoviadou *et al*. (2009) who reported that the incorporation of oregano essential oil (0.5 - 1.5% w/w in FFS) decreased EM and TS with the concomitant increase in EAB of whey protein isolate films. The changes were in dose-dependent manner. Tongnuanchan *et al.* (2013a) also reported the decrease in tensile resistance and the increase in stretch-ability of gelatin-based film when root essential oils were added. Atarés *et al.* (2010b) studied the mechanical properties of soy protein isolate incorporated with cinnamon and ginger essential oils at different concentrations (protein to oil mass ratios of 1:0.025, 1:0.050, 1:0.075 and 1:0.100). A slight decrease in elastic modulus (EM) was observed as the oil content increased. Normally, simple protein-based films are stronger and higher extensibility than protein/lipid emulsion films (Chen, 1995). Interference of protein-protein interaction by the replacement of lipids occurred in film network. According to Yang and Paulson (2000), the interactions between non-polar molecules and polar polymers molecules are much lower than those between polar polymer molecules. Essential oil contains highly amount of non-polar molecules or hydrophobic compounds, especially monoterpene hydrocarbon, which could reduce the compactness of film network as evidenced by the decreased TS and EM. With high proportions of essential oil used, they showed high ability to reduce the rigidity of film or acted as plasticizer. Increasing plasticizing agent content yields a decrease in the resistance to breakage with the increased deformability. However, the incorporation of cinnamon essential oil resulted in an increase in TS of soy protein isolate film (Atarés *et al.*, 2010b). Different types of essential oils plausibly affected the rearrangement of protein molecule in film matrix differently as evidenced by varying TS, EM and EAB. Essential oils contain aldehyde, ketone or phenolic compounds as major constituents (Bakkali *et al.*, 2008). Those compounds were reported to interact with protein and enhanced the mechanical properties of film (Hernández-Muñoz *et al.*, 2004; Hoque *et al.*, 2011). Essential oils containing various compounds might interact with protein, thereby affecting characteristic of film network. In the present study, film containing lemon essential oil was more stretchable, compared with those containing basil and plai essential oils ($p < 0.05$). Lipid addition generally could not enhance the

cohesiveness and uniformity of film network formation (Péroval *et al.*, 2002). Therefore, essential oils from different sources affected the mechanical property of film differently.

6.4.1.3 Water vapor permeability (WVP)

WVP of films incorporated with three essential oils is presented in Table 24. Films containing all essential oils had lower WVP than the control film, irrespective with types of essential oil added ($p < 0.05$). WVP of films containing basil, plai and lemon essential oils were decreased by 61.4, 45.1 and 59.2%, respectively, compared with that of control film. These results suggested that the increasing amount of hydrophobic substance such as essential oils more likely increased the hydrophobicity of film. As a result, the adsorption and permeation of water vapor through the films containing essential oils were lowered. The rate of adsorptivity as well as diffusivity of water vapor in the films depends on the hydrophilicity/hydrophobicity ratio of the film components. It has been known that protein-based films had relatively poor water vapor barrier properties because of their hydrophillicity of polar amino acids in protein molecules (Krochta, 2002). Monoterpenes (C_{10}) and sesquiterpenes (C_{15}) are highly hydrophobic substances found in essential oils, in which the content varied with types of essential oils (Turina *et al.*, 2006). Among all essential oils incorporated, basil essential oil had the highest efficiency in lowering WVP of film ($p < 0.05$), followed by lemon and plai essential oils, respectively. The difference in WVP among films might be due to the difference in hydrophobic substances in essential oils. Thus, WVP of films was varied with types of essential oils used. Hydrophobic materials such as essential oils has been incorporated to improve water vapor barrier property of protein-based films, e.g. film from hake protein by thyme essential oil (Pires *et al.*, 2011), film from soy protein isolate by cinnamon and ginger essential oils (Atarés *et al.*, 2010b). Tongnuanchan *et al*. (2012) reported that WVP of fish skin gelatin film decreased markedly from 3.11 to 1.88, 1.89 and 2.45 × 10^{-11} $gm^{-1}s^{-1}Pa^{-1}$ ($p < 0.05$), when films were incorporated with ginger, turmeric and plai, respectively, at a level of 100 %, in which WVP of film was reduced by 39.5, 39.2 and 21.2%, respectively. No difference in WVP was found among films from sodium casinate added with cinnamon and ginger essential

oils at the concentration of 2.5 - 7.5 % (w/w) (Atarés *et al.*, 2010a). Moreover, dispersion of essential oil in hydrophillic based film could limit the diffusion of water vapor through the film via increasing tortuosity factor for water transfer by the discontinuities in film matrix. Thus, incorporation of essential oil could markedly enhance water vapor barrier property of gelatin film, in which the capability was dependent on types of essential oils used.

6.4.1.4 Color

Table 25 presents the color (L^*-, a^*-, b^*-, ΔE^*- and WI values) of gelatin films incorporated with three types of essential oils. Films containing all essential oils showed the lower L^*-value (lightness) and WI (whiteness) with the coincidental increases in b^*- and ΔE^*-values ($p < 0.05$). Film added with basil essential oil had the highest WI, compared with those incorporated with other essential oils ($p < 0.05$). Among all film samples, that incorporated with plai essential oil had the highest of b^*- and ΔE^*-values with the lowest L^*-value and WI ($p < 0.05$). Higher ΔE^*-value was in agreement with higher b^*-value. This might be associated with the yellowish color of plai essential oil. Plai essential oil had the highest yellowness as evidenced by the highest b^*-value, compared with other essential oils (data not shown). The difference of coloring pigments/compounds and their contents in essential oils might determine the different color of resulting films. Therefore, incorporation of essential oils from different plants and parts had direct impact on the color of resulting film. This was in agreement with Salarbashi *et al.* (2014) who reported that films prepared from soluble soybean polysaccharide and incorporated with both *Zataria multiflora Boiss* and *Mentha pulegium* essential oils had lower L^*-value and WI but higher b^*- and ΔE^*-values, especially with increasing oil concentration. Peng and Li (2014) also reported that the incorporation of lemon, thyme, cinnamon and their mixtures in chitosan-based film could increase b^*- and ΔE^*-values, whilst L^*-value decreased.

Table 24. Mechanical properties, water vapor permeability and thickness of films from fish skin gelatin containing basil, plai and lemon essential oils.

Essential oils	TS (MPa)	EAB (%)	EM (MPa)	WVP ($x10^{-11}$ $gm^{-1}s^{-1}Pa^{-1}$)	Thickness (mm)
Control	33.13 ± 2.13 [a]*	51.10 ± 6.66 [d]	910.03 ± 76.09 [a]	1.84 ± 0.06 [a]	0.055 ± 0.002 [d]
Basil	23.34 ± 0.64 [b]	127.53 ± 9.16 [b]	367.10 ± 23.63 [b]	0.71 ± 0.01 [c]	0.115 ± 0.008 [b]
Plai	19.61 ± 2.16 [c]	140.80 ± 5.31 [a]	294.10 ± 32.34 [c]	1.01 ± 0.01 [b]	0.106 ± 0.007 [c]
Lemon	15.27 ± 0.75 [d]	113.78 ± 3.43 [c]	277.51 ± 34.63 [c]	0.75 ± 0.04 [c]	0.125 ± 0.004 [a]

* Mean ± SD (n=3).
Different letters in the same column indicate significant differences ($p < 0.05$).
Control film was prepared from FFS without addition of essential oils.

Table 25. Color and transparency value of films from fish skin gelatin containing basil, plai and lemon essential oils.

Essential oils	Color					Transparency value
	*L**	*a**	*b**	Δ*E**	WI	
Control	90.76 ± 0.06 [a]*	-1.36 ± 0.02 [a]	0.93 ± 0.04 [d]	2.13 ± 0.05 [d]	90.61 ± 0.06 [a]	0.86 ± 0.06 [d]
Basil	89.38 ± 0.09 [b]	-2.81 ± 0.02 [b]	11.28 ± 0.06 [c]	11.46 ± 0.08 [c]	84.26 ± 0.10 [b]	1.09 ± 0.04 [c]
Plai	88.42 ± 0.17 [c]	-3.52 ± 0.02 [d]	20.25 ± 0.13 [a]	20.39 ± 0.10 [a]	76.41 ± 0.08 [d]	2.32 ± 0.06 [b]
Lemon	89.34 ± 0.07 [b]	-2.84 ± 0.03 [c]	13.34 ± 0.08 [b]	13.15 ± 0.07 [b]	82.69 ± 0.03 [c]	4.49 ± 0.10 [a]

* Mean ± SD (n=3).
Different letters in the same column indicate significant differences ($p < 0.05$).
Control film was prepared from FFS without addition of essential oils.

6.4.1.5 Light transmittance and transparency

Transmission of UV and visible light in the wavelength range of 200 - 800 nm of gelatin films incorporated with different essential oils is shown in Figure 24. The transmission of UV light was not observed at 200 nm for films incorporated with and without essential oils. However, films containing essential oils had much lower light transmittance at wavelength lower than 280 nm, as compared to control film, regardless of types of essential oils added. It was suggested that the incorporation of essential oils into gelatin film could enhance the barrier property against UV light. Therefore, gelatin films effectively protected the transmission of UV light, regardless of essential oils incorporated. Generally, protein films exhibited the excellent UV barrier properties due to their high amount of aromatic amino acids that absorb UV light (Hamaguchi *et al.*, 2007). Jongjareonrak *et al.* (2006) also reported higher UV light barrier capacity of gelatin film from cuttlefish and brownstripe red snapper and bigeye snapper, respectively. Light transmission of visible range (350-800 nm) of film without essential oil incorporated (control) ranged from 42.84 % to 89.12 %, whereas lower values were observed for film incorporated with essential oils (6.13 - 80.38 %), regardless of essential oil types. This result suggested that the light transmission of films were considerably decreased by the incorporation of essential oils. Essential oil droplets localized in film matrix possibly inhibited the light transmission for both UV and visible ranges of resulting films. Among all types of essential oils used, lemon essential oil yielded the film with the lowest light transmittance in visible range, indicating the highest opacity. Essential oils in films might cause light scattering to different degrees. Moreover, light transmission of film was most likely governed by the arrangement or alignment of polymer in film network (Limpan *et al.*, 2010).

The transparency value of all films is shown in Table 25. Films incorporated with essential oils had the higher transparency values than the control film, regardless of essential oil types ($p < 0.05$). The lower transparency value indicated that the film was more transparent. Thus, films containing essential oils were more opaque and less transparent, compared with the control film. For the films prepared with various essential oils, those incorporated with lemon essential oil

showed the highest transparency value ($p < 0.05$), followed by those added with plai and basil essential oils, respectively. It was noted that film containing lemon essential oil had the highest opaqueness, compared with others essential oils used. Peng and Li (2014) also reported that lemon essential oil had the most efficiency in lowering the opacity of chitosan film. Transparency value of films incorporated with essential oils varied, depending on types of essential oils. This might be governed by the differences in size or the distribution of essential oil droplets localize in the film network. This result was in agreement with Shojaee-Aliabadi *et al.* (2013) who reported that emulsified films based on carrageenan and essential oil turned more opaqueness, probably associated with the higher light scattering of lipid droplets. Therefore, the incorporation of essential oils had the pronounced impacts on light transmittance and transparency of films.

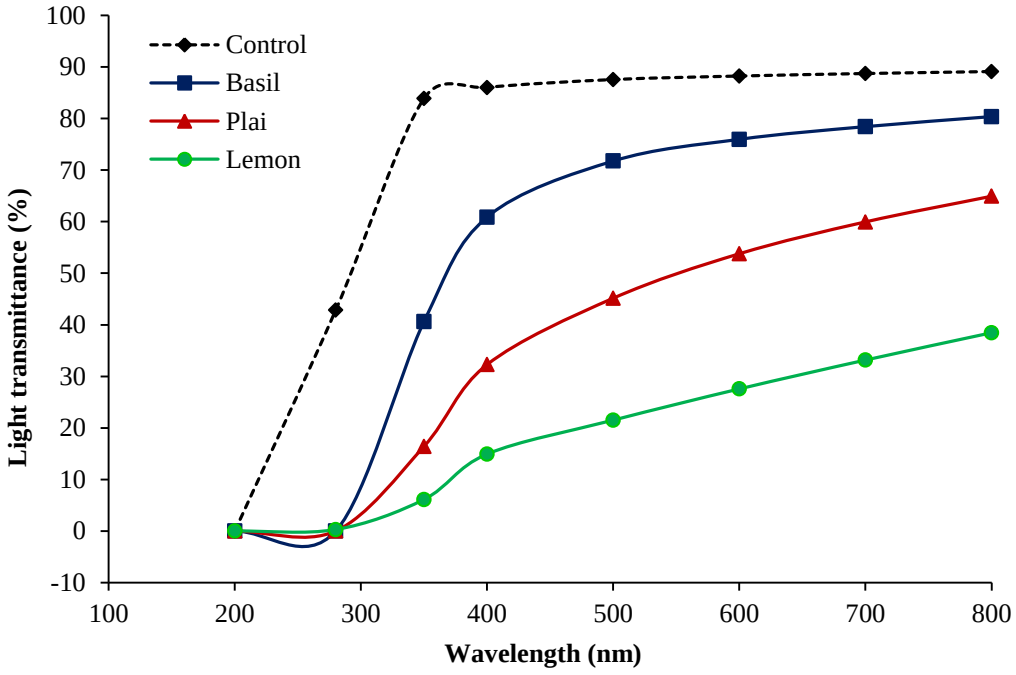


Figure 24. Light transmittance at wavelengths ranging from 200 to 800 nm of films from fish skin gelatin containing different essential oils.

6.4.2 Antioxidative activities

Antioxidative activities expressed as DPPH radical scavenging activity, ABTS radical scavenging activity and chelating activity of basil, plai and lemon essential oils are presented in Table 26. Basil essential oil had the highest DPPH and ABTS radical scavenging activities ($p < 0.05$), followed by plai and lemon essential oils, respectively. Nevertheless, chelating activity of lemon essential oil was highest ($p < 0.05$), followed by plai and basil essential oils, respectively. In general, plant essential oils have been known as antioxidant (Wu *et al.*, 1982). Several compounds in essential oils have the structure mimicing the well known plant phenols with antioxidant activity. Phenolics are organic compounds consisting of hydroxyl group (-OH) attached directly to a carbon atom that is a part of aromatic ring. The hydrogen atom of hydroxyl group can be donated to free radicals, thereby preventing other compounds to be oxidized (Nguyen *et al.*, 2003). Lemon essential oil was mainly composed of monoterpene hydrocarbons (α-pinene, α-fenchene, limonene and camphene) and oxygenated monoterpenes (citronellal, *cis*-carveol, α-citral, carvacol, terpniol, thymol, carvacrol and citral) (Mohamed *et al.*, 2010). Methylchavicol, 3-methoxycinnamaldehyde, methyleugenol, γ-cadinene and γ-muurolene were the dominant compounds identified in basil essential oil (Teixeira *et al.*, 2013). Thymol and carvacrol were reported to possess the highest antioxidant activity (Dapkevicius *et al.*, 1998). Methylchavicol also has been reported to present high antioxidant activity (Teixeira *et al.*, 2013). The antioxidant activity is generally related with the major active compounds in essential oils. However, the other antioxidant compounds in essential oils have also been reported to exhibit antioxidant activity, but their amounts were probably too low to exhibit antioxidant activity (Ruberto and Baratta, 2000).

Table 26. Antioxidant activity of basil, plai and lemon essential oils.

Essential oils	Antioxidant activity (µmol Trolox equivalents/ml)		Chelating activity (µmol EDTA equivalents/ml)
	DPPH	ABTS	
Basil	34.23 ± 0.05 [a]*	1921.27 ± 14.40 [a]	1.53 ± 0.18 [a]
Plai	3.03 ± 0.02 [b]	60.12 ± 3.18 [b]	3.91 ± 0.47 [b]
Lemon	0.49 ± 0.09 [c]	3.96 ± 0.85 [c]	30.51 ± 0.54 [c]

* Mean ± SD (n=3).

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

Table 27. Antioxidant activity of film from fish skin gelatin containing basil, plai and lemon essential oils.

Essential oils	Antioxidant activity (µmol Trolox equivalents/g dried film)		Chelating activity (µmol EDTA equivalents/g dried film)
	DPPH	ABTS	
Control	0.09 ± 0.02 [d]*	0.84 ± 0.16 [d]	0.25 ± 0.01 [d]
Basil	10.38 ± 0.06 [a]	129.65 ± 1.69 [a]	21.72 ± 0.51 [c]
Plai	0.64 ± 0.01 [b]	10.93 ± 0.08 [b]	55.02 ± 0.03 [b]
Lemon	0.53 ± 0.01 [c]	5.05 ± 0.53 [c]	58.12 ± 0.13 [a]

* Mean ± SD (n=3).

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

Control film was prepared from FFS without addition of essential oils.

The control film showed radical scavenging activities and chelating activity to some extent (Table 27). Gelatin exhibited antioxidant activities, in which peptide fraction containing particular amino acids such as glycine and proline had high activity (Kim *et al.*, 2001). Gómez-Estaca *et al.* (2009) also reported that edible film from tuna-skin and bovine-hide gelatin exhibited antioxidant activities. Films incorporated with essential oils had the marked increase in antioxidative activities and chelating activity ($p < 0.05$). DPPH and ABTS radical scavenging activities of film incorporated with basil essential oil was highest ($p < 0.05$), followed by those added with plai and lemon essential oils, respectively. For chelating activity, film containing

lemon essential oil exhibited the highest activity ($p < 0.05$), followed by those added with plai and basil essential oils. Different active compounds in various essential oils affected antioxidative activities and chelating activity of resulting films. Furthermore, some active compounds in different essential oils could interact with gelatin film matrix differently. This led to the varying release of antioxidant compounds from films. The availability of free active compounds in those films matrix could be varied. Thus, the incorporation of selected essential oils from various sources such as leaf, root and peel into gelatin-based film could enhance antioxidative activity.

6.5 Conclusion

Incorporation of different essential oils from various sources including basil, plai and lemon into fish gelatin film decreased TS and EM with coincidentally increased EAB via plasticizing effect. Essential oils effectively improved the water vapor barrier property of film, especially that containing basil and lemon essential oils. However, those essential oils could affect the color and light transmittance of resulting films. Among essential oils added, basil essential oil could be appropriately used to enhance water vapor barrier property and yielded film with antioxidative activity. Therefore, plant essential oils could be used as natural additives, which could improve physical and functional properties of gelatin film.

6.6 References

Arnao, M.B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73: 239-244.

ASTM. 1989. Standard test methods for water vapor transmission of materials. Standard designation E96-E80. *In* Annual book of ASTM standards. p. 730-739. ASTM. Philadelphia.

Atarés, L., Bonilla, J. and Chiralt, A. 2010a. Characterization of sodium caseinate-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 100: 678-687.

Atarés, L., De Jesús, C., Talens, P. and Chiralt, A. 2010b. Characterization of SPI-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 99: 384-391.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils - A review. Food Chem. Toxicol. 46: 446-475.

Binsan, W., Benjakul, S., Visessanguan, W., Roytrakul, S., Tanaka, M. and Kishimura, H. 2008. Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). Food Chem. 106: 185-193.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods - A review. Int. J. Food Microbiol. 94: 223-253.

Chen, H. 1995. Functional properties and applications of edible films made of milk proteins. J. Dairy Sci. 78: 2563-2583.

Dapkevicius, A., Venskutonis, R., van Beek, T.A. and Linssen, J.P.H. 1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J. Sci. Food Agr. 77: 140-146.

Gennadios, A., Ghorpade, V.M., Weller, C.L. and Hanna, M.A. 1996. Heat curing of soy protein films. T. ASABE. 39: 575-579.

Ghanbarzadeh, B., Almasi, H. and Entezami, A.A. 2010. Physical properties of edible modified starch/carboxymethyl cellulose films. Innov. Food Sci. Emerg. 11: 697-702.

Gómez-Estaca, J., Bravo, L., Gómez-Guillén, M.C., Alemán, A. and Montero, P. 2009. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chem. 112: 18-25.

Gontard, N., Duchez, C., Cuq, J.-L. and Guilbert, S. 1994. Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties. Int. J. Food Sci. Technol. 29: 39-50.

Guerrero, P., Nur Hanani, Z.A., Kerry, J.P. and de la Caba, K. 2011. Characterization of soy protein-based films prepared with acids and oils by compression. J. Food Eng. 107: 41-49.

Hamaguchi, P.Y., WuYin, W. and Tanaka, M. 2007. Effect of pH on the formation of edible films made from the muscle proteins of blue marlin (*Makaira mazara*). Food Chem. 100: 914-920.

Han, J.H. and Floros, J.D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. J. Plast. Film Sheet. 13: 287-298.

Hernández-Muñoz, P., Villalobos, R. and Chiralt, A. 2004. Effect of cross-linking using aldehydes on properties of glutenin-rich films. Food Hydrocolloid. 18: 403-411.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011. Properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin incorporated with cinnamon, clove and star anise extracts. Food Hydrocolloid. 25: 1085-1097.

Iwata, K., Ishizaki, S., Handa, A. and Tanaka, M. 2000. Preparation and characterization of edible films from fish water-soluble proteins. Fisheries Sci. 66: 372-378.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid. 20: 492-501.

Kim, S.-K., Kim, Y.-T., Byun, H.-G., Nam, K.-S., Joo, D.-S. and Shahidi, F. 2001. Isolation and characterization of antioxidative peptides from gelatin hydrolysate of alaska pollack skin. J. Agr. Food Chem. 49: 1984-1989.

Krochta, J.M. 1997. Edible protein films and coatings. *In* Food proteins and their applications. (Damodaran, S. and Paraf, A., eds.). p. 529-549. Marcel Dekker. New York.

Krochta, J.M. 2002. Protein as raw materials for films and coatings: definitions, current status, and opportunities. *In* Protein-Based Films and Coating (Gennadios, A., ed.). p. 1-39. CRC Press. New York.

Limpan, N., Prodpran, T., Benjakul, S. and Prasarpran, S. 2010. Properties of biodegradable blend films based on fish myofibrillar protein and polyvinyl alcohol as influenced by blend composition and pH level. J. Food Eng. 100: 85-92.

Limpisophon, K., Tanaka, M. and Osako, K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionace glauca*) skin gelatin. Food Chem. 122: 1095-1101.

Mohamed, A.A., El-Emary, G.A. and Ali, H.F. 2010. Influence of some citrus essential oils on cell viability, glutathione-s-transferase and lipid peroxidation in *Ehrlich ascites* Carcinoma Cells. J. Am. Sci. 6: 820-826.

Nguyen, M.T., Kryachko, E.S. and Vanquickenborne, L.G. 2003. General and theoretical aspects of phenols. *In* The Chemistry of Phenols. (Rappoport, Z., ed.). p. 1-198. John Wiley & Sons Ltd. Chichester, West Sussex, UK.

Núñez-Flores, R., Giménez, B., Fernández-Martín, F., López-Caballero, M.E., Montero, M.P. and Gómez-Guillén, M.C. 2012. Role of lignosulphonate in properties of fish gelatin films. Food Hydrocolloid. 27: 60-71.

Peng, Y. and Li, Y. 2014. Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films. Food Hydrocolloid. 36: 287-293.

Péroval, C., Debeaufort, F., Despré, D. and Voilley, A. 2002. Effects of lipid type on water vapor permeability, film structure, and other physical characteristics. J. Agr. Food Chem. 50: 3977-3983.

Pires, C., Ramos, C., Teixeira, G., Batista, I., Mendes, R., Nunes, L. and Marques, A. 2011. Characterization of biodegradable films prepared with hake proteins and thyme oil. J. Food Eng. 105: 422-428.

Ruberto, G. and Baratta, M.T. 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem. 69: 167-174.

Salarbashi, D., Tajik, S., Shojaee-Aliabadi, S., Ghasemlou, M., Moayyed, H., Khaksar, R. and Noghabi, M.S. 2014. Development of new active packaging film made from a soluble soybean polysaccharide incorporated *Zataria multiflora Boiss* and *Mentha pulegium* essential oils. Food Chem. 146: 614-622.

Shahidi, F., Botta, J.R. and Shahidi, F. 1994. Seafood processing by-products. *In* Seafoods: Chemistry, Processing Technology and Quality. p. 320-334. Springer US.

Shiku, Y., Hamaguchi, P.Y., Benjakul, S., Visessanguan, W. and Tanaka, M. 2004. Effect of surimi quality on properties of edible films based on Alaska pollack. Food Chem. 86: 493-499.

Shojaee-Aliabadi, S., Hosseini, H., Mohammadifar, M.A., Mohammadi, A., Ghasemlou, M., Ojagh, S.M., Hosseini, S.M. and Khaksar, R. 2013. Characterization of antioxidant-antimicrobial k-carrageenan films containing *Satureja hortensis* essential oil. Int. J. Biol. Macromol. 52: 116-124.

Soazo, M., Pérez, L.M., Rubiolo, A.C. and Verdini, R.A. 2013. Effect of freezing on physical properties of whey protein emulsion films. Food Hydrocolloid. 31: 256-263.

Teixeira, B., Marques, A., Ramos, C., Neng, N.R., Nogueira, J.M.F., Saraiva, J.A. and Nunes, M.L. 2013. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Ind. Crop. Prod. 43: 587-595.

Thiansilakul, Y., Benjakul, S. and Shahidi, F. 2007. Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). Food Chem. 103: 1385-1394.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2012. Properties and antioxidant activity of fish skin gelatin film incorporated with citrus essential oils. Food Chem. 134: 1571-1579.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2013a. Physico-chemical properties, morphology and antioxidant activity of film from fish skin gelatin incorporated with root essential oils. J. Food Eng. 117: 350-360.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2013b. Characteristics and antioxidant activity of leaf essential oil–incorporated fish gelatin films as affected by surfactants. Int. J. Food Sci. Tech. 48: 2143–2149.

Turina, A.V., Nolan, M.V., Zygadlo, J.A. and Perillo, M.A. 2006. Natural terpenes: self-assembly and membrane partitioning. Biophys. Chem. 122: 101-113.

Wasswa, J., Tang, J. and Gu, X. 2007. Utilization of fish processing by-products in the gelatin industry. Food Rev. Int. 23: 159-174.

Wu, J., Lee, M.-H., Ho, C.-T. and Chang, S. 1982. Elucidation of the chemical structures of natural antioxidants isolated from rosemary. J. Am. Soc. 59: 339-345.

Yang, L. and Paulson, A.T. 2000. Effects of lipids on mechanical and moisture barrier properties of edible gellan film. Food Res. Int. 33: 571-578.

Zinoviadou, K.G., Koutsoumanis, K.P. and Biliaderis, C.G. 2009. Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. Meat Sci. 82: 338-345.

CHAPTER 7

EMULSION FILM BASED ON FISH SKIN GELATIN AND PALM OIL: PHYSICAL, STRUCTURAL AND THERMAL PROPERTIES

7.1 Abstract

Physical, structural and thermal properties of emulsion film based on fish skin gelatin containing palm oil at different levels (25%, 50%, 75% and 100%, based on protein), were tested. Particle size distribution (d_{32} and d_{43}) of film-forming emulsion (FFE) at all oil levels was similar after preparation (0 h) ($p > 0.05$) and slightly increased with increasing storage time, especially at 12 h ($p < 0.05$). Films incorporated with palm oil had lower tensile strength (TS) and elastic modulus (EM) but higher elongation at break (EAB) as the amount of palm oil increased ($p < 0.05$). Decreased water vapor permeability (WVP) and moisture content (MC) were observed for films having the increasing amount of palm oil ($p < 0.05$). Emulsion gelatin films with higher levels of palm oil had lower L^*-value, whiteness index (WI), light transmittance but higher b^*- and ΔE^*-values ($p < 0.05$). The addition of palm oil generally resulted in the increased hydrophobicity and the weaker protein-protein interaction in film network, leading to the lower thermal stability. This was indicated by lower glass-transition and degradation temperatures of the resulting films. Most of oil droplets were imbedded in the matrix of emulsion films, regardless of palm oil levels used. Therefore, the levels of palm oil directly had the impact on physical, molecular structure and thermal properties of resulting emulsion gelatin film.

7.2 Introduction

Biodegradable films and coatings provide many benefits in terms of convenience and protective function for foods, food ingredients and drugs (Gennadios and Weller, 1990). In addition to the physical and chemical quality enhancement, edible films and coatings contribute to visual and marketing related quality factors. The oxygen-barrier properties of film and coating layers can prevent oxidation of lipid ingredients, colorants and flavors of food products such as nuts, confectionary, fried products and colored produces (Baldwin *et al.*, 1997).

Gelatin is one of the important biopolymers widely used in the manufacture of gel desserts, hard/soft capsules in pharmaceutical industries and edible films in food industries (Choi and Regenstein, 2000). Gelatin is derived from the fibrous insoluble protein or collagen, which is the principal constituent of animal skin, bone, and connective tissue. Gelatin is produced via the partial hydrolysis of native collagen with molecular weights from 3 to 200 kDa depending on the raw material used and the extraction conditions (Lacroix and Cooksey, 2005). Gelatin edible films, with high puncture strength and low puncture deformation, prepared from bovine and porcine skin were reported (Sobral *et al.*, 2001). Fish skin gelatin can be used as film forming material, but properties of film vary, depending on the source of gelatin, plasticizer and other factors (Vanin *et al.*, 2005). However, gelatin-based film is still encountering poor barrier property toward water vapor, due to the hydrophilic nature of gelatin and hydrophilic plasticizer required for film production. This restricts their uses as packing materials (Hoque *et al.*, 2011a; Limpisophon *et al.*, 2010).

To improve the water vapor barrier property, the incorporation of hydrophobic substances such as lipids and oils has been implemented to improve water vapor barrier property due to the hydrophobic nature of the oils (Limpisophon *et al.*, 2010; Tongnuanchan *et al.*, 2013a). Nevertheless, fatty acids with high price can be the constraint for applications. The use of cheap hydrophobic substances, e.g. palm oil, which is abundant in the Southeast Asia including Thailand, could pave a way for property improvement of gelatin-based film. Palm oil is an edible vegetable oil, derived from the fruit of the oil palm tree, which is reddish in color and contains several saturated and unsaturated fats (Edem, 2002). Palm oil is high in saturated fatty acids, which have been widely used as common food ingredients, e.g. margarines, instant noodles, chocolates, etc. Therefore, palm oil can serve as hydrophobic substances to improve the water vapor barrier property of edible/biodegradable protein based films. However, the incorporation of palm oil at different levels into the gelatin-based film could affect the properties of resulting film differently. The objective of this investigation was to study the effect of palm oil concentrations on physical properties, mechanical property, water vapor permeability, structural, morphological and thermal properties of emulsion gelatin-based film.

7.3 Materials and Methods

7.3.1 Chemicals

Glycerol was obtained from Merck (Darmstadt, Germany). Nile blue A and soy lecithin (l-α-phosphatidylcholine, HLB = 4.0) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals are of analytical grade.

7.3.2 Fish gelatin and palm oil

Fish gelatin produced from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Palm oil was obtained from Oleen Co., Ltd. (Samutsakorn, Thailand).

7.3.3 Preparation of film from fish gelatin incorporated with palm oil at different levels

7.3.3.1 Preparation of film forming emulsion

Gelatin powder was mixed with distilled water to obtain the protein concentration of 3.5 % (w/v). The mixture was heated at 70 °C for 30 min. Glycerol at 30 % (w/w) of protein content was used as a plasticizer. The obtained solution was referred to as film-forming solution (FFS). To prepare emulsion film, palm oil previously mixed with soy lecithin at 25% (w/w, based on palm oil) was added into FFS at levels of 25%, 50%, 75% and 100% (w/w, based on protein content). The film-forming emulsion (FFE) was homogenized at 22,000 rpm for 3 min using a homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The dissolved air in FFE was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature.

7.3.3.2 Characterization of film-forming emulsion

7.3.3.2.1 Particle size distribution

Particle size distribution of emulsions will be determined using a ZetaPlus zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA). The surface-weighted mean (d_{32}) and the volume-weighted mean particle diameter (d_{43}) of the emulsion droplets were measured as described by Palazolo *et al.* (2011).

7.3.3.2.2 Confocal scanning laser microscopy (CSLM)

The distributions of palm oil at different levels in film forming emulsions were determined by a CSLM (Fluoview FV300, Olympus, Tokyo, Japan). The fluorochromes were immediately applied to the FFE samples. Nile blue A (0.01%w/v, Sigma Chemical) was directly mixed with FFE with a ratio of 1:20 (v/v) for lipid marking. Samples with Nile blue A were elucidated with the helium neon-green laser at 543 nm. The laser was adjusted to the red fluorescence mode, which yielded a single excitation wavelength (590 nm). The Olympus objective lens was used at x20/0.7N.A./UPlanApo. The laser was set at 10% of their maximum power, whereas the barrier filter BA550-600 was selected. The confocal aperture number 2 was used to cut off the emission wavelength before through PMT detector and dichroic mirror DM570 was set. Each sample was placed on a coverslips 20x50 mm^2 and the magnification of 200x was applied in all images.

7.3.3.3 Preparation of film

To prepare the films, the FFE (4 g) was cast onto a rimmed silicone resin plate (50×50 mm^2) and air-blown for 12 h at room condition (27±2 °C and 75±10 % relative humidity (RH)). The films were further dried 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 were prepared from the FFS containing gelatin and glycerol without palm oil and surfactant.

7.3.3.4 Determination of film properties

Prior to testing of film thickness, moisture content, mechanical properties, water vapor permeability, color and transparency value, films were conditioned for 48 h at 50 ± 5% relative humidity (RH) and 25 ± 0.5 °C. For the rest of characterizations, films were conditioned in a desiccator containing dried silica gel for 2 weeks, followed by placing for 1 week in a desiccator containing P_2O_5 at room temperature (25-30 °C) to obtain the most dehydrated films prior to analyses.

7.3.3.4.1 Film thickness

The thickness of films 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.

7.3.3.4.2 Mechanical properties

Tensile strength (TS), elastic modulus (EM) 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 film 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.

7.3.3.4.3 Water vapor permeability (WVP)

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

$$WVP\ (gm^{-1}s^{-1}Pa^{-1}) = wlA^{-1}t^{-1}(P_2 - P_1)^{-1}$$

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 vapour pressure difference across the film (Pa).

7.3.3.4.4 Color

Film samples were subjected to color measurement using a CIE colorimeter (Hunter associates laboratory, Inc., 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), b^*-value (yellowness/blueness), total difference of color (ΔE^*) and whiteness (WI) were calculated as follows (Gennadios *et al.*, 1996; Ghanbarzadeh *et al.*, 2010):

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

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences between the color parameter of the samples and the color parameters of the white standard (L^* = 92.84, a^* = - 1.23, b^* = 0.47).

7.3.3.4.5 Transparency value

Films were measured at the wavelength of 600 nm using a UV–vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The transparency value was calculated using the following equation (Han and Floros, 1997):

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

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.

7.3.3.4.6 Scanning electron microscopy (SEM)

Morphology of surface and cross-section of film samples were visualized using a scanning electron microscope (SEM) (Quanta 400, FEI, Eindhoven, the Netherlands). For cross-section of film, 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.

7.3.3.4.7 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 -40 to 150 °C, with a heating rate of 5 °C/min. Dry ice was used as a cooling medium and the system was equilibrated at -40 °C for 5 min prior to the scan. An empty aluminium pan was used as the reference. The maximum transition temperature was estimated from the endothermic peak of the DSC thermogram and transition enthalpy was determined from the area under the endothermic peak. A second scan was also performed in the same manner, followed by quench-cooling of the sample after completing the first scan. Glass transition temperatures (T_g) of palm oil and glycerol were also evaluated over the temperature range of -150 to 150 °C using liquid nitrogen as a cooling medium with a heating rate of 5 °C/min.

7.3.3.4.8 Thermo-gravimetric analysis (TGA)

Films were scanned using a thermo-gravimetric analyzer (TGA7, PerkinElmer, Norwalk, CT, USA) from 25 to 1000 ºC at a rate of 10 ºC/min (Nuthong *et al.*, 2009). Nitrogen was used as the purge gas at a flow rate of 20 ml/min.

7.3.3.4.9 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 (45º ZnSe; 80 mm long, 10 mm wide and 4 mm thick) (PIKE Technology Inc., Madison, WI, USA) at 25 ºC as described by Nuthong *et al.* (2009). Films were placed onto the crystal cell and the cell was 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 were ratioed against a background spectrum recorded from the clean empty cell at 25 ºC.

7.3.4 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, SPSS Inc., Chicago, IL, USA).

7.4 Results and Discussion

7.4.1 Distribution of oil droplets in FFE

7.4.1.1 Particle size distribution

d_{32} and d_{43} of oil droplets in film-forming emulsion (FFE) containing fish skin gelatin and palm oil at different levels (25-100%) as a function of storage time are shown in Table 28. After emulsification (0 h), similar d_{32} and d_{43} of FFE containing palm oil at different levels were noticeable ($p > 0.05$). During emulsification, soy lecithin used as the surfactant could quickly migrate and absorb at the surface of the newly formed oil droplets, thereby lowering the surface tension and forming the strong outer layers around droplets. This can retard the flocculation or coalescence due to steric or electrostatic repulsions (Dickinson, 2009). Soy lecithin (phospholipid) has been reported to provide high stability of emulsion as well as homogeneous oil distribution in protein-lipid emulsion film network (Tongnuanchan

et al., 2014). For FFE containing the same palm oil level, no changes in d_{32} were observed during the first 6 h of storage ($p > 0.05$). Similar result was found for d_{43} of FFE containing 25 and 50% palm oil. The d_{32} is inversely related to specific surface area. The smaller d_{32} means the higher specific surface area (Huck-Iriart *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 (Huck-Iriart *et al.*, 2013; McClements, 2005). However, the continuous increases in d_{43} were observed after 6 h of storage in FFE containing 75 and 100% palm oil ($p < 0.05$). This suggested that the coalescence and flocculation took place after 6 h in those two samples. Overall, the increases in d_{32} and d_{43} were noticeable in all FFE after 12 h of storage. This clearly indicated the instability of emulsion, in which the collapse of emulsion by coalescence or flocculation mechanisms might occur (Bonilla *et al.*, 2012). Among all FFE samples, that containing 100% palm oil had the highest d_{32} and d_{43} after 12 h of storage, followed by those containing 25%, 75 and 50% palm oil, respectively. The result implied that the size of oil droplets was plausibly increased during casting and drying to some degree, particularly when palm oil at high level was incorporated into FFE. The changes in oil droplet size might affect the distribution of droplets throughout the film, thereby governing the water vapor barrier property of gelatin film.

7.4.1.2 CSLM

CSLM images of FFS and FFE containing palm oil at different levels are illustrated in Figure 25. CSLM was used to visualize the distribution of palm oil droplets (red) in the continuous protein phase (black). Obviously, oil droplets were homogeneously dispersed in the protein phase, regardless of level of oil incorporated. As the levels of palm oil increased, higher number of oil droplets was observed in CSLM images. However, no large coalescence of oil droplets was found in FFE at all levels of palm oil used. This indicated that, soybean lecithin used as the surfactant was able to stabilize oil droplets after homogenization. Lecithin was localized at oil-water interface, in which polar head was extended to aqueous phase, while non-polar domain was located at oil surface (Friberg *et al.*, 2004). With sufficient amount of surfactant, oil droplets could be dispersed in FFS without coalescence.

Table 28. Oil droplet size distribution of film-forming emulsion from fish skin gelatin incorporated with palm oil at different levels at different storage time.

Oil levels (% based on protein content)	Storage time (h)	d_{32} (μm)	d_{43} (μm)
25	0	0.197 ± 0.006 ^{bA}*	0.213 ± 0.004 ^{bA}
	6	0.206 ± 0.009 ^{bA}	0.223 ± 0.007 ^{bA}
	12	0.248 ± 0.008 ^{aB}	0.275 ± 0.008 ^{aB}
50	0	0.188 ± 0.015 ^{bA}	0.205 ± 0.011 ^{bA}
	6	0.193 ± 0.007 ^{bA}	0.210 ± 0.005 ^{abB}
	12	0.213 ± 0.013 ^{aD}	0.221 ± 0.009 ^{aD}
75	0	0.185 ± 0.012 ^{bA}	0.207 ± 0.007 ^{cA}
	6	0.201 ± 0.007 ^{bA}	0.222 ± 0.007 ^{bA}
	12	0.230 ± 0.010 ^{aC}	0.244 ± 0.007 ^{aC}
100	0	0.197 ± 0.004 ^{bA}	0.208 ± 0.007 ^{cA}
	6	0.202 ± 0.008 ^{bA}	0.221 ± 0.008 ^{bA}
	12	0.304 ± 0.009 ^{aA}	0.310 ± 0.005

* Mean ± SD (n=3).

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

Different capital letters in the same column under the same storage time indicate significant differences ($p < 0.05$).

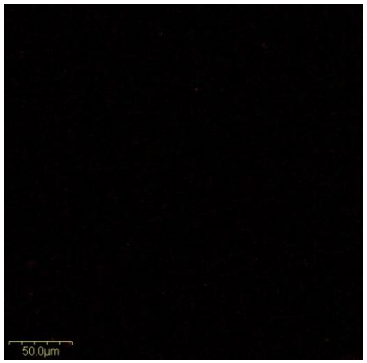
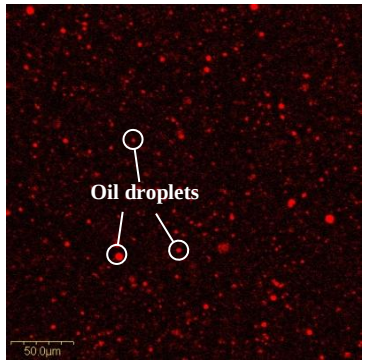
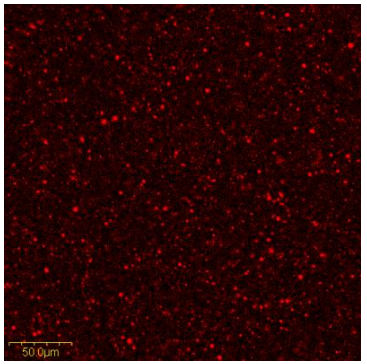
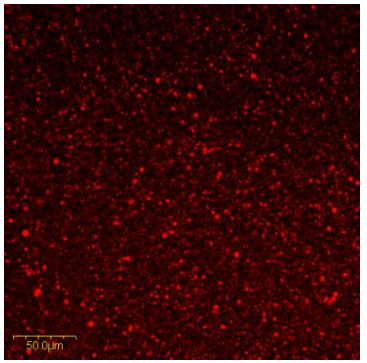
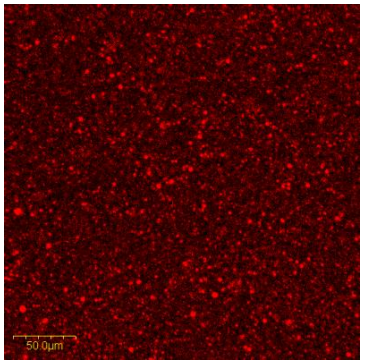
Control (Without palm oil added)	Palm oil concentration (% based on protein content)			
	25	50	75	100
				

Figure 25. CSLM images of film forming emulsion at different levels of palm oil.

7.4.2 Properties and characteristics of emulsion gelatin film

7.4.2.1 Thickness

The thickness of fish skin gelatin films incorporated with palm oil at different levels (25, 50, 75 and 100%, based on protein content) was higher than that of the control film (without incorporated palm oil) ($p < 0.05$) (Table 29). Thickness of films increased with increasing levels of palm oil ($p < 0.05$). This was simply due to increasing solid content in the film. Moreover, the interaction and alignment of gelatin molecules in film matrix could also be impeded by oil droplets. As a result, the compact and ordered network was not be developed. Interfering effect might be more pronounced as the amount of oil increased. As a consequence, the protruded network was formed with increasing amount of palm oil, as evidenced by the increased thickness.

7.4.2.2 Mechanical properties

Mechanical properties expressed as tensile strength (TS), elastic modulus (EM) and elongation at break (EAB) of fish skin gelatin films incorporated with palm oil at different levels are shown in Table 29. The control film had the highest TS and EM, but lowest EAB ($p < 0.05$), compared with those incorporated with palm oil. This indicated that the control gelatin film was shifter and less extensible than the emulsion gelatin films. As the level of palm oil increased, lower TS and EM were observed in resulting films ($p < 0.05$). However, no differences in TS and EM were observed between films containing palm oil at 75% and 100% ($p > 0.05$). In general, when the concentration of palm oil increased from 25% up to 75%, EAB of resulting films increased ($p < 0.05$). Soy lecithin with low hydrophobic-lipophilic balance (HLB) was the appropriate surfactant for stabilizing emulsion, which rendered the homogeneous oil distribution in protein/lipid emulsion film (Tongnuanchan *et al.*, 2014). As a result, droplets of palm oil were dispersed uniformly inside the film network and lowered protein-protein interaction in film network, as indicated by the lower TS and EM with concomitantly higher EAB. Nevertheless, EAB of film containing palm oil at the highest level (100%, based on protein) was lower than that of film incorporated with oil at 75% ($p < 0.05$). It was

suggested that the increase in film thickness might possibly decrease the extensibility of film to some degree. Among all films incorporated with palm oil, the lowest TS and EM were found in films added with 75% or 100% palm oil and the highest EAB was obtained in film incorporated with palm oil at 75% ($p < 0.05$).

The incorporation of hydrophobic substances (lipids, fatty acids, wax, oils, essential oils etc), particularly at higher concentration generally decreased TS and EM of gelatin-based films from various sources including porcine-hide gelatin (Andreuccetti *et al.*, 2009), bovine-hide gelatin (Ma *et al.*, 2012b) and fish skin gelatin (Jongjareonrak *et al.*, 2006b; Limpisophon *et al.*, 2010). The addition of palm oil reduced film strength but increased the flexibility, as indicated by decreased TS and EM with the concomitant increased in EAB of gelatin film. The result suggested the plasticizing effect of palm oil in film matrix. Palm oil droplets more likely hindered or interfered protein–protein interaction in film network, leading to the discontinuity of film matrix (Prodpran *et al.*, 2007). The reduced continuity and lack of cohesive structure integrity of film network therefore lowered the strength of film.

Table 29. Mechanical properties and thickness of films from fish skin gelatin incorporated with palm oil at different levels.

Film samples	Oil levels (% based on protein content)	TS (MPa)	EM (MPa)	EAB (%)	Thickness (mm)
Control film	0	28.77 ± 1.31 [a]*	705.94 ± 44.34 [a]	44.09 ± 5.57 [d]	0.048 ± 0.002 [e]
Emulsion film	25	21.39 ± 2.03 [b]	378.70 ± 39.39 [b]	108.45 ± 4.33 [c]	0.063 ± 0.004 [d]
	50	15.75 ± 1.69 [c]	259.20 ± 23.02 [c]	125.34 ± 11.21 [b]	0.085 ± 0.004 [c]
	75	11.98 ± 1.30 [d]	157.47 ± 22.01 [d]	143.30 ± 8.99 [a]	0.105 ± 0.004 [b]
	100	12.85 ± 0.91 [d]	177.40 ± 14.30 [d]	106.61 ± 7.75 [c]	0.120 ± 0.002 [a]

* Mean ± SD (n=3).

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

7.4.2.3 Water vapor permeability (WVP) and moisture content (MC)

WVP of films from fish skin gelatin incorporated with palm oil at different levels is shown in Table 30. Films incorporated with palm oil significantly had the lower WVP than the control films ($p < 0.05$). The lower WVP was obtained in resulting films with increasing level of palm oil ($p < 0.05$). WVP of gelatin film decreased markedly from 2.54 to 1.63, 1.18, 1.18 and 0.70 × 10^{-11} $gm^{-1}s^{-1}Pa^{-1}$ ($p < 0.05$), when palm oil was incorporated into gelatin films at the levels of 25%, 50%, 75% and 100%, which reduced WVP of films by 35.83%, 53.54%, 56.30% and 72.52%, respectively. Nevertheless, films added with palm oil at 50% and 75% had no difference in WVP ($p > 0.05$). In general, gelatin-based film had poor water vapor barrier properties because of its hydrophilicity of gelatin molecules (Krochta, 2002). The incorporation of nonpolar or hydrophobic substances with uniform distribution in film matrix could increase hydrophobicity of the film as well as lower adsorption and diffusion of water vapor through the film as evidenced by the lower WVP. Triglycerides in palm oil consist of a mixture of monounsaturated, polyunsaturated and saturated fatty acids (Jeirani *et al.*, 2013), but saturated fatty acid constitutes at higher content than others (Phan *et al.*, 2011). Furthermore, surfactant might affect water vapor barrier properties of films. Tongnuanchan *et al.* (2013b) reported that gelatin/lipid emulsion film prepared using soy lecithin as surfactant had the lowest WVP, since lecithin could stabilize and facilitate oil droplets to disperse uniformity in film network (Dickinson, 2003). Those oil droplets could provide tortuosity for water vapor migration in film matrix.

Moisture content (MC) of films decreased with increasing amount of palm oil ($p < 0.05$) (Table 30). However, no difference in MC was observed between films incorporated with palm oil at levels of 50%, 75% and 100% ($p > 0.05$). Hydrophobic nature of palm oil distributed throughout the film more likely impeded the water vapor adsorption to the film. The similar results were observed in emulsified protein-based films from bovine-hide gelatin film (Ma *et al.*, 2012b). Protein-palm oil interactions with the concomitant lowered protein-water interactions also led to the lower MC of resulting films. Therefore, the addition of palm oil was able to reduce

hygroscopic nature of film and retard the migration of water vapor from surrounding atmosphere through the films.

Table 30. Water vapor permeability and moisture content of films from fish skin gelatin incorporated with palm oil at different levels.

Film samples	Oil levels (% based on protein content)	WVP ($x10^{-11}$ $gm^{-1}s^{-1}Pa^{-1}$)	MC (%)
Control film	0	2.54 ± 0.05 [a]*	20.18 ± 1.30 [a]
Emulsion film	25	1.63 ± 0.12 [b]	18.49 ± 1.46 [b]
	50	1.18 ± 0.02 [c]	16.17 ± 0.46 [c]
	75	1.11 ± 0.06 [c]	15.45 ± 0.64 [c]
	100	0.70 ± 0.02 [d]	15.07 ± 0.54 [c]

* Mean ± SD (n=3).

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

7.4.2.4 Color and film transparency

Table 31 presents the color (L^*-, a^*-, b^*-, ΔE^*- and WI values) of gelatin films incorporated with palm oil at various levels. Films incorporated with palm oil showed the lower L^*-value and WI with the coincidentally higher b^* and ΔE^*-values, compared with control film ($p < 0.05$). However, lower a^*-value was also observed in resulting films but no difference in a^*-value was found among films incorporated with palm oil at all levels used ($p > 0.05$). Film incorporated with the highest level of palm oil (100%, based on protein) had the highest b^* and ΔE^*-values with the concomitant lower L^*-value and WI, compared with others ($p < 0.05$). Moreover, higher ΔE^*-value was associated with higher b^*-value, especially with increasing amount of palm oil. Palm oil had yellowish color due to its coloring compounds or pigments. The pale yellow color in palm oil was due to its high beta-carotene content (Manorama and Rukmini, 1992). Therefore, the color in palm oil directly affected the color of resulting films. Additionally, all films incorporated with palm oil were prepared from emulsion, in which the appropriate surfactant was

required to stabilize the system. Soy lecithin with dark brown color also contributed to higher yellowness of gelatin films. This result was in agreement with Tongnuanchan *et al.* (2013b) who reported that films with soy lecithin as surfactant had the highest b^* and ΔE^*-values in comparison with those using other surfactants. Therefore, the colors of palm oil and surfactant directly determined color of resulting films.

Based on transparency value (Table 31), the lowest transparency value was obtained in control film, compared with those of films incorporated with palm oil ($p < 0.05$). The transparency value of films containing palm oil increased as the amount of palm oil increased ($p < 0.05$). However, no difference in transparency value was observed between films incorporated with palm oil at the levels of 50% and 75% ($p > 0.05$). The lower transparency value indicated that the film was more transparent. Films containing palm oil were generally more turbid or opaque than the control film. The increases in turbidity of film was more likely caused by oil droplets distributed throughout the film as well as the decreased ordered structure in film matrix. The higher transparency value was in agreement with lower L^*-value and WI of films as oil level increased. Therefore, the incorporation of palm oil directly influenced transparency of resulting films.

Table 31. Color and transparency value of films from fish skin gelatin incorporated with palm oil at different levels.

Film samples	Oil levels (% based on protein content)	*L**	*a**	*b**	*ΔE**	WI	Transparency value
Control film	0	91.32 ± 0.01 [a]*	-1.19 ± 0.04 [a]	1.58 ± 0.08 [e]	1.88 ± 0.05 [e]	91.10 ± 0.03 [a]	1.16 ± 0.07 [d]*
Emulsion film	25	90.65 ± 0.05 [c]	-1.37 ± 0.28 [ab]	4.81 ± 0.32 [d]	4.87 ± 0.31 [d]	89.39 ± 0.21 [b]	3.48 ± 0.17 [c]
	50	90.89 ± 0.01 [b]	-1.40 ± 0.04 [b]	5.87 ± 0.06 [c]	5.74 ± 0.06 [c]	89.08 ± 0.04 [c]	6.22 ± 0.07 [b]
	75	90.48 ± 0.07 [d]	-1.41 ± 0.04 [b]	7.91 ± 0.13 [b]	7.54 ± 0.14 [b]	87.54 ± 0.13 [d]	6.29 ± 0.13 [b]
	100	90.36 ± 0.10 [e]	-1.23 ± 0.04 [ab]	8.96 ± 0.10 [a]	8.60 ± 0.10 [a]	86.78 ± 0.10 [e]	6.55 ± 0.08 [a]

* Mean ± SD (n=3).

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

7.4.2.5 Film morphology

SEM micrographs of the surface and freeze-fractured cross-section of gelatin films incorporated without and with palm oil at different levels are shown in Figure 26. The surface of control film (without incorporated palm oil) was smoother and more homogeneous than those of films incorporated with palm oil, regardless of concentration of palm oil. Films incorporated with palm oil at all levels had rougher surface than control film, especially when the highest level (100%) was used. Obviously, the small oil droplets were more concentrated at the top layer of film incorporated with palm oil at a level of 100%. However, this phenomenon did not take place when palm oil at lower levels was used. The results suggested that palm oil droplets with the large number plausibly floated towards the upper surface of film during film drying process. With the limited capacity of film matrix to hold those droplets, all oil droplets might not be able to insert themselves in the film network. As a result, some of them were expelled from film network and localized at the upper surface of film. However, the majority of palm oil was still remained and localized inside the film matrix. Those droplets with hydrophobic nature might serve as barrier for water vapor adsorption and migration as indicated by low WVP. It was noted that some crack or discontinuous zone was observed on the surface of the control film and that containing palm oil at the level of 25%. However, there was no crack on the surface of films incorporated with palm oil at the level of 50% up to 100%. The result suggested that the control film which had the highest protein-protein interaction might possibly possess the enhanced shrinkage of film network. On the other hand, when palm oil was incorporated, more loosen and flexible structure was formed, in which no crack of film network was obtained.

The cross-section of control film was smoother and more compact than those found in films incorporated with palm oil. This revealed that control film had more homogeneous or uniform structure than other films. When palm oil at different levels was incorporated into gelatin film, the differences in cross-section morphology were observed, in comparison with control film. It was noted that, films containing palm oil exhibited similar morphology, regardless of concentration of oil added. With the incorporation of palm oil at all levels, the cross-section of films became rougher

than the control film. The addition of palm oil into gelatin film more likely interrupted the alignment of protein molecules in film matrix, thereby lowering the protein-protein interaction and causing the roughness of film. However, there was no macroscopic separation of oil droplet as large agglomeration in protein matrix in the films incorporated with palm oil, except at high level (100% oil), where higher intensity of oil droplet was noticeable at the upper layer. The result indicated that films were prepared from the stable emulsion system. It was noted that there were some pores in the film, which could allow the moisture to migrate through the films. The high proportion of oil to protein might impair the internal three-dimensional structure of resulting films as shown by the presence of pore or cavity. This partially resulted in the weakened barrier property of films. However, those pores were embedded in the film matrix and the long distance between pores might not facilitate such a migration, especially in the presence of hydrophobic oil droplets distributed throughout the films.

Obviously, the increases in film thickness were observed at the microscopic level with increasing amount from 25% to 100%, basically due to higher solid content in the films. Film network was enlarged by the dispersion of oil droplets, thereby decreasing the compactness and cohesiveness of polymer chain-to-chain interactions. Additionally, palm oil with the low density preferably located at the near-surface of the air rather than the interior of the films, more likely due to the creaming during casting. This coincided with the slight increase in oil droplets in FFE as the storage time increased (Table 1). Those changes might enhance the water vapor barrier property of the resulting emulsion films.

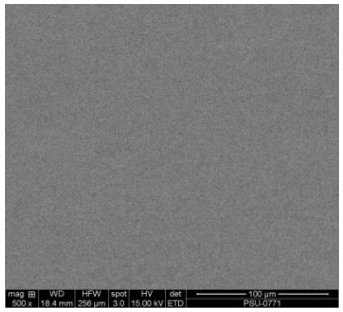
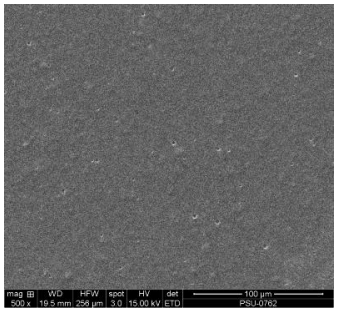
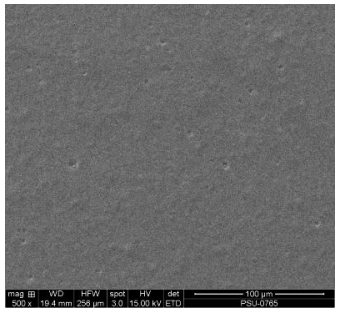
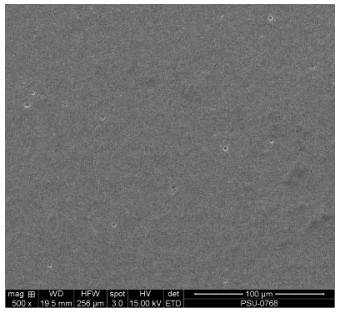
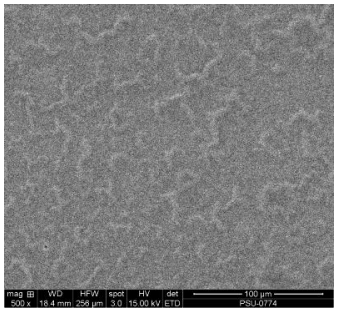
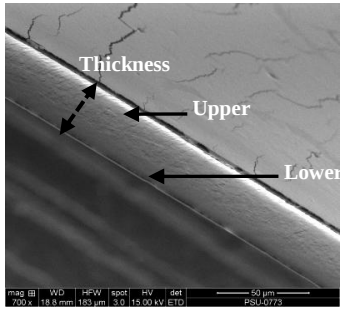
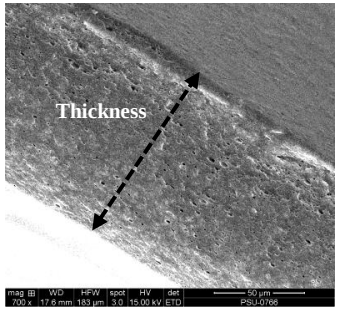
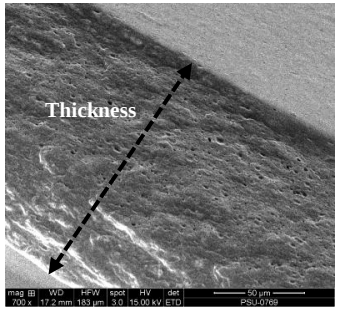
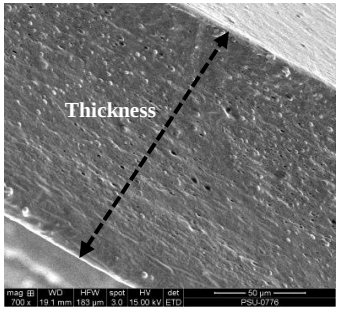
Position	Control (Without palm oil added)	Palm oil concentration (% based on protein content)			
		25	50	75	100
Surface					
Cross-section					

Figure 26. SEM micrographs of surface (500x) and cross-section (700x) of films from fish skin gelatin containing palm oil at different levels. In cross-sectional images, the upper surface represents the film surface exposed to the air during film casting.

7.4.2.6 Differential scanning calorimetry (DSC)

DSC thermograms of the 1st and 2nd-heating scans of gelatin films incorporated without and with palm oil at different levels are illustrated in Figure 27A and 27B, respectively. Glass transition temperature (T_g), melting transition temperature (T_{max}) and the melting enthalpy (ΔH) of all film samples are summarized in Table 32.

From thermogram of the 1st heating scan (from -30 to 120 ºC), the control film (without palm oil) showed two step-like transitions, indicating the glass transition temperature (T_g), and an endothermic melting transition (T_{max}). Thermal transitions indicate the changes in the physical state of material due to changes in temperature or pressure (Ghanbarzadeh and Oromiehi, 2008). In general, the first-order transitions in food materials are melting, crystallization, vaporization, condensation, sublimation, transitions between polymorphic states in fats, starch gelatinization, and protein denaturation (Aguilera and Stanley, 1999), and a second-order phase transition is a glass transition in amorphous or partially amorphous food materials (Kalichevsky *et al.*, 1992). For protein-based film, the glass transition is associated with molecular segmental motion of disordered (amorphous phase) structure which undergo 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 (crystalline phase) as well as changes from the native state of protein to denatured one, which were stabilized by various protein interactions during film formation (Tang *et al.*, 2009). T_g of the control gelatin film was found at temperature of 41.02 ºC, which was more likely associated with T_g of plasticized gelatin-rich phase. For gelatin-based films, different T_g was observed, depending upon gelatin sources, compositions of film and process used, e.g. 45.65 ºC for cuttlefish skin gelatin film (Hoque *et al.*, 2011b), 41.30 ºC for tilapia skin gelatin film (Tongnuanchan *et al.*, 2014), 99 ºC for cod gelatin film and 109 ºC for porcine gelatin (Staroszczyk *et al.*, 2012). In addition, it was reported that T_g was also found at very low temperature (> -20 ºC) in tilapia skin gelatin film (Tongnuanchan *et al.*, 2014). This T_g was more likely due to the Tg of glycerol-rich phase, in which T_g of pure glycerol were found at around -93 ºC (Pol *et al.*, 2002).

Generally, films containing palm oil had lower T_g than did the control film, irrespective of oil level. This result 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. Generally, T_g is increased by increasing the amount of chains stiffness and bonds, crosslinking between chains and the degree of crystallinity, whereas T_g is decreased as the amount of low molecular plasticizers is increased (Rogers, 1985). T_g of gelatin film was gradually decreased from 41.02 ºC (control film) to 40.52, 39.18, 36.77 and 35.85 ºC, when palm oil at the levels of 25, 50, 75 and 100% was incorporated, respectively. It was noted that the incorporation of palm oil shifted the T_g to lower temperature and T_g decreased with increasing amount of palm oil. Oil has been used as hydrophobic plasticizer in protein-based film (Sobral *et al.,* 2001). Palm oil droplets could interact with protein chains and simultaneously interrupt protein-protein interaction, thereby yielding structural relaxation. Plasticizer may depress the glass transition temperature by increasing polymer free volume and mobility of polymeric chains in film matrix (Sothornvit and Krochta, 2005). Additionally, water molecules in the films also function as a plasticizer, depressing T_g (Gontard and Ring, 1996). The decrease in T_g of cod skin gelatin and bovine skin gelatin films was demonstrated by the effect of plasticization, induced by the water molecules (Staroszczyk *et al.*, 2012).

For endothermic/melting transition, the control film showed endothermic peak with T_{max} of 117.43 ºC. This endothermic transition appeared 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 molecular structure. Gelatin chains could undergo partial renaturation during film formation process (Hoque *et al.*, 2011b). T_{max} was reported at various temperatures in gelatin-based films from different sources including bigeye snapper skin (53.14 - 96.42 ºC), brownstrip red snapper skin (59.89 - 100.28 ºC) (Jongjareonrak *et al.,* 2006a), cuttlefish skin (78.37 ºC) (Hoque *et al.*, 2011b), cod skin (161 ºC), pig skin (109 ºC) (Staroszczyk *et al.*, 2012). This was mainly dependent on amount of imino acid composition (proline and hydroxyproline) of gelatin, which directly determine thermal stability of protein via hydrogen bond (Sikorski *et al.*, 1984). The result

showed that melting/ordered-phase transition peak decreased in intensity when palm oil was incorporated, especially with increasing amount of palm oil, suggesting a decrease in ordered-phase structure in the film samples. T_{max} of control film was gradually shifted from 117.43 ºC to 117.77, 120.85, 123.18 and 123.52 ºC when palm oil at the levels of 25, 50, 75 and 100% were added, respectively. The shift of T_{max} to higher values might be associated with the lower water content in film (Guerrero and de la Caba, 2010). The control film exhibited the highest ΔH, compared with those incorporated with palm oil. ΔH of films were decreased from 11.76 J/g (control film) to 9.53, 6.91, 3.67 and 2.45 J/g upon the incorporation of palm oil at the levels of 25, 50, 75 and 100%, respectively. It was noted that the addition of palm oil lowered the enthalpy for disruption of the inter-chain interaction of resulting film. Gelatin underwent the formation of a three-dimensional network, in which zones of intermolecular microcrystalline junctions were developed (Arvanitoyannis, 2002). When palm oil droplets were dispersed in the gelatin film matrix, the weaker film structure was obtained, particularly with increasing oil amount. The weaker film structure had the lower thermal stability, which required a lower enthalpy for destroying the inter-molecular interaction. The number of order-phase fraction in the film matrix was evaluated from ΔH or the area under endothermic melting peak (Tongnuanchan *et al.*, 2014). This result reconfirmed that the fraction of ordered-phase structure became lowered with increasing amount of palm oil droplets. The lower renaturation of gelatin via recovered helical structure in films containing palm oil was postulated in comparison with the control film, which was mainly stabilized by hydrogen bonds. The presence of palm oil at higher levels directly influenced the conformation of gelatin molecule in the film via plasticizing effect as evidenced by lower ΔH value.

Furthermore, the thermograms of films incorporated with palm oil at all levels exhibited one more endothermic transition peak (T_{max1}), which was visible before glass transition. This endothermic transition peak was observed at temperature of -2.35, 0.17, 0.18 and 0.52 ºC for films incorporated with palm oil at the levels of 25, 50, 75 and 100%, respectively. 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. Endothermic transition of pure palm oil was found at 2.68 ºC. T_{max1} was generally shifted to higher temperature with increasing amount of palm oil. The result suggested that the higher content of palm oil could enhance the oil-rich phase, which might undergo the stronger ordered-phase fraction along with gelatin molecules. This result was in agreement with Ma *et al.* (2012a) who reported that bovine gelatin film incorporated with olive oil exhibited an additional endothermic peak at -8 ºC, which was more likely due to the melting peak of olive oil. ΔH of films containing palm oil at the levels of 25, 50, 75 and 100% were 2.61, 7.29, 8.97 and 12.49 J/g, respectively. It was noted that the ΔH of films containing palm oil increased with increasing amount of palm oil.

From the thermograms of the 2nd-heating scan (Figure 27B), 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. However, the endothermic peak, which related to gelatin-rich phase transition (T_{max2}), was not observed for all film samples, suggesting that gelatin was not able to rearrange themselves into ordered structure upon quench cooling during DSC scan. For the control gelatin film, the T_g of 41.02 ºC obtained in 1st-heating scan was shifted to 81.58 ºC in the 2nd-heating scan. Moreover, for films incorporated with palm oil at all levels, T_g of gelatin-rich phase was not clearly observed. These results were due to the rearrangement of gelatin molecules along with loss of adsorbed water was more enhanced after the first- heating scan. Thus, the incorporation of palm oil at different levels directly affected the thermal behavior of emulsion gelatin film.

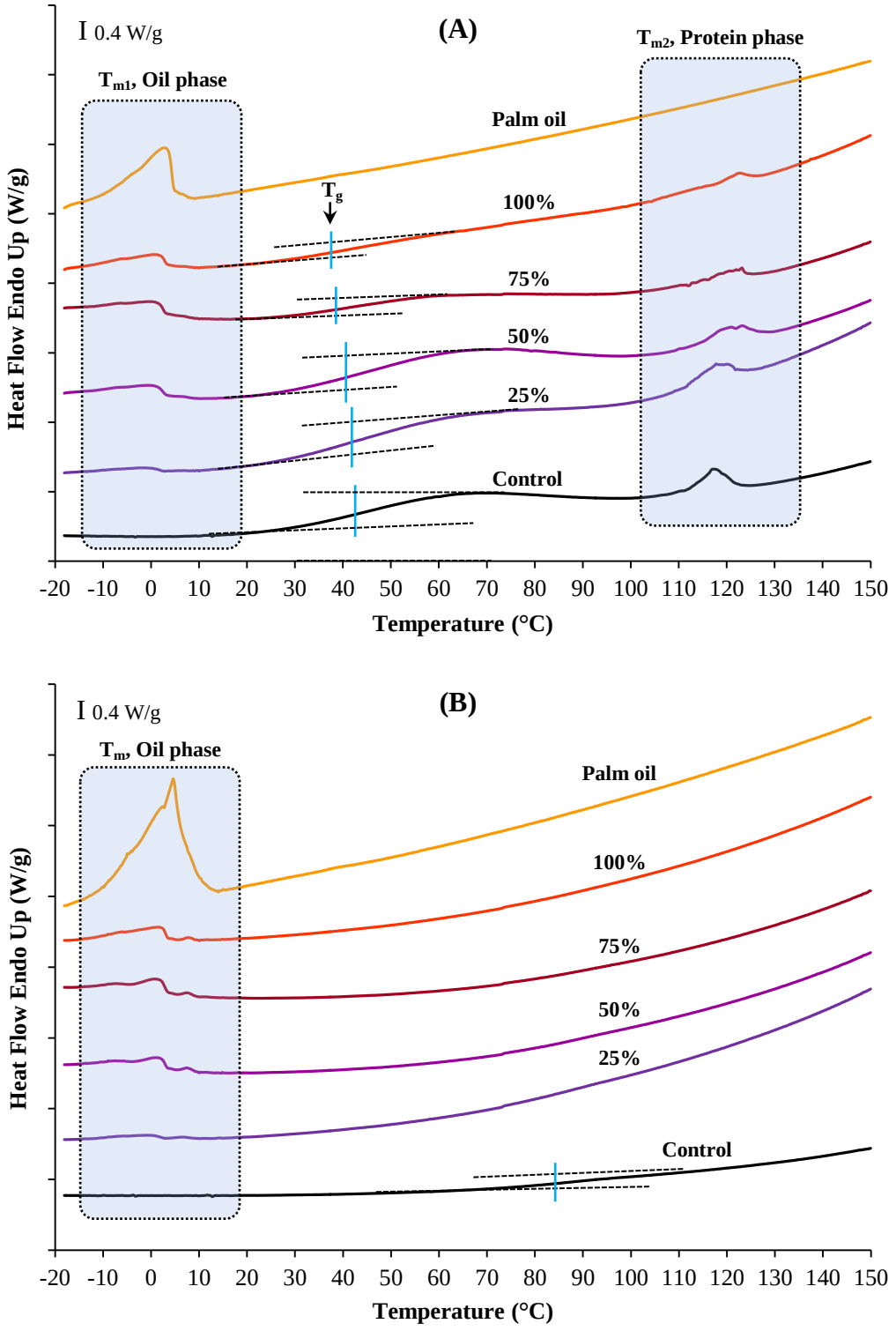


Figure 27. DSC thermograms of 1st-heating scan (A) and 2nd-heating scan (B) of films from fish skin gelatin containing palm oil at different levels. Control: without addition of palm oil and surfactant.

Table 32. Glass transition temperature (T_g), melting/order-phase transition temperature (T_{max}) and enthalpy (ΔH) of films from fish skin gelatin incorporated with palm oil at different levels.

Film Samples	Oil levels (based on protein content)	1st - Heating								
		Melting/order-phase transition								Glass 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)
Control film	0	-	-	-	-	113.19	117.43	122.20	11.76	41.02
Emulsion film	25	-12.93	-2.32	2.51	2.61	111.08	117.77	122.51	9.53	40.52
	50	-14.35	0.17	3.32	7.29	114.38	120.85	124.94	6.91	39.18
	75	-12.76	0.18	3.22	8.97	112.52	123.18	124.10	3.67	36.77
	100	-13.89	0.52	3.68	12.49	119.19	123.52	125.77	2.45	35.85
Palm oil		-8.41	2.68	4.93	32.49	-	-	-	-	-
Glycerol		-	-	-	-	-	-	-	-	-85.04
Film Samples	Oil levels (based on protein content)	2nd - Heating								Glass 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)
Control film	0	-	-	-	-	-	-	-	-	81.58
Emulsion film	25	-11.26	-0.96	2.76	2.39	-	-	-	-	-
	50	-11.89	1.02	3.49	6.15	-	-	-	-	-
	75	-10.08	1.10	3.53	8.79	-	-	-	-	-
	100	-14.03	1.52	3.58	11.95	-	-	-	-	-
Palm oil		-9.96	4.60	7.56	43.59	-	-	-	-	-
Glycerol		-	-	-	-	-	-	-	-	-81.13

Control: without addition of palm oil and surfactant.

7.4.2.7 Thermo-gravimetric analysis (TGA)

TGA thermograms revealing thermal degradation behavior of gelatin films incorporated with and without palm oil are illustrated in Figure 28. The degradation temperatures (T_d), weight loss (Δw) and residue of all film samples are presented in Table 33. Among all films, the control film (without palm oil added) exhibited three main stages of weight loss, while those incorporated with palm oil at all levels had four main stages of weight loss. For all films, the first stage weight loss (Δw_1 = 3.99-7.36 %) was observed over the temperature (T_{d1}) ranging from 26.47-34.70 ºC. The weight loss at this temperature range was more likely associated with the loss of free and bound water absorbed in the film. The similar result was found in gelatin-based film from several sources including cuttlefish skin gelatin (Hoque *et al.*, 2011b) and splendid squid skin gelatin (Nagarajan *et al.*, 2012). Additionally, films incorporated with palm oil had lower weight loss than the control film, but the weight loss became lowered with increasing amount of palm oil incorporated. This might be due to the lower amount of water in film matrix, associated with higher hydrophobicity of film. This result was also in agreement with decreased moisture content of films with increasing amount of palm oil added (Table 30).

The second stage of weight loss of films appeared approximately at the onset temperature of 180.47-200.79 ºC (T_{d2}) with Δw_2 of 10.18-20.98 %. Weight loss at this stage was mostly associated with loss of low molecular weight protein fraction and glycerol compounds (plasticizer) as well as structural bound water (Hoque *et al.*, 2011a). It was noted that T_{d2} and Δw_2 of films incorporated with palm oil at all levels were lower than the control film. Δw_2 of those films containing palm oil decreased with increasing amount of oil. This might be due to lower proportion of glycerol in film matrix when palm oil was incorporated. Hoque *et al.* (2011a) and Nagarajan *et al.* (2012) reported the T_{d2} in the range of 196.30-216.71 ºC and 191.58–211.52 ºC for cuttlefish skin gelatin film and squid skin gelatin film, respectively. However, this range of temperature was higher than the boiling point of glycerol (182 ºC) (Guerrero *et al.*, 2011), in which some kinds of interaction such as hydrogen bound could be formed between protein fractions and glycerol (Guerrero *et al.*, 2011). For the third stage of weight loss, Δw_3 of 34.89-70.36 % and T_{d3} of 301.29-334.48 ºC were

observed for all films incorporated without and with palm oil. This stage of weight loss was most likely associated with the degradation or decomposition of larger size or higher interacted protein fractions. Guerrero *et al.* (2011) reported that Δw_1, Δw_2 and Δw_3 were related with loss of free water, glycerol and degradation of major protein fractions in film matrix, respectively. The result indicated that the degradation of control film and films added with palm oil began at ≈180 °C. In general, the T_{d2} and T_{d3} for films incorporated with palm oil were lower than the control film but varied with concentrations of palm oil added. The results revealed that film incorporated with palm oil exhibited lower heat resistance than did the control film. Incorporation palm oil yielded a poorer film network, thereby lowering the inter/intra-molecular protein interaction in film matrix, leading to lower heat resistance of resulting films. Thus, the addition of palm oil exhibited a plasticizing effect on gelatin film, which was in accordance with the decreases in TS and EM but the increase in EAB, in comparison with control film (Table 29). Moreover, T_{d3} of films containing palm oil decreased with the concomitant increases in Δw_3 as the amount of palm oil increased, suggesting the decrease in heat resistance of films as the increasing amount of palm oil was added. Lower thermal stability can be governed by changes in the protein structure and provoked by the rupture of low energy intermolecular bonds stabilizing film network (Kaminska and Sionkowska, 1999). Nevertheless, T_{d3} was higher than the smoke point of palm oil (≈235 °C) (Guzman *et al.*, 2010), which possibly indicated that the loss of volatile compounds in palm oil, such as free fatty acids or short-chain degradation products of oxidation at high temperature possibly took place.

For the fourth stage of weight loss, Δw_4 of 4.02-12.16 % with T_{d4} of 566.61-693.17 ºC was obtained for films incorporated with palm oil. However, the fourth stage of weight loss (Δw_4) was not detectable for the control film. Films added with palm oil at different levels exhibited the fourth stage of weight loss, suggesting the loss of high temperature stable components. Moreover, the increases in T_{d4} and Δw_4 of films containing palm oil were observed with increasing amount of palm oil added.

In general, gelatin films incorporated with palm oil had lower residue mass (representing char content) 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 and EM (Table 29). As a consequence, the residue or char in films added with palm oil was found at a lower extent. This result suggested that the increased amount of palm oil could loosen film network, leading to higher degree of thermal degradation. Therefore, the addition of palm oil had the pronounced impact on thermal stability of gelatin film.

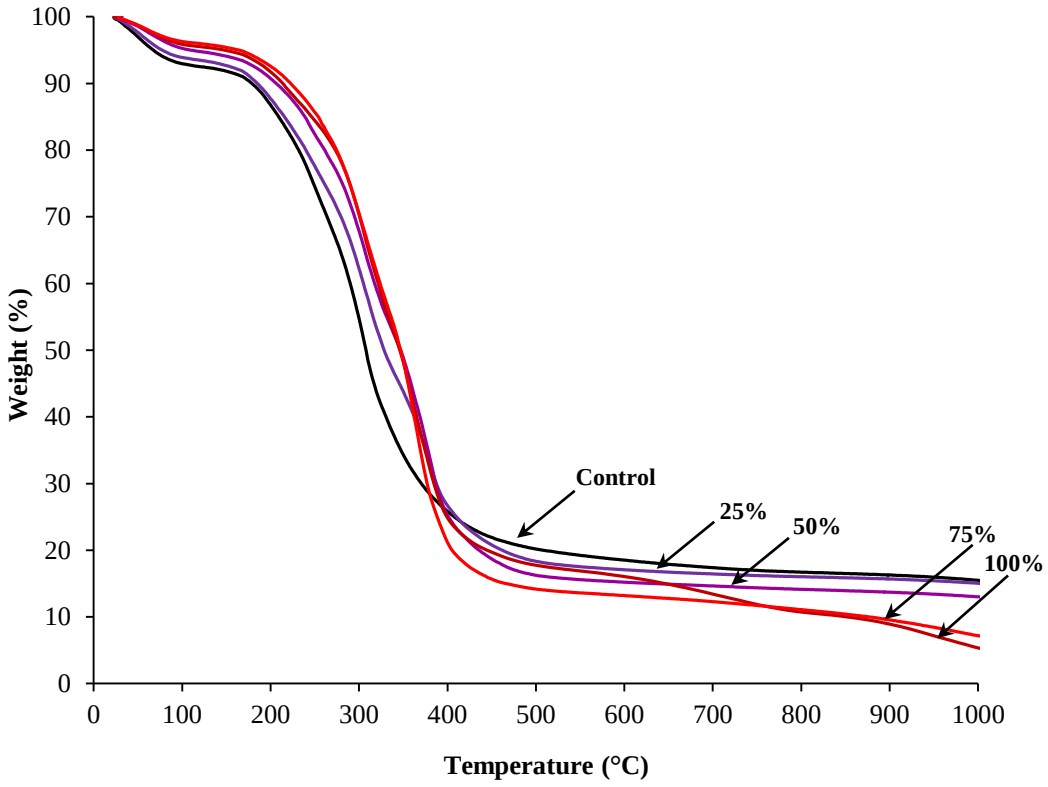


Figure 28. Thermo-gravimetric curves of films from fish skin gelatin containing palm oil at different levels. Control: without addition of palm oil and surfactant.

Table 33. Thermal degradation temperatures (T_d, ºC) and weight loss (Δw, %) of films from fish skin gelatin incorporated with palm oil at different levels.

Film Samples	Oil levels (based on protein content)	Δ_1		Δ_2		Δ_3		Δ_4		Residue (%)
		$Td_{1,onset}$	Δw_1	$Td_{2,onset}$	Δw_2	$Td_{3,onset}$	Δw_3	$Td_{4,onset}$	Δw_4	
Control film	0	26.47	7.36	200.74	20.98	287.49	52.50	-	-	19.16
Emulsion film	25	30.51	6.48	188.74	18.98	282.92	57.20	566.61	4.02	13.32
	50	34.70	5.28	189.27	13.88	282.11	65.43	569.94	4.19	11.22
	75	29.54	4.52	180.47	11.52	281.26	66.97	607.45	11.14	5.85
	100	30.16	3.99	186.43	10.18	275.92	72.37	693.17	12.16	1.30

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.

Control: without addition of palm oil and surfactant.

7.4.2.8 Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra of gelatin films incorporated with palm oil at different levels are illustrated in Figure 29. Generally, FTIR spectra of control film and film containing palm oil exhibited the similar major peaks but the amplitudes of peaks varied, depending on the level of palm oil incorporated. The peak situated at the wavenumber of 1034-1043 cm^{-1} was found in all 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 this peak decreased with increasing concentration of palm oil incorporated. Oil addition might show the dilution effect on glycerol in the resulting films. All films had the similar spectra in the range of 1700-700 cm^{-1}, covering amide-I, II and III bands. All films had the major bands at 1630 cm^{-1} (amide-I, illustrating C=O stretching/hydrogen bonding coupled with C–N stretch and CCN deformation), 1538 cm^{-1} (amide-II, presenting the bending vibrations of N-H groups and stretching vibrations of C-N groups) and 1237 cm^{-1} (amide-III, illustrating the vibrations in-plane of C-N and N-H groups of bound amide as well as absorptions arising from wagging vibrations from CH_2 groups from the glycine backbone and proline side-chains) (Muyonga *et al.*, 2004). This result was in agreement with Núñez-Flores *et al.* (2013) who reported the similar spectra of gelatin film from type A warm-water fish, where amide-I, II, and III bands were found at wavenumbers of 1631, 1534 and 1238 cm^{-1}, respectively. Nur Hanani *et al.* (2013) also reported the amide-I, II and III bands of gelatin-based film from beef skin at wavenumbers of 1629, 1540 and 1237 cm^{-1}, respectively. It was noted that the amplitude of peaks of amide-I, II, and III of films containing palm oil were lower than the control film, but those peaks had the decrease in amplitude when the increasing levels of palm oil were used. The results were simply due to the lower protein content of films containing palm oil.

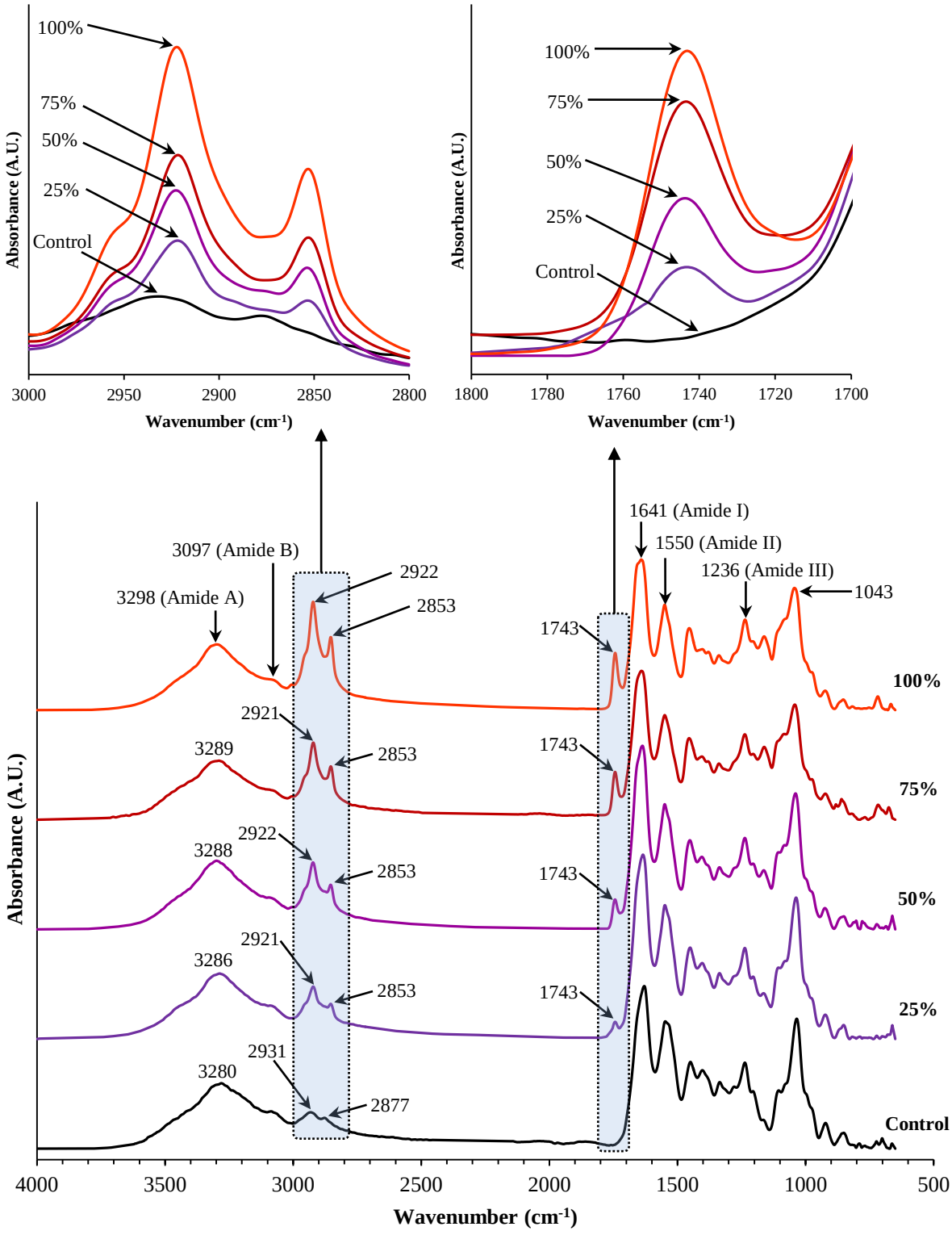


Figure 29. ATR-FTIR of films from fish skin gelatin containing palm oil at different levels. Control: without addition of palm oil and surfactant.

Amide-A and amide-B bands were observed at the wavenumber of 3280-3298 cm^{-1} and 3086-3097 cm^{-1} for all film samples, respectively. The amide-A band represents the NH-stretching coupled with hydrogen bonding and amide-B band illustrates NH-stretching vibration and asymmetric CH-stretching vibration at wavenumber of ~3300 and ~3100 cm^{-1}, respectively (Kong and Yu, 2007; Krimm and Bandekar, 1986). When palm oil was incorporated, amide-A and amide-B peaks of control film found at wavenumber of 3280 and 3086 cm^{-1}, respectively, were gradually shifted to higher wavenumbers as the concentration of palm oil increased. Wavenumbers of amide-A peak shifted to 3286, 3288, 3289 and 3297 cm^{-1} and amide-B peak shifted to 3289, 3293, 3297 and 3297 cm^{-1} for films incorporated with palm oil at levels of 25, 50, 75 and 100%, respectively. The changes in wavenumber and their amplitude peaks might be correlated with the interaction between the functional groups of protein and palm oil (Bahram *et al.*, 2014). Normally, the existing hydrogen bonding between protein molecules was evidenced by the shift of amide-A to the lower wavenumber (Xie *et al.*, 2006). As a result, the control film exhibited the lowest wavenumber of amide-A peak and the highest wavenumber was found in film containing palm oil at a level of 100%. This result revealed that the incorporation of palm oil could decrease protein-protein interaction. Furthermore, the amplitude of amide-A peaks decreased when being incorporated with palm oil at higher levels. Moreover, the amplitude of amide-B band of films also decreased with increasing amount of palm oil. The decrease in amplitude of amide-B peak suggested hydrophobic interaction between –CH of protein molecules and palm oil.

Moreover, the peaks at wavemunbers of 2931 cm^{-1} and 2877 cm^{-1} were observed in the control film. Those peaks were shifted to lower wavenumbers (2921-2922 cm^{-1} and 2853 cm^{-1}) when palm oil was incorporated into film matrix, respectively, regardless of oil concentration. Peaks at wavenumbers around 2853 cm^{-1} and 2924 cm^{-1} represent the methylene asymmetrical and symmetrical stretching vibration of the aliphatic C-H in CH_2 and CH_3 groups, respectively (Guillén and Cabo, 1997). Both methylene asymmetrical stretching bands at approximately 2853 cm^{-1} and methylene symmetrical stretching band near 2924 cm^{-1} were found in most lipids and hydrophobic substances (Guillén and Cabo, 2004). Both peaks had the increase in amplitude with increasing concentrations of palm oil. Furthermore, the

peak at wavenumber of 1743 cm^{-1} was observed in films containing palm oil at all levels. However, there was no peak at 1743 cm^{-1} found in the control film. The carbonyl absorption of triglyceride ester linkage was observed at 1746 cm^{-1} (Setiowaty *et al.*, 2000). For films incorporated with palm oil, the amplitude of stretching vibration peak assignable to the C=O group of triglycerides increased with increasing levels of palm oil. Palm-kernel oil contains high amount of triglyceride, composed of high saturated fatty acids and glycerol (Bezard, 1971). Valenzuela *et al.* (2013) also reported similar peak for quinoa protein–chitosan film incorporated sunflower oil, where carbonyl group (C=O) was observed at wavenumber of 1737 cm^{-1}.

7.5 Conclusion

Addition of palm oil had the impact on the properties of gelatin films. Oil droplets size of FFE increased during 12 h of storage. Decreases in TS and EM with the concomitant increase in EAB were observed, especially with increasing levels of palm oil. WVP of film was effectively improved with addition of palm oil. Nevertheless, the incorporation of palm oil directly affected colors and transparency of films. Films incorporated with palm oil at all levels tested and stabilized with soy lecithin exhibited a homogeneous morphological microstructure without bilayer form, excepted for those added with 100% oil, where oil droplets were preferably localized at the upper layer of film. The incorporation of palm oil had marked influence on the thermal property of gelatin film. Therefore, the use of hydrophobic substance such palm oil at an appropriate level could improve water vapor barrier properties of gelatin-based film, which could be potentially used as an alternative packaging to plastics.

7.6 References

Aguilera, G.M. and Stanley, D.W. 1999. Microstructural Principals of Food Processing and Engineering. 2nd Ed. Aspen Publishers. Gaithersburg, MD.

Andreuccetti, C., Carvalho, R. and Grosso, C.R.F. 2009. Effect of hydrophobic plasticizers on functional properties of gelatin-based films. Food Res. Int. 42: 1113-1121.

Arvanitoyannis, I.S. 2002. Formation and properties of collagen and gelatin films and coatings. *In* Protein-based films and coatings. (Gennadios, A., ed.). p. 275-304. CRC Press. Boca Raton.

ASTM. 1989. Standard test methods for water vapor transmission of materials. Standard designation E96-E80. *In* Annual book of ASTM standards. p. 730-739. ASTM. Philadelphia.

Bahram, S., Rezaei, M., Soltani, M., Kamali, A., Ojagh, S.M. and Abdollahi, M. 2014. Whey protein concentrate edible film activated with cinnamon essential oil. J. Food Process. Pres. 38: 1251-1258.

Baldwin, E.A., Nispero-Carriedo, M.O., Hagenmaier, R.D. and Baker, R.A. 1997. Use of lipids in coatings for food products. Food Technol. 51: 56-64.

Bergo, P. and Sobral, P.J.A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. Food Hydrocolloid. 21: 1285-1289.

Bezard, J. 1971. The component triglycerides of palm-kernel oil. Lipids. 6: 630-634.

Bonilla, J., Atarés, L., Vargas, M. and Chiralt, A. 2012. Effect of essential oils and homogenization conditions on properties of chitosan-based films. Food Hydrocolloid. 26: 9-16.

Choi, S.S. and Regenstein, J.M. 2000. Physicochemical and Sensory Characteristics of Fish Gelatin. J. Food Sci. 65: 194-199.

Dickinson, E. 2003. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocolloid. 17: 25-39.

Dickinson, E. 2009. Hydrocolloids as emulsifiers and emulsion stabilizers. Food Hydrocolloid. 23: 1473-1482.

Edem, D.O. 2002. Palm oil: Biochemical, physiological, nutritional, hematological and toxicological aspects: A review. Plant Food. Hum. Nutr. 57: 319-341.

Friberg, S., Larsson, K. and Sjoblom, J. 2004. Food emulsions. Marcel Dekker. New York.

Gennadios, A. and Weller, C.L. 1990. Edible films and coating from wheat and corn proteins. Food Technol. 44: 63-69.

Gennadios, A., Ghorpade, V.M., Weller, C.L. and Hanna, M.A. 1996. Heat curing of soy protein films. T. ASABE. 39: 575-579.

Ghanbarzadeh, B. and Oromiehi, A.R. 2008. Studies on glass transition temperature of mono and bilayer protein films plasticized by glycerol and olive oil. J. Appl. Polym. Sci. 109: 2848-2854.

Ghanbarzadeh, B., Almasi, H. and Entezami, A.A. 2010. Physical properties of edible modified starch/carboxymethyl cellulose films. Innov. Food Sci. Emerg. 11: 697-702.

Gontard, N., Duchez, C., Cuq, J.-L. and Guilbert, S. 1994. Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties. Int. J. Food Sci. Tech. 29: 39-50.

Gontard, N. and Ring, S. 1996. Edible wheat gluten film: influence of water content on glass transition temperature. J. Agr. Food Chem. 44: 3474-3478.

Guerrero, P. and de la Caba, K. 2010. Thermal and mechanical properties of soy protein films processed at different pH by compression. J. Food Eng. 100: 261-269.

Guerrero, P., Nur Hanani, Z.A., Kerry, J.P. and de la Caba, K. 2011. Characterization of soy protein-based films prepared with acids and oils by compression. J. Food Eng. 107: 41-49.

Guillén, M.D. and Cabo, N. 1997. Infrared spectroscopy in the study of edible oils and fats. J. Sci. Food Agr. 75: 1-11.

Guillén, M.D. and Cabo, N. 2004. Study of the effects of smoke flavourings on the oxidative stability of the lipids of pork adipose tissue by means of Fourier transform infrared spectroscopy. Meat Sci. 66: 647-657.

Guzman, A., Torres, J.E., Prada, L.P. and Nuñez, M.L. 2010. Hydroprocessing of crude palm oil at pilot plant scale. Catal. Today. 156: 38-43.

Han, J.H. and Floros, J.D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. J. Plast. Film Sheet. 13: 287-298.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011a. Effects of partial hydrolysis and plasticizer content on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. Food Hydrocolloid. 25: 82-90.

Hoque, M.S., Benjakul, S., Prodpran, T. and Songtipya, P. 2011b. Properties of blend film based on cuttlefish (*Sepia pharaonis*) skin gelatin and mungbean protein isolate. Int. J. Biol. Macromol. 49: 663-673.

Huck-Iriart, C., Pizones Ruiz-Henestrosa, V.M., Candal, R.J. and Herrera, M.L. 2013. Effect of aqueous phase composition on stability of sodium caseinate/sunflower oil emulsions. Food Bioprocess Tech. 6: 2406-2418.

Iwata, K., Ishizaki, S., Handa, A. and Tanaka, M. 2000. Preparation and characterization of edible films from fish water-soluble proteins. Fisheries Sci. 66: 372-378.

Jeirani, Z., Mohamed Jan, B., Si Ali, B., Noor, I.M., See, C.H. and Saphanuchart, W. 2013. Formulation, optimization and application of triglyceride microemulsion in enhanced oil recovery. Ind. Crop. Prod. 43: 6-14.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006a. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid. 20: 492-501.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006b. Fatty acids and their sucrose esters affect the properties of fish skin gelatin-based film. Eur. Food Res. Technol. 222: 650-657.

Kalichevsky, M.T., Jaroszkiewicz, E.M. and Blanshard, J.M.V. 1992. Glass transition of gluten. 1: Gluten and gluten—sugar mixtures. Int. J. Biol. Macromol. 14: 257-266.

Kaminska, A. and Sionkowska, A. 1999. The effect of UV radiation on the values of thermal parameters of collagen containing β-carotene. Polym. Degrad. Stabil. 65: 87-90.

Kong, J. and Yu, S. 2007. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Bioch. Bioph. Sin. 39: 549-559.

Krimm, S. and Bandekar, J. 1986. Vibrational spectroscopy and conformation of peptides, polypeptides, and proteins. Adv. Protein Chem. 38: 181−364.

Krochta, J.M. 2002. Protein as raw materials for films and coatings: definitions, current status, and opportunities. *In* Protein-Based Films and Coating (Gennadios, A., ed.). p. 1-39. CRC Press. New York.

Lacroix, M. and Cooksey, K. 2005. Edible films and coatings from animal-origin proteins. *In* Innovations in food packaging. Vol. 301-317. (Han, J. H., ed.). Elsevier Academic Press. San Diego, Calif.

Limpisophon, K., Tanaka, M. and Osako, K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionace glauca*) skin gelatin. Food Chem. 122: 1095-1101.

Ma, W., Tang, C.-H., Yin, S.-W., Yang, X.-Q., Qi, J.-R. and Xia, N. 2012a. Effect of homogenization conditions on properties of gelatin–olive oil composite films. J. Food Eng. 113: 136-142.

Ma, W., Tang, C.-H., Yin, S.-W., Yang, X.-Q., Wang, Q., Liu, F. and Wei, Z.-H. 2012b. Characterization of gelatin-based edible films incorporated with olive oil. Food Res. Int. 49: 572-579.

Manorama, R. and Rukmini, C. 1992. Crude palm oil as a source of beta-carotene. Nutr. Res. 12: 223-232.

McClements, D.J. 2005. Food emulsions: Principles, practice and techniques. 2nd Ed. CRC Press. Florida.

Muyonga, J.H., Cole, C.G.B. and Duodu, K.G. 2004. Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chem. 85: 81-89.

Nagarajan, M., Benjakul, S., Prodpran, T. and Songtipya, P. 2012. Properties of film from splendid squid (*Loligo formosana*) skin gelatin with various extraction temperatures. Int. J. Biol. Macromol. 51: 489-496.

Núñez-Flores, R., Giménez, B., Fernández-Martín, F., López-Caballero, M.E., Montero, M.P. and Gómez-Guillén, M.C. 2013. Physical and functional characterization of active fish gelatin films incorporated with lignin. Food Hydrocolloid. 30: 163-172.

Nur Hanani, Z.A., McNamara, J., Roos, Y.H. and Kerry, J.P. 2013. Effect of plasticizer content on the functional properties of extruded gelatin-based composite films. Food Hydrocolloid. 31: 264-269.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009. Characterization of porcine plasma protein-based films as affected by pretreatment and cross-linking agents. Int. J. Biol. Macromol. 44: 143-148.

Palazolo, G.G., Sobral, P.A. and Wagner, J.R. 2011. Freeze-thaw stability of oil-in-water emulsions prepared with native and thermally-denatured soybean isolates. Food Hydrocolloid. 25: 398-409.

Phan, T.T., Attaphong, C. and Sabatini, D.A. 2011. Effect of extended surfactant structure on interfacial tension and microemulsion formation with triglycerides. J. Am. Soc. 88: 1223-1228.

Pol, H., Dawson, P., Acton, J. and Ogale, A. 2002. Soy protein isolate/corn-zein laminated films: transport and mechanical properties. J. Food Sci. 67: 212-217.

Prodpran, T., Benjakul, S. and Artharn, A. 2007. Properties and microstructure of protein-based film from round scad (*Decapterus maruadsi*) muscle as affected by palm oil and chitosan incorporation. Int. J. Biol. Macromol. 41: 605-614.

Rogers, C.E. 1985. Permeation of gases and vapors in polymers. *In* Polymer Permeability. (Comyn, J., ed.). p. 11-73. Elsevier Applied Science. London, U.K.

Setiowaty, G., Che Man, Y.B., Jinap, S. and Moh, M.H. 2000. Quantitative determination of peroxide value in thermally oxidized palm olein by Fourier transform infrared spectroscopy. Phytochem. Analysis. 11: 74-78.

Shiku, Y., Hamaguchi, P.Y., Benjakul, S., Visessanguan, W. and Tanaka, M. 2004. Effect of surimi quality on properties of edible films based on Alaska pollack. Food Chem. 86: 493-499.

Sikorski, Z.E., Scott, D.N., Buisson, D.H. and Love, R.M. 1984. The role of collagen in the quality and processing of fish. Cr. Rev. Food Sci. 20: 301-343.

Sobral, P.J.A., Menegalli, F.C., Hubinger, M.D. and Roques, M.A. 2001. Mechanical, water vapor barrier and thermal properties of gelatin based edible films. Food Hydrocolloid. 15: 423-432.

Sothornvit, R. and Krochta, J.M. 2005. Plasticizers in edible films and coatings. *In* Innovations in food packaging. Vol. 403-433. (Han JH, ed.). Elsevier Academic Press. San Diego, Calif.

Staroszczyk, H., Pielichowska, J., Sztuka, K., Stangret, J. and Kołodziejska, I. 2012. Molecular and structural characteristics of cod gelatin films modified with EDC and TGase. Food Chem. 130: 335-343.

Tang, C.-H., Xiao, M.-L., Chen, Z., Yang, X.-Q. and Yin, S.-W. 2009. Properties of cast films of vicilin-rich protein isolates from *Phaseolus* legumes: Influence of heat curing. LWT - Food Sci. Technol. 42: 1659-1666.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2013a. Physico-chemical properties, morphology and antioxidant activity of film from fish skin gelatin incorporated with root essential oils. J. Food Eng. 117: 350-360.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2013b. Characteristics and antioxidant activity of leaf essential oil–incorporated fish gelatin films as affected by surfactants. Int. J. Food Sci. Tech. 48: 2143–2149.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2014. Structural, morphological and thermal behaviour characterisations of fish gelatin film incorporated with basil and citronella essential oils as affected by surfactants. Food Hydrocolloid. 41: 33-43.

Valenzuela, C., Abugoch, L. and Tapia, C. 2013. Quinoa protein–chitosan–sunflower oil edible film: Mechanical, barrier and structural properties. LWT - Food Sci. Technol. 50: 531-537.

Vanin, F.M., Sobral, P.J.A., Menegalli, F.C., Carvalho, R.A. and Habitante, A.M.Q.B. 2005. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. Food Hydrocolloid. 19: 899-907.

Xie, Y.-L., Zhou, H.-M. and Qian, H.-F. 2006. Effect of addition of peach gum on physicochemical properties of gelatin-based microcapsule. J. Food Biochem. 30: 302-312.

CHAPTER 8

MECHANICAL, THERMAL AND HEAT SEALING PROPERTIES OF FISH SKIN GELATIN FILM CONTAINING PALM OIL AND BASIL ESSENTIAL OIL WITH DIFFERENT SURFACTANTS

8.1 Abstract

Fish skin gelatin films plasticized with glycerol and incorporated with basil essential oil, palm oil or a mixture of basil essential oil/palm oil (1:1), in the presence of different surfactants (soy lecithin and Tween-20) were characterized. Films incorporated with various oils had lower tensile strength (TS) and elastic modulus (EM) with the higher elongation at break (EAB) in comparison with the control film (without oil and surfactant incorporated) ($p < 0.05$). TS and EM of films varied with oils and surfactants used. Films prepared with Tween-20 as surfactant showed higher EAB than those added with soy lecithin, regardless of oil types ($p < 0.05$). DSC analysis revealed that the incorporation of oils resulted in gelatin-based films with the decreases in both glass transition temperature (T_g) and extent of ordered-phase. All films added with different oils and surfactants had lower seal strength than the control film ($p < 0.05$). Film containing palm oil exhibited the higher seal strength and seal efficiency than those with basil essential oil or the mixture ($p < 0.05$), regardless of surfactants. Tween-20 yielded the film with the higher heat sealability than soy lecithin ($p < 0.05$). Good quality seal (complete fusion) was observed via SEM in control film and films containing various oils using Tween-20 as surfactant. Therefore, both oil and surfactants could affect the mechanical properties, thermal properties as well as heat sealability of resulting films.

8.2 Introduction

The development of biodegradable films from bio-based materials has gained increasing interest since the commercial plastic packaging produced from petrochemical products are associated with environmental pollution and serious ecological problems (Arvanitoyannis, 1999). Therefore, biodegradable or edible films with environmentally friendly aspect can overcome those problems and can be used as

alternative packaging to synthetic polymer packaging films. Proteins from various sources have been used as packaging material due to good film formation as well as excellent barrier properties against gas and volatile compounds, oils and UV light (Cho *et al.*, 2007; Krochta, 2002). Gelatin is produced by partial hydrolysis or thermal degradation of collagen, and is mainly extracted from bones, and connective tissues of mammalian sources. Currently, marine gelatin sources particularly from fish skin have been focused as alternative to mammalian counterpart, which can be used without religious constraint (Aewsiri *et al.*, 2009; Jongjareonrak *et al.*, 2010; Rouhi *et al.*, 2013; Yi *et al.*, 2006). Gelatin from fish skin has been used for preparing biodegradable or edible films. However, gelatin films have poor water vapor barrier properties (Hoque *et al.*, 2011; Núñez-Flores *et al.*, 2013; Rattaya *et al.*, 2009; Tongnuanchan *et al.*, 2012).

Gelatin/lipid emulsion film has been developed to enhance the water vapor barrier properties of gelatin-based film (Cao *et al.*, 2009; Sztuka and Kołodziejska, 2009; Tongnuanchan *et al.*, 2015). Lipids from several types and sources have been used to incorporate in protein-based film (Atarés *et al.*, 2010; Bahram *et al.*, 2014; Ma *et al.*, 2012; Valenzuela *et al.*, 2013). Essential oils with antimicrobial and antioxidant properties, e.g basil essential oil, could be added into gelatin film to lower water vapor permeability (Atarés *et al.*, 2010; Tongnuanchan *et al.*, 2014b). Due to the high price of essential oil and strong smell, its application is limited. Therefore, the use of cheap hydrophobic materials such as palm oil or their mixtures could pave the way for property improvement with reasonable price. Additionally, surfactant is an important parameter for stabilizing the emulsion during film formation, in which emulsion without macroscopic separation between oil phase and protein phase is generally required for obtaining emulsion films with good properties (Tongnuanchan *et al.*, 2014a).

Sealing ability is one of important characteristics for the application of those films as starting material for making sachets, pouches or bags to package liquids or dry food ingredients. Basically, the seal strength of material must be strong enough to keep the products (liquids or solids) inside the package without leakage during handling or storage (Kim and Ustunol, 2001). In general, heat sealing is widely used for joint polymer films in the packaging industry (Dodin, 1981; Meka and Stehling,

1994). Heat sealing properties of polymers depend on surface chemistry of the materials (Allen, 1987). Heat sealing condition such as temperature, pressure, heating time and cooling time are important parameters which directly impact on seal strength (Theller, 1989). However, no information on the heat sealing properties of emulsion film based on fish skin gelatin film has been reported. Thus, the objective of this investigation was to determine the mechanical and thermal properties as well as heat seal ability of emulsion gelatin-based film.

8.3 Materials and Methods

8.3.1 Chemicals

Glycerol was obtained from Merck (Darmstadt, Germany). Tween-20 (HLB = 16.7) and soy lecithin (l-α-phosphatidylcholine, HLB = 4.0) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade.

8.3.2 Fish gelatin and palm oil

Fish gelatin produced from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Palm oil was obtained from Oleen Co., Ltd. (Samutsakorn, Thailand). Basil essential oil (*Ocimum basilicum*) was purchased from *Botanicessence* (Bangkok, Thailand).

8.3.3 Preparation of film from fish gelatin incorporated with different oils and surfactants

8.3.3.1 Preparation of film forming emulsion

Gelatin powder was mixed with distilled water to obtain the protein concentration of 3.5 % (w/v). The mixture was heated at 70 °C for 30 min. Glycerol at 30 % (w/w) of protein content was used as a plasticizer. The obtained solution was referred to as film-forming solution (FFS). To prepare emulsion film, palm oil, basil essential oil and their mixture (ratio 1:1) previously mixed with different surfactants (Tween-20 and soy lecithin) at 25 % (w/w, based on oil) were added into FFS at levels of 50 % (w/w, based on protein content). The film-forming emulsion (FFE) was homogenized at 22,000 rpm for 3 min using a homogenizer (IKA Labortechnik

homogenizer, Selangor, Malaysia). The dissolved air in FFE was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature (28 – 30 °C).

8.3.3.2 Preparation of film

To prepare the films, the FFE (4 g) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown for 12 h (27 ± 2 °C and 75 ± 10 % relative humidity (RH)). The films were further dried at 25 °C and 50 ± 5 % RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). Control film was prepared from the FFS containing gelatin and glycerol without palm oil and surfactant. The resulting films were manually peeled off and subjected to analyses.

8.3.3.3 Determination of film properties

Prior to determination of film thickness, mechanical properties, seal strength and seal efficiency, films were conditioned for 48 h at 50 ± 5 % relative humidity (RH) and 25 ± 0.5 °C. For the rest of testing, films were conditioned in a desiccator containing dried silica gel for 2 weeks, followed by placing for 1 week in a desiccator containing P_2O_5 at room temperature (25 - 30 °C) to obtain the most dehydrated films.

8.3.3.3.1 Film thickness

The thickness of films 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.

8.3.3.3.2 Mechanical properties

Tensile strength (TS), elastic modulus (EM) 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 film 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.

8.3.3.3.3 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 -40 to 150 °C, with a heating rate of 5 °C/min. Dry ice was used as a cooling medium and the system was equilibrated at -40 °C for 5 min prior to the scan. An empty aluminium pan was used as the reference. The maximum transition temperature was estimated from the endothermic peak of the DSC thermogram and transition enthalpy was determined from the area under the endothermic peak. A second scan was also performed in the same manner, followed by quench-cooling of the sample after completing the first scan. Glass transition temperatures (T_g) of palm oils and glycerol were also evaluated over the temperature range of -150 to 150 °C using liquid nitrogen as a cooling medium and a heating rate of 5 °C/min.

8.3.3.3.4 Seal strength and seal efficiency

Film samples were cut into strips of 25 x 20 mm^2. One strip was placed on the top of another (Figure 30). Those two strips were heat-sealed using impulse sealer with magnet Model ME-300HIM (S.N.MARK Ltd., Park, Nonthaburi, Thailand) at 150 ± 0.5 ºC for 1.00, 1.25 and 1.50 s of heating time and 1.50 s of cooling time. The width of seal area was 2 mm.

All sealed film samples were conditioned at 25 ± 0.5 °C and 50 ± 5 % relative humidity (RH) for 48 h before testing seal strength. The heat-seal strength was estimated using the peel and the lap-shear tests. The peel strength, lap-shear strength and seal efficiency of the heat-sealed films were determined according to Standard ASTM F-88 (ASTM, 2001) with some modifications, using Universal Testing Machine (Lloyd Instruments, Hampshire, UK), at 25 ± 0.5 °C and 50 ± 5 % RH. Each leg of the sealed film was clamped to the machine, with each end of the sealed film was held perpendicularly to the direction of the pull. The distance between the clamps was 50 mm. A 100 N static load cell and crosshead speed of 30 mm/min

were used. The maximum force required to cause seal failure was reported as seal strength in newtons/meter (N/m). Seal strength and seal efficiency of some synthetic films including polypropylene (PP), low-density polyethylene (LDPE) and nylon/low-density polyethylene (Nylon/LDPE) were also determined as for comparison. Ten specimens of each sample were used for testing. Seal strength and seal efficiency were calculated as follows:

Seal strength = Peak force/film width

Seal efficiency (%) = (Peak force/tensile force) x100

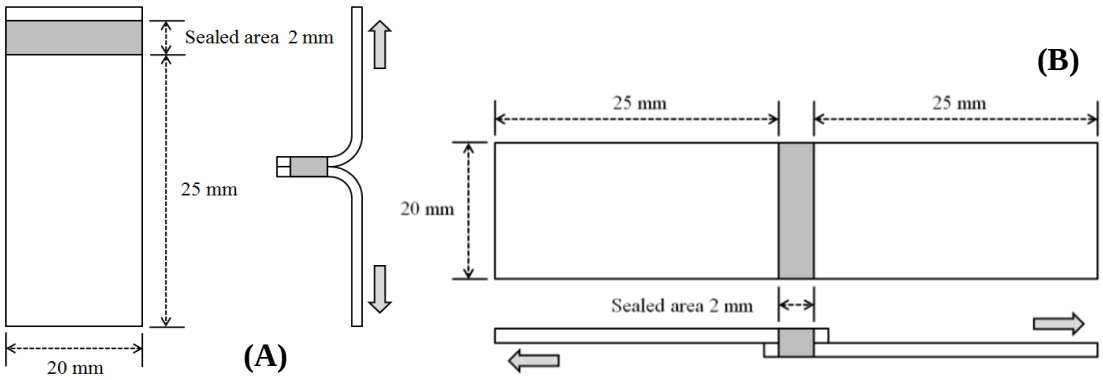


Figure 30. Simplified illustration for the testing direction and the test specimen dimensions for peel test (A) and lab-shear test (B).

8.3.3.3.5 Scanning electron microscopy (SEM)

Morphology of cross-section of the heat sealing areas of film samples were visualized using a scanning electron microscope (SEM) (Quanta 400, FEI, Eindhoven, the Netherlands). For cross-section of film, 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.

8.3.4 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, SPSS Inc., Chicago, IL, USA).

8.4 Results and Discussion

8.4.1 Film appearance and thickness

Photographs of gelatin films incorporated without and with basil essential oil, palm oil and oil mixture using Tween-20 as surfactant are shown in Figure 31. When all types of oils were incorporated in gelatin film, films became more opaque, compared with the control film. Film containing palm oil had the highest opaqueness, followed by those added with oil mixture and basil essential oil, respectively. Thus, transparency and color of films varied, depending on types of oils incorporated.

Thickness of emulsion films based on fish skin gelatin incorporated with basil essential oil, palm oil, and oil mixture (1:1) is shown in Table 34. Regardless of types of surfactants used, all emulsion films had higher thickness than the control film (without oils and surfactants incorporated) ($p < 0.05$). Among all emulsion films, those incorporated with palm oil had the highest thickness ($p < 0.05$), followed by those added with oil mixture and basil essential oil, respectively, irrespective of surfactants. The increase in film thickness was plausibly associated with the higher solid content via oil addition. This result also suggested that the alignment of gelatin molecule in film matrix was formed with lower degree of compactness, when all types of oils were incorporated. Those oil droplets were dispersed and localized in the protein film network, thereby lowering the interaction between protein molecules. As a result, the protruded film network was formed as indicated by the increased film thickness. When the same type of oil was used, films prepared using soy lecithin as surfactant had higher thickness than those added with Tween-20 ($p < 0.05$), excepted for films incorporated with palm oil, which had no difference in thickness ($p > 0.05$). The bulky structure of soy lecithin might impede

the ordered alignment of gelatin chain and the higher protrusion of film network was obtained. Therefore, different types of oils and surfactants directly affected the alignment between peptide chains in film network and thus the thickness of resulting films.

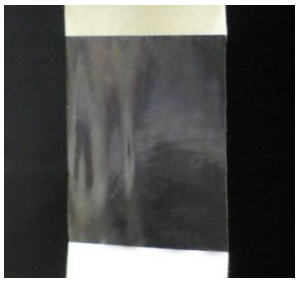
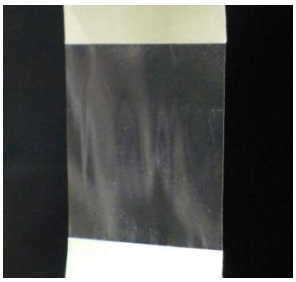
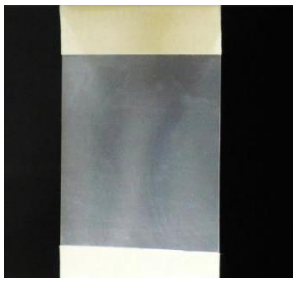
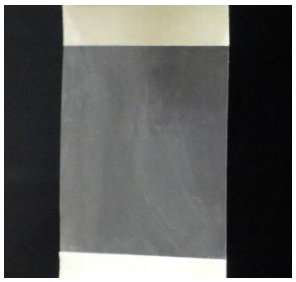
Control (Without oil added)	Oils		
	Basil essential oil	Palm oil	Mixture
			

Figure 31. Photographs of films from fish skin gelatin containing basil essential oil, palm oil and oil mixture in the presence of Tween-20 as surfactant. Black background was used.

8.4.2 Mechanical properties

Mechanical properties expressed as tensile strength (TS), elastic modulus (EM) and elongation at break (EAB) of fish skin gelatin films incorporated with various oils in the presence of different surfactants are shown in Table 34. Films incorporated with various oils exhibited the lower TS and EM than the control film (without incorporated oils and surfactants), irrespective of surfactants used ($p < 0.05$). For emulsified films prepared using soy lecithin as surfactant, that incorporated with oil mixture showed the highest TS ($p < 0.05$), followed by those added with basil essential oil and palm oil, respectively. However, film incorporated with palm oil showed the highest TS ($p < 0.05$), followed by those containing oil mixture and basil essential oil, respectively, when Tween-20 was used as surfactant. When comparing the effect of surfactant on TS of resulting film, film prepared using soy lecithin had the lower TS than that containing Tween-20 when palm oil was incorporated ($p < 0.05$). Nevertheless, films incorporated with basil essential oil and oil mixture had no difference in TS between those containing Tween-20 and soy lecithin ($p > 0.05$). Moreover, film prepared with soy lecithin as surfactant showed the highest EM when

basil essential oil was used and had the lowest EM when palm oil and oil mixture were present ($p < 0.05$). No difference in EM was observed between films containing different oils when Tween-20 was used as surfactant ($p > 0.05$). When palm oil and oil mixture were incorporated, no difference in EM was observed between films having soy lecithin and Tween-20 ($p > 0.05$), excepted for those added with basil essential oil ($p < 0.05$). The decreases in both TS and EM reflected the reduced interaction between gelatin molecules. As a result, the looser network of film was developed. It was noted that various oils and surfactants affected TS and EM of resulting films differently. In general, films incorporated with various oils and surfactants had much higher EAB than did the control film ($p < 0.05$). Tween-20 rendered the films with higher EAB than soy lecithin ($p < 0.05$), regardless of oil types. In general, the higher EAB reflected the increased flexibility. The increased EAB of gelatin film containing various oils was more likely caused by the lower interaction between protein chains in film matrix. Hydrogen bond and hydrophobic interaction were reported as the main associative forces in the gelatin film network (Hoque *et al.*, 2010; Park *et al.*, 2014). Furthermore, the different hydrophobic-lipophilic balance (HLB) between both surfactants might determine the stabilizing effect of oil droplets from various sources differently. HLB values of soy lecithin and Tween-20 are 4.0 and 16.7, respectively. As a result, both soy lecithin and Tween-20 could maintain the emulsion in different fashions. Oil droplets in FFE were able to insert themselves between gelatin chains, thereby lowering the intra and intermolecular bondings as indicated by lowered TS and EM. The result suggested that the addition of basil essential oil, palm oil or mixed oil exhibited the plasticizing effect in resulting film to some extent.

Films incorporated with hydrophobic substances generally had the decreases in TS and EM with the concomitant increase in EAB (Andreuccetti *et al.*, 2010; Ma *et al.*, 2012). Various oils incorporated in film matrix could increase the free volume between protein molecules and provide the greater mobility, thereby enhancing the higher extensibility of resulting films (Sothornvit and Krochta, 2001). Nevertheless, different chemical compositions of basil essential oil and palm oil might affect the bondings or interactions with protein molecules in film matrix, in which the varying mechanical properties of emulsion films were observed.

Table 34. Mechanical properties and thickness of films from fish skin gelatin incorporated with different oils in the presence of soy lecithin and Tween-20 as surfactants.

Film samples	Surfactants	Oils	TS (MPa)	EM (MPa)	EAB (%)	Thickness (mm)
Control film	Without surfactant	Without oil	32.91 ± 0.55 ^{a,w}*	933.80 ± 20.94 ^{a,w}	35.56 ± 7.60 ^{c,z}	0.054 ± 0.003 ^{d,z}
Emulsion films	Soy lecithin	Basil essential oil	14.11 ± 1.12 ^{c,A}	320.83 ± 24.84 ^{b,A}	94.20 ± 7.79 ^{b,B}	0.073 ± 0.003 ^{c,A}
		Palm oil	11.95 ± 0.81 ^{d,B}	281.78 ± 18.59 ^{c,A}	104.95 ± 2.67 ^{b,B}	0.101 ± 0.004 ^{a,A}
		Mixture	16.28 ± 1.74 ^{b,A}	269.58 ± 20.75 ^{c,A}	124.04 ± 16.72 ^{a,B}	0.086 ± 0.003 ^{b,A}
	Tween-20	Basil essential oil	14.66 ± 0.51 ^{z,A}	250.04 ± 10.22 ^{x,B}	127.16 ± 8.26 ^{y,A}	0.060 ± 0.003 ^{y,B}
		Palm oil	21.81 ± 1.49 ^{x,A}	273.07 ± 11.82 ^{x,A}	143.19 ± 10.74 ^{x,A}	0.102 ± 0.003 ^{w,A}
		Mixture	16.62 ± 1.26 ^{y,A}	263.25 ± 21.66 ^{x,A}	158.51 ± 14.51 ^{w,A}	0.083 ± 0.002 ^{x,B}
Thermoplastic films	Single-layer	PP	40.96 ± 3.18	834.87 ± 14.59	720.90 ± 9.99	0.033 ± 0.000
		LDPE	32.33 ± 2.67	242.53 ± 14.59	454.32 ± 9.86	0.040 ± 0.000
	Multi-layer	Nylon/LDPE	30.73 ± 1.15	613.74 ± 18.09	96.86 ± 12.24	0.066 ± 0.000

* Mean ± SD (n=3).

Different letters (abc) in the same column of control film and films prepared with soy lecithin indicate significant differences ($p < 0.05$). Different letters (wxy) in the same column of control film and films prepared with Tween 20 indicate significant differences ($p < 0.05$). Different capital letters (ABC) in the same column under the same oil indicate significant differences ($p < 0.05$).

PP = Polypropylene; LDPE = low-density polyethylene; Nylon/LDPE = nylon /low-density polyethylene

8.4.3 Thermal properties

DSC thermograms of the 1st and 2nd-heating scans of gelatin films incorporated without and with basil essential oil, palm oil and oil mixture (1:1) using soy lecithin and Tween-20 as surfactants are illustrated in Figure 32 and 33, respectively. Table 35 shows the melting transition temperature (T_{max}), melting enthalpy (ΔH) and the glass transition temperature (T_g) of those film samples. Changes in thermal transition (heat absorbed or released) of film samples were determined by DSC.

From thermogram of the 1st heating scan (from -20 to 150 ºC), all film samples exhibited a step-like transition, indicating the glass transition temperature (T_g) and an endothermic melting transition (T_{max}). Generally, glass transition of a polymer is associated with the molecular mobility of the polymer chains, particularly the amorphous materials or in amorphous regions (Fernández-García *et al.*, 2000). This transition is an important phenomenon, which influences its material properties and potential applications. Glass transition is a primary relaxation due to the initiation of the molecular motions. T_g of the control film (without incorporated oils and surfactants) was observed at temperature of 49.77 ºC, which was likely associated with T_g of plasticized gelatin-rich phase. Similar T_g values were observed in pig skin gelatin films plasticized with glycerol (41.4 ºC) and ethylene glycol (39.9 ºC) (30 g/100 g gelatin), while films plasticized with other plasticizers (propylene glycol and diethylene glycol) exhibited different T_g values (61.9 ºC and 13.4 ºC, respectively), in which the values decreased with increasing amount of plasticizer (Vanin *et al.*, 2005). It was reported that the T_g of kafirin protein film plasticized with glycerol decreased when higher amount glycerol was incorporated as measured by both DSC and DMTA (Gao *et al.*, 2006). The addition of plasticizer, particularly with increasing level, increases the motion of protein chains in film matrix as well as changes in the protein formation (Gao *et al.*, 2006). However, no phase separation between glycerol and gelatin occurred. The lower value of T_g of plasticizer-rich phase compared to that of plasticized protein-rich phase was reported for various plasticized protein-based films. Those films included sorbitol-plasticized bovine hide gelatin (45-65 g/100 g gelatin)

and pigskin gelatin (35-65 g/100 g gelatin) (Sobral *et al.*, 2001) and glycerol-plasticized kafirin protein film (40 g/100 g gelatin) (Gao *et al.*, 2006).

Films incorporated with various oils, regardless of surfactant types used, had lower T_g, compared with the control film (Table 35). With the incorporation of oils, the inflexion of the base lines became broader, caused by glass transition and longer distance from melting transition. This result suggested that the higher plasticizing effect was formed when various types of oils were incorporated. In general, hydrophobic substances such as lipids (fats and oils) could act as plasticizer in protein-based films, which disrupted intra and intermolecular interactions of protein, leading to the increased chain mobility of gelatin in film matrix (Sothornvit and Krochta, 2005). Thus, the loosen structure of emulsion film was formed. Normally, the reduction of T_g indicates plasticization of polymer molecules, while the shift of T_g to higher temperature indicates anti-plasticization by chain stiffness or ordering of polymer molecules (Rahman *et al.*, 2008). As a result, the decrease in T_g might be associated with the breaking restriction of chain movement of gelatin in film matrix. This corresponded to a decrease in rigidity with the concomitant increase in flexibility of film, as evidenced by the decrease in mechanical strength of films (Table 34). Nevertheless, T_g associated with immiscible oil phase was not found in the thermogram of all emulsion films within temperature range tested (-20 to 150 ºC). T_g of those oils were most likely lower than temperature range tested.

For films prepared using soy lecithin as surfactant, that incorporated with basil essential oil showed the lowest T_g, followed by those containing oil mixture and palm oil, respectively (Table 35). The similar result was also observed with films having Tween-20 as surfactant. Basil essential oil, which yielded the gelatin film with the highest molecular flexibility, had very low temperature T_g (-93.72 ºC) (data not shown). It was noted that T_g of films incorporated with various oils shifted to different lower temperature as compared to that of control film, depending on type of oil used. For films incorporated with the same oil, those having soy lecithin as a surfactant had lower T_g than those using Tween-20 as surfactant. Additionally, type of oil and surfactant affected T_g of resulting films. Surfactant played a role in stabilization of oil droplets, which localized in film matrix. As a result, different arrangement and orientation of gelatin chains to form the film network were presumed. Additionally,

T_g values of all film samples were higher than room temperature, suggesting the low humidity of films after conditioning in dried atmosphere (Vanin *et al.*, 2005). Films conditioned under the relative humidity at least 60 % had T_g at temperature lower than room temperature (Sobral *et al.*, 2002).

For endothermic/melting transition (Figure 32, 33 and Table 35), the control film showed endothermic peak with T_{max} of 117.10 ºC. The melting transition is generally associated with the disruption of ordered or aggregated molecular structure (Jongjareonrak *et al.*, 2006; Tang *et al.*, 2009). The observed endothermic peak was possibly associated with the helix-coil transition of gelatin (Rahman *et al.*, 2008). Similar observations were reported by Vanin *et al.* (2005) and Staroszczyk *et al.* (2012). This result suggested that the gelatin molecules could undergo partial renaturation, thereby transforming themselves into a more ordered structure during casting and drying (Hoque *et al.*, 2011; Rahman *et al.*, 2008). The DSC thermograms of films incorporated with various oils, irrespective of surfactants used, exhibited wider melting peak and decreased peak intensity in comparison with the control film. The oils incorporated, which acted also as plasticizer, might impede the renaturation (coil-to-helix transformation) of gelatin molecules during film formation. It was noted that the macromolecular network was broken by oils incorporated. This could result in the decrease in ΔH of emulsion gelatin films, compared to the control film. However, all film samples incorporated without and with various oils and surfactants generally exhibited similar $T_{max,\ peak}$, excepted for that containing palm oil. The control film exhibited the highest ΔH in comparison with those containing various oils and surfactants. ΔH of films were decreased from 12.39 J/g (control film) to 4.05, 3.36 and 3.56 J/g when basil oil, palm oil and their mixture were added and soy lecithin was used as surfactant, while those prepared with Tween-20 were 5.29, 4.82 and 3.71 J/g, respectively. This result indicated that the lesser portion of ordered structure formed in the film upon oils incorporated, which required the lower enthalpy for disruption of the inter-chain interaction in the matrix of films. This was possibly owing to the presence of oil droplets in film structure which could lower the interaction of gelatin chains. Enthalpy or area under endothermic melting peak generally corresponded with the amount of ordered phase structure (Tongnuanchan *et al.*, 2014a; Vanin *et al.*, 2005). This result reconfirmed that the lower fraction of

ordered-phase was formed in films containing oils, irrespective of surfactants. It was postulated that gelatin molecules could less undergo partial renaturation to helical structure in films to a lower extent in the presence of oil. Moreover, films ingredients such as essential oil, palm oil, glycerol, surfactants and water could enhance the non-ordered phase fraction (amorphous) in film structure, with the lower ordered-phase fraction in films incorporated with various oils, resulted in lower ΔH. This was associated with lower TS and EM but higher EAB of emulsified gelatin films in comparison with control film.

In addition, the thermograms of films incorporated with palm oil and oil mixture showed one more endothermic transition peak at temperature (T_{max1}) of -0.73 and -9.15 ºC for those using soy lecithin as surfactant. Those having Tween-20 had the transition peak at -0.07 and -9.15 ºC, respectively. This suggested that the existence of two different ordered structures in those film matrixes was mostly mediated by those separated phase between formed by palm oil of the lower T_{max1} and gelatin of the higher T_{max2}. This endothermic transition appeared before glass transition, suggesting the melting transition of palm oil droplets dispersed throughout the gelatin network of film. This result was confirmed by the endothermic transition of pure palm oil, which appeared at 2.68 ºC (data not shown). Nevertheless, the endothermic transition in this range of temperature was undetectable for film containing basil essential oil. It was noticed that no melting transition was observed for pure basil essential oil (data not shown).

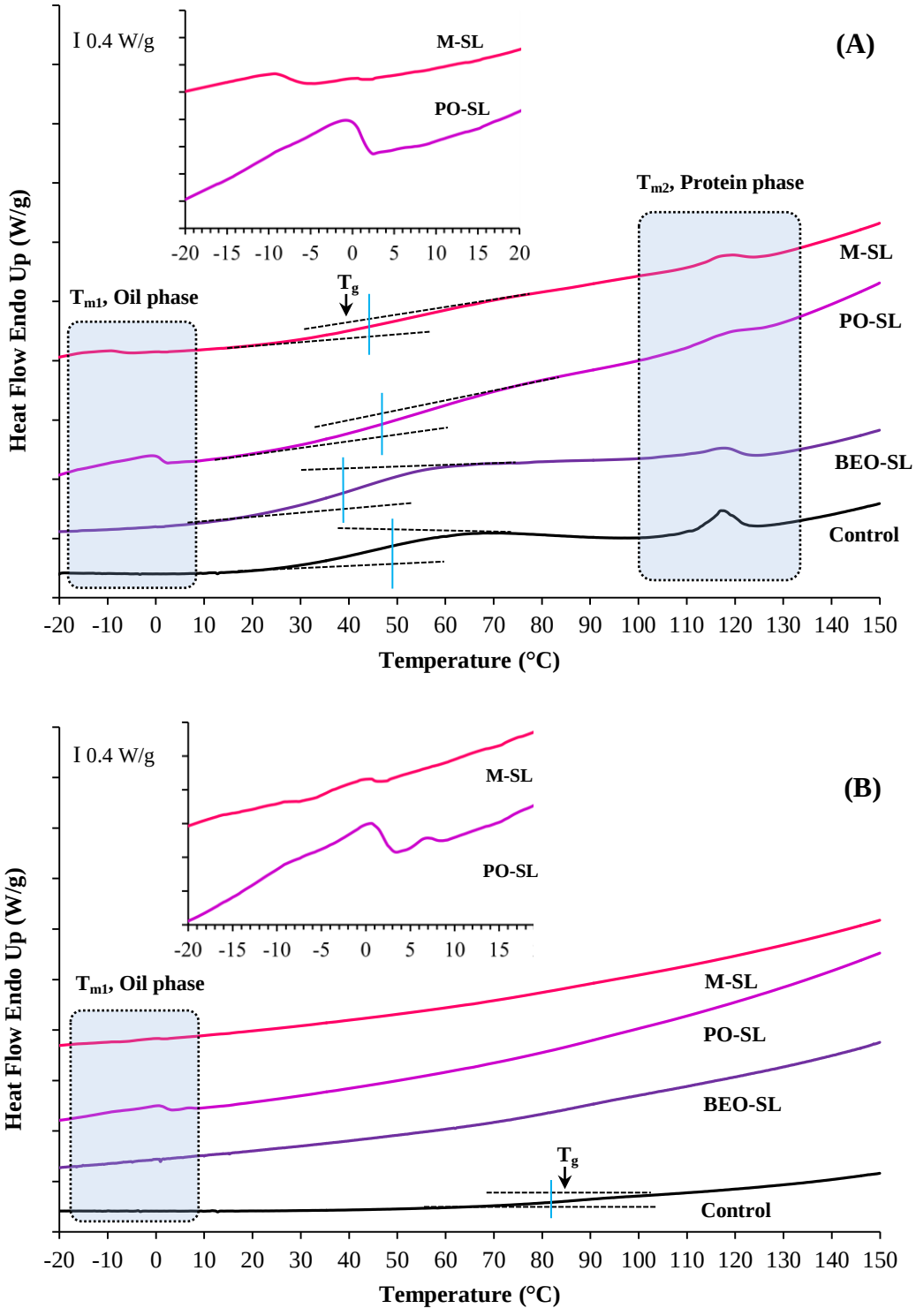


Figure 32. DSC thermograms of 1st-heating scan (A) and 2nd-heating scan (B) of films from fish skin gelatin containing basil essential oil, palm oil and oil mixture in the presence of Tween-20 as surfactant. Control: without addition of oil and surfactant.

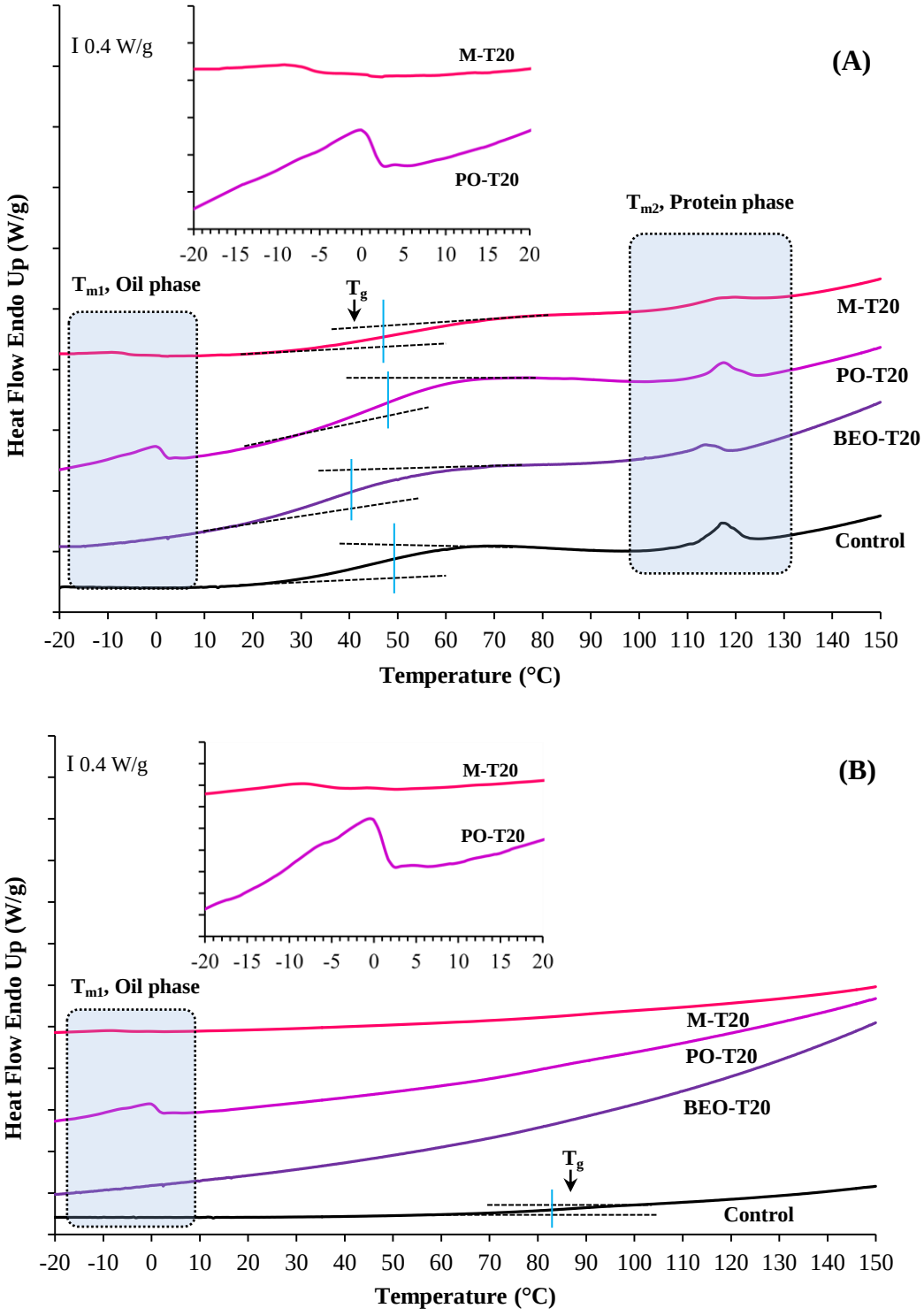


Figure 33. DSC thermograms of 1st-heating scan (A) and 2nd-heating scan (B) of films from fish skin gelatin containing basil essential oil, palm oil and oil mixture in the presence of soy lecithin as surfactant. Control: without addition of oil and surfactant.

Table 35. Glass transition temperature (T_g), melting/order-phase transition temperature (T_{max}) and enthalpy (ΔH) of films from fish skin gelatin incorporated with palm oil at different levels.

Film Samples	Surfactants	Type of Oils	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)
Control film	Without surfactant	Without oil	-	-	-	-	113.71	117.10	122.39	12.39	49.77
Emulsion film	Soy lecithin	Basil essential oil	-	-	-	-	110.79	118.02	122.61	4.05	39.85
		Palm oil	-17.23	-0.73	1.97	5.09	109.85	118.85	126.93	3.36	46.93
		Mixture	-15.32	-9.15	-6.58	0.54	112.93	117.52	123.57	3.56	44.68
	Tween 20	Basil essential oil	-	-	-	-	109.12	113.52	118.22	5.29	40.27
		Palm oil	-9.16	-0.07	2.22	4.49	113.04	117.43	121.90	4.82	47.92
		Mixture	-14.94	-9.15	-5.26	1.03	109.92	116.85	118.68	3.71	46.51
Film Samples	Surfactants	Type of Oils	2nd - Heating								Glass 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)
Control film	Without surfactant	Without oil	-	-	-	-	-	-	-	-	81.55
Emulsion film	Soy lecithin	Basil essential oil	-	-	-	-	-	-	-	-	-
		Palm oil	-17.55	0.18	2.87	4.48	-	-	-	-	-
		Mixture	-5.42	-0.57	1.43	0.48	-	-	-	-	-
	Tween 20	Basil essential oil	-	-	-	-	-	-	-	-	-
		Palm oil	-13.01	-0.32	1.91	3.59	-	-	-	-	-
		Mixture	-14.67	-8.07	-4.22	0.63	-	-	-	-	-

From the thermograms of the 2nd-heating scan of gelatin films without and with various oils (Figure 32B and 33B), the endothermic melting peak was found at low temperatures (-8.07 ºC – 0.18 ºC) (T_{max1}) for films incorporated with palm oil and oil mixture, which was more likely associated with the melting transition of palm oil as described above. However, it was not observed for the control film and films containing basil essential oil, irrespective of surfactants. Additionally, no endothermic peak of gelatin-rich phase transition (T_{max2}) was observed for all film samples. After 1st-heating scan and followed by fast cooling, the gelatin molecule was not able to rearrange themselves into ordered structure before starting 2nd-heating scan. Furthermore, T_g of the control film was shifted from 49.77 ºC (1st-heating scan) to 81.55 ºC in the 2nd-heating scan, while T_g of plasticized gelatin-rich phase was not clearly observed for films incorporated with various oils and surfactants. After 1st-heating scan, the absorbed and bounded water was eliminated and therefore the gelatin molecules could undergo more interaction, which resulted in stiffer matrix. Therefore, both oils and surfactants had the influence on phase structure as well as thermal characteristics of gelatin-based film.

8.4.4 Seal strength, seal efficiency and morphology of seal

8.4.4.1 Peel test

Seal strength and seal efficiency of gelatin films incorporated without and with different oils and surfactants, heat sealed at 150 ± 0.5 ºC with different sealing times are shown in Table 36. All film samples were sealed close to or higher temperature for the melting transition of protein in film. Thermal transition temperatures are potentially used in determining sealing properties of film from several materials (Hernandez, 1997). During heat sealing process, the surface of 2 films underwent melting via applied heat, which was potentially enhanced by pressure and time. The optimum condition of heat sealing could promote the interfacial interactions across the contact surfaces, yielding the sufficient seal strength to the sealed film. It was observed that heating time below 1 s did not provide insufficient melting and fusion, while heating time above 1.5 s burned the sealing area. Therefore, heating temperature of 150 ± 0.5 ºC for 1 s, 1.25 s and 1.5 s was selected for this

study. The control film (without oils and surfactants incorporated) showed the highest seal strength at all sealing times used, compared with those films incorporated with various oils and surfactants ($p < 0.05$). This might be due to the stronger seal, which was in agreement with higher TS and EM of control films (Table 34).

When considering seal efficiency, films incorporated with palm oil using Tween-20 and soy lecithin as surfactant showed high sealing efficiency when sealed for 1 and 1.5 s. It was noted that the control film showed the highest seal efficiency at sealing time of 1.25 s ($p < 0.05$). For films prepared with soy lecithin as a surfactant, the highest seal strength and seal efficiency of films incorporated with basil essential oil, palm oil and mixture were observed at sealing times of 1.5 s, 1.5 s and 1.25 s, respectively. When Tween-20 was used as a surfactant, films incorporated with basil essential oil, palm oil and oil mixture exhibited the highest seal strength and seal efficiency when sealing times were 1.25 s, 1 s and 1.25 s, respectively.

Heat sealability of films increased with increasing sealing time ($p < 0.05$), especially for films incorporated with basil essential oil and palm oil using soy lecithin as surfactant. When soy lecithin was used as surfactant, the decreases in seal strength and seal efficiency were observed as higher heating time (1.5 s) was used for control film and film incorporated with oil mixture. The similar result was found for films containing basil essential oil and oil mixture when Tween-20 was present. However, film added with palm oil using Tween-20 as surfactant showed the highest seal strength and seal efficiency when sealing time of 1 s was used. The decreased values were obtained with increasing sealing time ($p < 0.05$). This was more likely due to excessive time of heat treatment, which yielded the distorted and weakened seals. In addition, the distorted or nonfunctional seal was induced when heat required to produce a seal was higher than the heat sealing temperature of film materials (Martin, 1986). As a result, the overheating during heat sealing process directly weakened the structure of film via thermal degradation of proteins. Thus, the heat sealing condition with appropriate heating time could improve the seal strength and seal efficiency of resulting films.

For films prepared using soy lecithin as surfactant, that incorporated with palm oil showed the highest seal strength and seal efficiency for all sealing times used ($p < 0.05$), followed by those containing oil mixture and basil essential oil,

respectively. The similar trend of heat sealability was also obtained for films containing Tween-20 as surfactant. The results indicated that palm oil rendered the film with the highest seal strength and seal efficiency in comparison with other oils ($p < 0.05$), irrespective of sealing time and surfactant used. Conversely, the basil essential oil yielded the films with the lowest heat sealability ($p < 0.05$). This result suggested that the structure of films containing palm oil, irrespective of surfactants, were more appropriate for heat sealing than those having essential oil. Different components in basil essential oil and palm oil might play a role on modifying the protein network of the film differently. Basil essential oil and palm oil contained monoterpenes hydrocarbon and triglyceride as major component, respectively (Teixeira *et al.*, 2013). Those compounds could interrupt or interact with gelatin molecules via hydrophobic interaction. Essential oils are concentrated hydrophobic liquid containing several compounds such as aldehydes, ketones and phenols (Bakkali *et al.*, 2008; Tongnuanchan and Benjakul, 2014). Aldehydes or ketones could interact with protein (De Carvalho and Grosso, 2004). Hydroxyl group and hydrophobic groups of polyphenol were able to combine with gelatin chains via hydrogen bond and hydrophobic interaction, respectively. Those compounds with protein cross-linking ability in basil essential oil could result in more rigid network of gelatin film. As a result, the rigid network of gelatin film did not favor the melting as the heat was applied for sealing. Moreover, the interacted gelatin molecules in film containing essential oil could impede molecular interdiffusion across the interface of molten surface of films upon heat sealing, resulting in lower seal strength and seal efficiency. Therefore, heat sealability for gelatin films varied with types of oils used.

Additionally, for films incorporated with the same oil and sealing time used, those having Tween-20 as surfactant had a higher heat sealability than those using soy lecithin as evidenced by higher seal strength and seal efficiency ($p < 0.05$), excepted for those containing palm oil with sealing time of 1.5 s, in which film using soy lecithin had higher values than that using Tween-20 ($p < 0.05$). Kim and Ustunol (2001) studied the chemical bonding formation of sealed and unsealed whey protein isolate emulsion films via electron spectroscopy for chemical analysis (ESCA). The higher formation of hydrogen and covalent bonds involving C-O-H and N-C bonds was observed upon heat sealing, which may be the major forces responsible for seal

formation. During sealing, plasticized protein-based film could form the molecular interdiffusion, stabilized mainly by hydrogen bond. Hydrogen bonding could also occur between protein and plasticizer, as well as between the plasticizer molecules or protein molecules (Kim and Ustunol, 2001). It has been reported that plasticizer such as glycerol could act as heat sealing promoters, thereby forming an adhesive force (Bundy *et al.*, 2000). Polar functional groups of polymers or compounds such as hydroxyl (OH), carboxyl (COOH) or aldehyde (CHO) were directly responsible for adhesion strength of film (Lee, 1994). C-O-Hs in hydrophilic head of Tween-20 could enhance the interaction via hydrogen bonding in sealed film, resulting in higher seal strength and seal efficiency. Therefore, type of surfactant also directly affected heat sealability of resulting films. Kim and Ustunol (2001) also reported that heat sealing temperature showed the highest significant influence on peel seal strength of whey protein isolate/lipid emulsion films plasticized with sorbitol and glycerol, followed by dwell time. However, they also reported that pressure variation did not has an impact on seal strength significantly. The optimum seal strengths of control film and films incorporated with various oils and surfactants were lower than seal strength and seal efficiency of heat sealed synthetic thermoplastic films (single-layer and multi-layer) (Table 36). It was noted that the maximum seal strengths of films in the present study were higher than that reported for whey protein isolate emulsion films plasticized with sorbitol and glycerol, which were 120-301 and 169-323 N/m, respectively (Kim and Ustunol, 2001). The similar observation was also reported for wheat gluten films plasticized with glycerol at different sealing and molding temperatures (Cho *et al.*, 2007) and sago starch films plasticized with sorbitol, glycerol and their mixtures with different levels (Abdorreza *et al.*, 2011). Nevertheless, fish gelatin films incorporated with ZnO nanorods (Rouhi *et al.*, 2013) possessed higher seal strength than those obtained in the present study.

Table 36. Seal strength and seal efficiency for peel test of films from fish skin gelatin incorporated with different oils in the presence of soy lecithin and Tween-20 as surfactants.-

Film samples	Surfactants	Oils	Heating time (s)	Seal strength (N/m)	Seal efficiency (%)
Control film	Without surfactant	Without oil	1	569.64 ± 28.64 ^{b,w,W}*	32.05 ± 1.60 ^{b,w,X}
			1.25	781.41 ± 39.51 ^{a,w,W}	43.97 ± 2.22 ^{a,w,W}
			1.5	602.00 ± 36.87 ^{b,w,W}	33.88 ± 2.07 ^{b,x,W}
Emulsion films	Soy lecithin	Basil essential oil	1	26.18 ± 4.98 ^{c,z,B}	1.26 ± 0.24 ^{c,z,B}
			1.25	50.96 ± 3.60 ^{b,z,B}	2.45 ± 0.17 ^{b,z,B}
			1.5	64.39 ± 5.55 ^{a,z,B}	3.10 ± 0.27 ^{a,z,B}
		Palm oil	1	204.00 ± 25.34 ^{b,x,B}	17.00 ± 2.11 ^{b,x,B}
			1.25	234.69 ± 18.82 ^{b,x,B}	19.56 ± 1.57 ^{b,x,B}
			1.5	478.22 ± 17.96 ^{a,x,A}	39.86 ± 1.50 ^{a,w,A}
		Mixture	1	63.64 ± 5.73 ^{c,y,B}	4.40 ± 0.40 ^{c,y,B}
			1.25	182.76 ± 21.66 ^{a,y,B}	12.65 ± 1.50 ^{a,y,B}
			1.5	103.87 ± 9.44 ^{b,y,B}	7.19 ± 0.65 ^{b,y,B}
	Tween-20	Basil essential oil	1	268.68 ± 17.16 ^{c,Z,A}	12.93 ± 0.83 ^{c,Z,A}
			1.25	379.52 ± 15.95 ^{a,Z,A}	18.27 ± 0.77 ^{a,Z,A}
			1.5	330.54 ± 13.44 ^{b,Y,A}	15.91 ± 0.65 ^{b,Z,A}
		Palm oil	1	496.86 ± 21.82 ^{a,X,A}	41.42 ± 1.82 ^{a,W,A}
			1.25	472.51 ± 25.84 ^{b,X,A}	39.39 ± 2.15 ^{b,X,A}
			1.5	376.23 ± 11.73 ^{c,X,B}	31.36 ± 0.98 ^{c,X,B}
		Mixture	1	389.59 ± 15.37 ^{b,Y,A}	26.94 ± 1.06 ^{b,Y,A}
			1.25	432.61 ± 8.26 ^{a,Y,A}	29.94 ± 0.57 ^{a,Y,A}
			1.5	291.26 ± 10.45 ^{c,Z,A}	20.16 ± 0.72 ^{c,Y,A}
Thermoplastic films	Single-layer	PP	1.25	1123.14 ± 84.47†	84.47 ± 3.43
		LDPE	1.25	677.71 ± 52.41	52.41 ± 1.50
	Multi-layer	Nylon/LDPE	1.5	1228.66 ± 60.57	60.57 ± 2.83

* Mean ± SD (n=3).

† Seal strength and seal efficiency of thermoplastic films was selected from the sealing time which yielded the highest value.

Different letters (abc) in the same column under the same surfactant and oil indicate significant differences ($p < 0.05$).

Different letters (wxy) in the same column under the same sealing time of control film and films prepared with soy lecithin indicate significant differences ($p < 0.05$).

Different capital letters (WXY) in the same column under the same sealing time of control film and films prepared with Tween 20 indicate significant differences ($p < 0.05$).

Different capital letters (ABC) in the same column under the same oil and sealing time indicate significant differences ($p < 0.05$).

PP = Polypropylene; LDPE = low-density polyethylene; Nylon/LDPE = nylon/low-density polyethylene

Mode of failure for peel test of those films is summarized in Table 37 and Figure 34A. There were more than one failure modes occurred for films incorporated with various oils and surfactants (Table 37). Mode of failure represented the quality of heat sealing. For film prepared using soy lecithin as surfactant, the adhesive failure was observed for film incorporated with basil essential oil, regardless of sealing time. It was indicated that the seal of film was generated, based on the incomplete fusion of protein molecules and the weaker of seal was developed. This result was associated with the lowest seal strength and seal efficiency of films. However, films added with palm oil exhibited adhesive failure or fracture at seal edge, while either the adhesive or cohesive failure was observed for that containing oil mixture when soy lecithin was used as a surfactant. It was found that types of oils affected heat sealability of film differently. This might be due to the different characteristics of oils, dispersed and localized in film matrix. As a result, sealing time did not affect the mode of failure of film containing basil essential oil, whereas this parameter had the significant influence for film added with palm oil when soy lecithin was used as surfactant. In contrast to those prepared with soy lecithin, the failure at the seal edge generally occurred for films prepared with Tween-20, regardless of types of oils. Also a control film seemed to favor failure at the seal edge. Sealing time had no impact on mode of failure of those films. The result indicated that all films prepared with Tween-20 had stronger seal than those using soy lecithin as surfactant, irrespective of sealing time. This result suggested that type of surfactant had the influence on the quality of heat seal of emulsion-based gelatin film. Moreover, both single-layer thermoplastic films (PP and LDPE) fractured at seal edge, whereas peel failure of multi-layer film (Nylon/LDPE) was adhesive type. As a result, film compositions and heat sealing conditions had the marked impact on heat sealing as well as properties of seals.

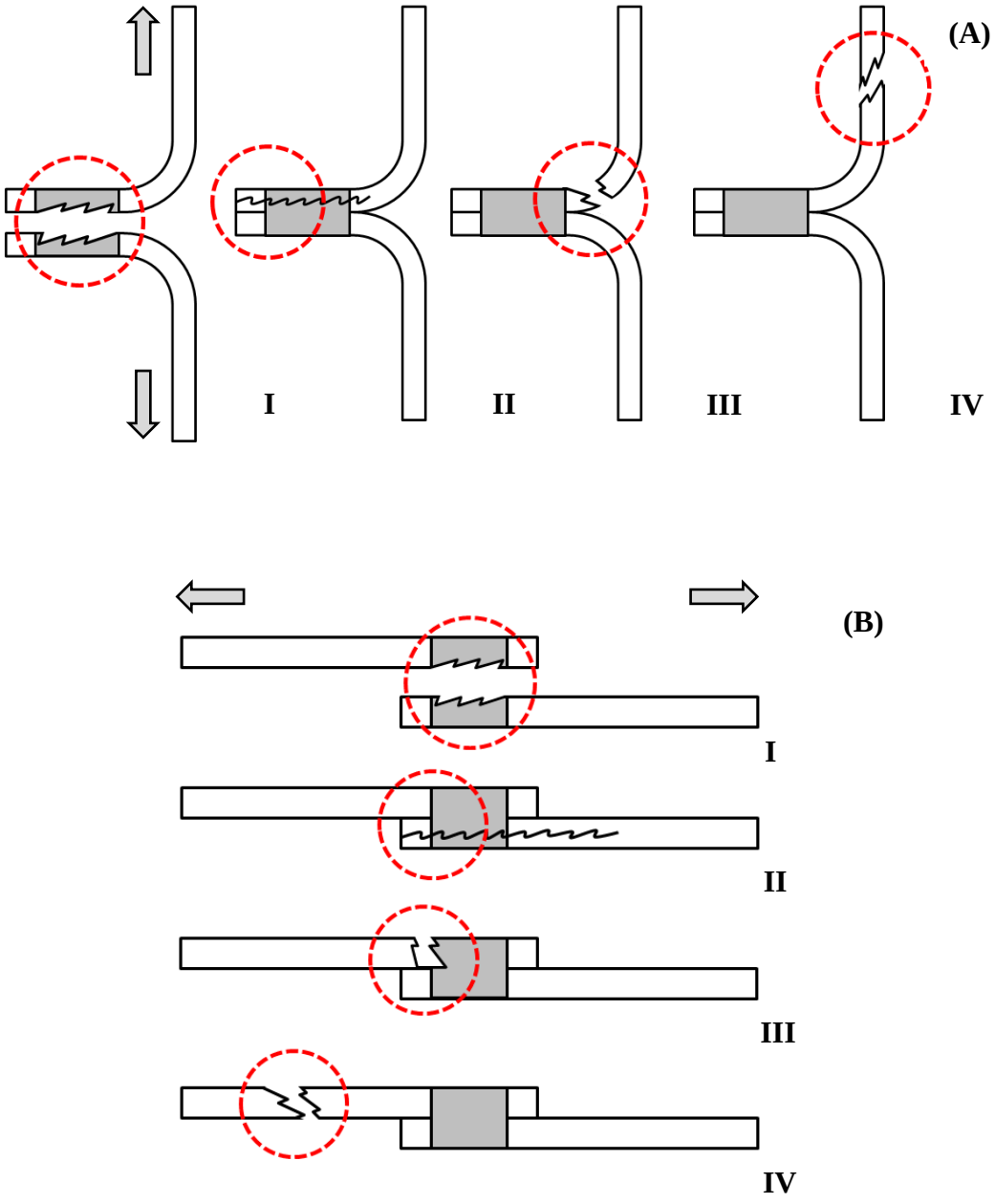


Figure 34. Failure modes illustrated for peel test (A) and lab-shear test (B).

Table 37. Mode of failure for peel test of films from fish skin gelatin incorporated with different oils in the presence of soy lecithin and Tween-20 as surfactants.

Film samples	Surfactants	Oils	Heating time (s)		
			1.00	1.25	1.50
Control film	Without surfactant	Without oil	III	III	III
Emulsion films	Soy lecithin	Basil essential oil	I	I	I
		Palm oil	I	I/III	III
		Mixture	I	I/II	I/II
	Tween-20	Basil essential oil	III	III	III
		Palm oil	III	III	III
		Mixture	III	III	III
Thermoplastic films	Single-layer	PP	-	III	-
		LDPE	-	III	-
	Multi-layer	Nylon/LDPE	-	-	I

(I) Adhesive seal failure; (II) Cohesive seal failure; (III) Failure at seal edge; (IV) Failure at film body (See Figure 34A for detail).

PP = Polypropylene; LDPE = low-density polyethylene; Nylon/LDPE = nylon/low-density polyethylene

8.4.4.2 Lap-shear test

Based on peel test, films prepared using Tween-20 as surfactant had higher heat sealability than those containing soy lecithin. Those films were subjected to lap-shear test and the result is presented in Table 38. Films incorporated with various oils exhibited the lower seal strength than the control film (without oil and surfactant), regardless of sealing time ($p < 0.05$) (Table 38). The control film had the highest seal strength for all sealing time, compared with others ($p < 0.05$). For seal efficiency, the control film showed the highest seal efficiency when sealing time of 1 s was used ($p < 0.05$), while the highest seal efficiency were found in films incorporated with palm oil when sealing time of 1.25 s and 1.5 s were used ($p < 0.05$).

The highest seal strength and seal efficiency of control film were obtained at 1 s of sealing time ($p < 0.05$), while no differences between 1.25 s and 1.5 s were observed ($p > 0.05$). The decreases in lap-shear strength and efficiency of the control film might be due to the excess heating time used for melting, which caused the partial degradation of film. This resulted in the weaker interactions after repolymerization, particularly during cooling. In general, films incorporated with basil essential oil and oil mixture had no difference in seal strength and seal efficiency when different sealing times were implemented ($p > 0.05$). Nevertheless, film containing palm oil showed the highest values when sealed for 1 s and 1.5 s of heating time ($p < 0.05$). The result suggested that seal strength and seal efficiency of lap-shear test was unaffected by the function of sealing time. Generally, seal strength and seal efficiency for lap-shear test of control film and films incorporated with various oils and prepared using Tween-20 as surfactant were greater than those obtained from peel test (Table 36). This result suggested that the seal zip of all emulsion-based gelatin films tested was less resistant to peeling mode than lap-shear mode. Under peeling mode of testing, the applied force might be directly transferred to the tip of the zip, thus allowing the zip to open up with more ease. Cho *et al.* (2007) reported that the sealing temperature had slight effect on lap-shear strength of wheat gluten film plasticized with glycerol, and peel strength increased with increasing temperature. The maximum seal strength and seal efficiency for lap-shear test of the control film and films added with various oils was mostly higher than those of thermoplastic films. The results obtained are comparable with other studies. The optimum seal strength of this work was higher than gelatin film incorporated with corn oil manufactured by extrusion with different temperatures and pH values (seal strength ≈ 231.78 - 604.59 N/m) (Nur Hanani *et al.*, 2014) and glycerol-plasticized wheat gluten films at different sealing and molding temperature (seal strength ≈ 0.070 - 0.229 N/m) (Cho *et al.*, 2007).

Table 38. Seal strength and seal efficiency for lab-shear test of films from fish skin gelatin incorporated with different oils in the presence of Tween-20 as surfactant.

Film samples	Oils		Heating time (s)	Seal strength (N/m)	Seal efficiency (%)
Gelatin film	Without oil		1.00	2214.22 ± 108.38 ^{a,w}*	124.60 ± 6.10 ^{a,w}
			1.25	1351.12 ± 52.91 ^{b,w}	76.03 ± 2.98 ^{b,x}
			1.50	1364.39 ± 90.52 ^{b,w}	76.78 ± 5.09 ^{b,x}
Emulsion films	Basil essential oil		1.00	1025.05 ± 80.75 ^{a,x}	49.33 ± 3.89 ^{a,z}
			1.25	1023.38 ± 66.85 ^{a,xy}	49.25 ± 3.22 ^{a,y}
			1.50	1059.31 ± 35.77 ^{a,x}	50.98 ± 1.72 ^{a,y}
	Palm oil		1.00	1057.69 ± 58.69 ^{ab,x}	88.17 ± 4.89 ^{ab,x}
			1.25	993.19 ± 39.26 ^{b,y}	82.79 ± 3.30 ^{b,w}
			1.50	1082.21 ± 39.26 ^{a,x}	90.21 ± 3.27 ^{a,w}
	Mixture		1.00	1039.64 ± 95.32 ^{a,x}	71.95 ± 6.60 ^{a,y}
			1.25	1083.17 ± 41.75 ^{a,x}	74.96 ± 2.89 ^{a,x}
			1.50	1125.52 ± 51.87 ^{a,x}	77.89 ± 3.59 ^{a,x}
Thermoplastic films	Single-layer	PP	1.25	996.17 ± 18.78	74.92 ± 1.41
		LDPE	1.25	721.98 ± 44.09	55.84 ± 3.41
	Multi-layer	Nylon/LDPE	1.50	1923.02 ± 79.85	94.80 ± 3.94

* Mean ± SD (n=3).

† Seal strength and seal efficiency of thermoplastic films was selected from the sealing time which yielded the highest value.

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

Different letters (wxy) in the same column under the same sealing time indicate significant differences ($p < 0.05$).

PP = Polypropylene; LDPE = low-density polyethylene; Nylon/LDPE = nylon/low-density polyethylene

Mode of failure of sealed film samples under lap-shear test is shown in Table 39 and Figure 34B. All film samples exhibited the same failure mode, which was failure at seal edge. This result suggested that the seal of film samples for lap-shear test were strong and the sealing time had no effect on mode of failure of resulting films. This result was in agreement with high seal strength and seal efficiency of films. Thus, single lap seal provided higher seal strength than peel seal. However, the same mode of failure of both peel test and lap-shear test of those films using Tween-20 as surfactant was noticeable. For the thermoplastic films, PP and LDPE fractured at the seal edge. In addition, the failure was found far from seal for the Nylon/LDPE, suggesting that the seal area was stronger than body of film.

Table 39. Mode of failure for lab-shear test of films from fish skin gelatin incorporated with different oils in the presence of Tween-20 as surfactant.

Film samples	Oils		Heating time (s)		
			1.00	1.25	1.50
Control film	Without oil		III	III	III
Emulsion films	Basil essential oil		III	III	III
	Palm oil		III	III	III
	Mixture		III	III	III
Thermoplastic films	Single-layer	PP	-	III	-
		LDPE	-	III	-
	Multi-layer	Nylon/LDPE	-	-	III/IV

(I) Adhesive seal failure; (II) Cohesive seal failure; (III) Failure at seal edge; (IV) Failure at film body (See Figure 34B for detail).

PP = Polypropylene; LDPE = low-density polyethylene; Nylon/LDPE = nylon/low-density polyethylene

8.4.4.3 Morphology of seal zip

Figure 35 shows SEM micrographs of the freeze-fractured cross-section of heat sealing areas of control film (without incorporated oils and surfactant) and films incorporated with palm oil, basil essential and oil mixture prepared with Tween-20 as surfactant and heat sealed at 150 ± 0.5 ºC for 1.25, 1, 1.25 and 1.25 s, which yielded the highest seal strength and seal efficiency, respectively. As observed in Figure 35A, the bulk of control film had smoother and more compact network than those incorporated with palm oil, basil essential oil and oil mixture. This demonstrated that the control film had more ordered-phase and homogeneous structure than other films. All emulsion films exhibited the coarser cross-sectional morphologies, in comparison with control film. Different oils used might contribute to film structure or morphology differently. Different arrangement and orientation of gelatin chains during film formation might take place in different fashions when different oils were incorporated. This might be mediated by the coexisting of two different phases of materials, which were the hydrophobic phase (oils) and hydrophilic phase (protein). Oil droplets localized in the protein film network could interrupt the protein-protein interaction between protein chains, thereby lowering the ordered alignment of proteins in film network. This led to higher roughness of film. Additionally, no phase separation between oil phase and protein phase or large agglomeration of oil droplets was observed in film matrix. Therefore, films incorporated with palm oil, basil essential oil and their mixture were prepared using the stable emulsion system. However, the small voids were observed in films containing palm oil and oil mixture, while this phenomenon did not occur when basil essential oil was used. Obviously, the increases in film thickness were observed in films incorporated with different oils. This result indicated the protrusion of film structure, which varied, depending on oils used. Oil droplets from different sources distributed, localized and interacted with gelatin in film matrix in different ways, thereby providing the different film networks.

The SEM morphologies of seal zip, both at the edge (Figure 35A) and in the middle (Figure 35B) of the zip of the control film and films incorporated with basil essential oil, palm oil and their mixture indicated that those films exhibited

complete fusion along sealed area (good quality of seal). This revealed that all film samples could melt and merge themselves together, which underwent entanglement and form bonding between gelatin chains that interdiffused across the sealed interface, particularly during both heating and cooling process. This was most likely associated with the strong seal strength, as indicated by film fractured at the sealed edge for all film samples. This indicated that control gelatin films could merge with each other, thereby allowing gelatin molecules to from entanglement upon heat sealing more effectively. Smoother and more continuous structure of the seal was observed in control film as compared to emulsion films. However, emulsion films containing different oils exhibited coarser structure of sealed areas, indicated looser network of the film matrix. The morphological results of sealed area could explain the seal strength and seal efficiently of films incorporated without and with different oils (Table 36 and 38). Thus, types of oils had the profound impact on microstructures of seal of emulsion-based gelatin films and their heat sealing ability.

Based on all supporting results, a simplified illustration of the matrix of films incorporated without and with various oils before and after heat sealing is gives in Figure 36. Generally, gelatin film without oil (Figure 36A) was postulated to have the higher molecular interdiffusion and degree of entanglement and interaction of gelatin molecules after heat sealing, in comparison with that containing oils (Figure 36B). Oil distributed in film matrix more likely impeded gelatin-gelatin interaction as indicated by the lower seal strength. However, crosslinking of gelatin induced by essential oil incorporated, which could previously occur in the film during film formation (before heat sealing), could potentially impede molecular interdiffusion and thus lowering the degree of gelatin chain entanglement and interaction upon heat sealing. This led to the lower seal strength of gelatin film incorporated with basil essential oil. Thus, oil and essential oil play a role in heat sealing mechanism and seal strength of emulsion-based gelatin film.

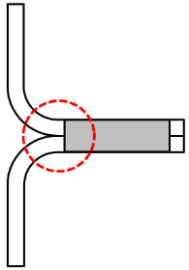
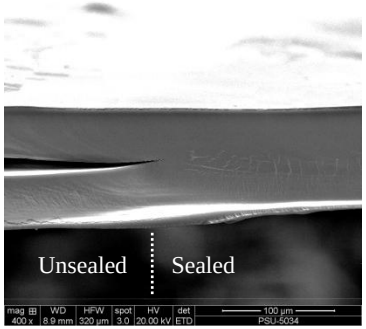
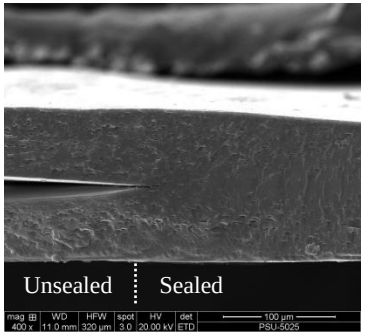
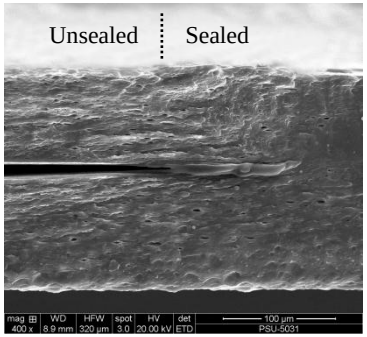
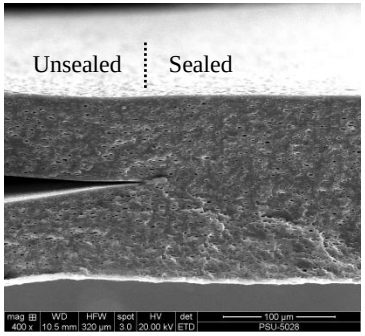
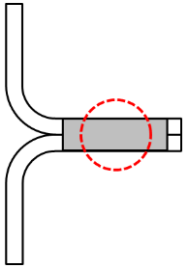
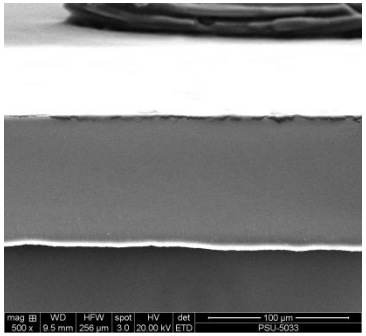
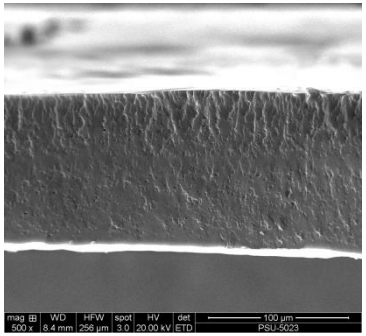
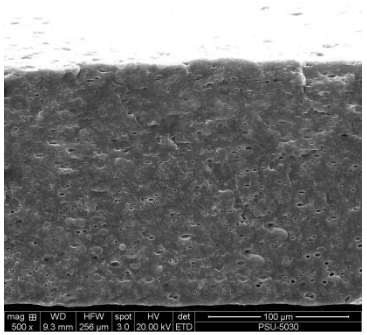
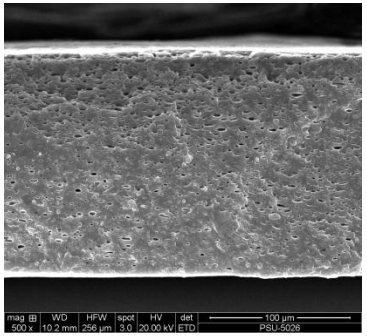
Position	Control (Without oil added)	Oils		
		Basil essential oil	Palm oil	Mixture
(A) Seal edge 				
(B) Middle 				

Figure 35. SEM micrographs of cross-section at seal edge (400x) (A) and middle of seal (500x) (B) of films from fish skin gelatin containing basil essential oil, palm oil and mixture in the presence of Tween-20 as surfactant.

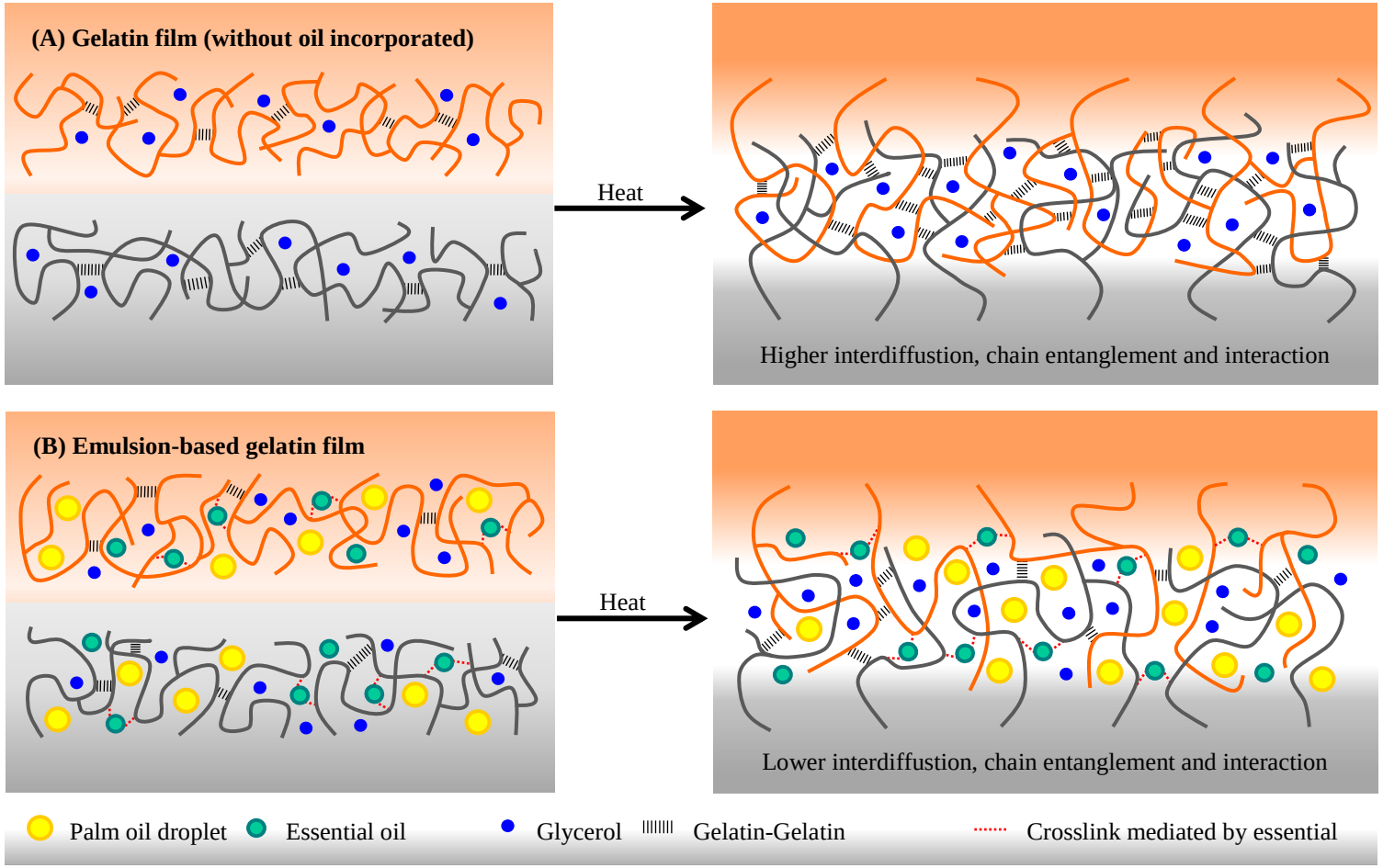


Figure 36. Simplified illustration for molecular interdiffusion and interaction possibly occurred in the heat sealed area of gelatin film matrix without and with oils.

8.5 Conclusion

Properties of glycerol-plasticized gelatin film was influenced by basil essential oil and palm oil as well as different types of surfactants (soy lecithin and Tween-20). Different types of oils directly had the impact on mechanical properties of film via plasticizing effect, as indicated by the decreased film strength with the concomitant increase in flexibility. Thermal properties of gelatin films were mainly influenced by the oils incorporated. Gelatin film incorporated with and without different oils were heat sealable. The seal strength and seal efficiency of film varied with the film compositions. In general, palm oil yielded the emulsion gelatin film with higher seal strength than others for peel test, regardless of surfactants used, but slight difference was observed for lap-shear test. Tween-20 was the appropriated surfactant, which potentially enhanced the heat sealability of emulsion gelatin films and provided the good quality (complete fusion) seal. Sealing time was also essential parameter to improve the heat sealability of emulsion gelatin films. Therefore, the emulsion gelatin films prepared using the appropriate compositions and sealing conditions could be used as an alternative film packaging.

8.6 References

Aguilera, G.M. and Stanley, D.W. 1999. Microstructural Principals of Food Processing and Engineering. 2nd Ed. Aspen Publishers. Gaithersburg, MD.

Andreuccetti, C., Carvalho, R. and Grosso, C.R.F. 2009. Effect of hydrophobic plasticizers on functional properties of gelatin-based films. Food Res. Int. 42: 1113-1121.

Arvanitoyannis, I.S. 2002. Formation and properties of collagen and gelatin films and coatings. *In* Protein-based films and coatings. (Gennadios, A., ed.). p. 275-304. CRC Press. Boca Raton.

ASTM. 1989. Standard test methods for water vapor transmission of materials. Standard designation E96-E80. *In* Annual book of ASTM standards. p. 730-739. ASTM. Philadelphia.

Bahram, S., Rezaei, M., Soltani, M., Kamali, A., Ojagh, S.M. and Abdollahi, M. 2014. Whey protein concentrate edible film activated with cinnamon essential oil. J. Food Process. Pres. 38: 1251-1258.

Baldwin, E.A., Nispero-Carriedo, M.O., Hagenmaier, R.D. and Baker, R.A. 1997. Use of lipids in coatings for food products. Food Technol. 51: 56-64.

Bergo, P. and Sobral, P.J.A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. Food Hydrocolloid. 21: 1285-1289.

Bezard, J. 1971. The component triglycerides of palm-kernel oil. Lipids. 6: 630-634.

Bonilla, J., Atarés, L., Vargas, M. and Chiralt, A. 2012. Effect of essential oils and homogenization conditions on properties of chitosan-based films. Food Hydrocolloid. 26: 9-16.

Choi, S.S. and Regenstein, J.M. 2000. Physicochemical and Sensory Characteristics of Fish Gelatin. J. Food Sci. 65: 194-199.

Dickinson, E. 2003. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocolloid. 17: 25-39.

Dickinson, E. 2009. Hydrocolloids as emulsifiers and emulsion stabilizers. Food Hydrocolloid. 23: 1473-1482.

Edem, D.O. 2002. Palm oil: Biochemical, physiological, nutritional, hematological and toxicological aspects: A review. Plant Food. Hum. Nutr. 57: 319-341.

Friberg, S., Larsson, K. and Sjoblom, J. 2004. Food emulsions. Marcel Dekker. New York.

Gennadios, A. and Weller, C.L. 1990. Edible films and coating from wheat and corn proteins. Food Technol. 44: 63-69.

Gennadios, A., Ghorpade, V.M., Weller, C.L. and Hanna, M.A. 1996. Heat curing of soy protein films. T. ASABE. 39: 575-579.

Ghanbarzadeh, B. and Oromiehi, A.R. 2008. Studies on glass transition temperature of mono and bilayer protein films plasticized by glycerol and olive oil. J. Appl. Polym. Sci. 109: 2848-2854.

Ghanbarzadeh, B., Almasi, H. and Entezami, A.A. 2010. Physical properties of edible modified starch/carboxymethyl cellulose films. Innov. Food Sci. Emerg. 11: 697-702.

Gontard, N., Duchez, C., Cuq, J.-L. and Guilbert, S. 1994. Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties. Int. J. Food Sci. Tech. 29: 39-50.

Gontard, N. and Ring, S. 1996. Edible wheat gluten film: influence of water content on glass transition temperature. J. Agr. Food Chem. 44: 3474-3478.

Guerrero, P. and de la Caba, K. 2010. Thermal and mechanical properties of soy protein films processed at different pH by compression. J. Food Eng. 100: 261-269.

Guerrero, P., Nur Hanani, Z.A., Kerry, J.P. and de la Caba, K. 2011. Characterization of soy protein-based films prepared with acids and oils by compression. J. Food Eng. 107: 41-49.

Guillén, M.D. and Cabo, N. 1997. Infrared spectroscopy in the study of edible oils and fats. J. Sci. Food Agr. 75: 1-11.

Guillén, M.D. and Cabo, N. 2004. Study of the effects of smoke flavourings on the oxidative stability of the lipids of pork adipose tissue by means of Fourier transform infrared spectroscopy. Meat Sci. 66: 647-657.

Guzman, A., Torres, J.E., Prada, L.P. and Nuñez, M.L. 2010. Hydroprocessing of crude palm oil at pilot plant scale. Catal. Today. 156: 38-43.

Han, J.H. and Floros, J.D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. J. Plast. Film Sheet. 13: 287-298.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011a. Effects of partial hydrolysis and plasticizer content on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. Food Hydrocolloid. 25: 82-90.

Hoque, M.S., Benjakul, S., Prodpran, T. and Songtipya, P. 2011b. Properties of blend film based on cuttlefish (*Sepia pharaonis*) skin gelatin and mungbean protein isolate. Int. J. Biol. Macromol. 49: 663-673.

Huck-Iriart, C., Pizones Ruiz-Henestrosa, V.M., Candal, R.J. and Herrera, M.L. 2013. Effect of aqueous phase composition on stability of sodium caseinate/sunflower oil emulsions. Food Bioprocess Tech. 6: 2406-2418.

Iwata, K., Ishizaki, S., Handa, A. and Tanaka, M. 2000. Preparation and characterization of edible films from fish water-soluble proteins. Fisheries Sci. 66: 372-378.

Jeirani, Z., Mohamed Jan, B., Si Ali, B., Noor, I.M., See, C.H. and Saphanuchart, W. 2013. Formulation, optimization and application of triglyceride microemulsion in enhanced oil recovery. Ind. Crop. Prod. 43: 6-14.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006a. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid. 20: 492-501.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006b. Fatty acids and their sucrose esters affect the properties of fish skin gelatin-based film. Eur. Food Res. Technol. 222: 650-657.

Kalichevsky, M.T., Jaroszkiewicz, E.M. and Blanshard, J.M.V. 1992. Glass transition of gluten. 1: Gluten and gluten—sugar mixtures. Int. J. Biol. Macromol. 14: 257-266.

Kaminska, A. and Sionkowska, A. 1999. The effect of UV radiation on the values of thermal parameters of collagen containing β-carotene. Polym. Degrad. Stabil. 65: 87-90.

Kong, J. and Yu, S. 2007. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Bioch. Bioph. Sin. 39: 549-559.

Krimm, S. and Bandekar, J. 1986. Vibrational spectroscopy and conformation of peptides, polypeptides, and proteins. Adv. Protein Chem. 38: 181−364.

Krochta, J.M. 2002. Protein as raw materials for films and coatings: definitions, current status, and opportunities. *In* Protein-Based Films and Coating (Gennadios, A., ed.). p. 1-39. CRC Press. New York.

Lacroix, M. and Cooksey, K. 2005. Edible films and coatings from animal-origin proteins. *In* Innovations in food packaging. Vol. 301-317. (Han, J. H., ed.). Elsevier Academic Press. San Diego, Calif.

Limpisophon, K., Tanaka, M. and Osako, K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionace glauca*) skin gelatin. Food Chem. 122: 1095-1101.

Ma, W., Tang, C.-H., Yin, S.-W., Yang, X.-Q., Qi, J.-R. and Xia, N. 2012a. Effect of homogenization conditions on properties of gelatin–olive oil composite films. J. Food Eng. 113: 136-142.

Ma, W., Tang, C.-H., Yin, S.-W., Yang, X.-Q., Wang, Q., Liu, F. and Wei, Z.-H. 2012b. Characterization of gelatin-based edible films incorporated with olive oil. Food Res. Int. 49: 572-579.

Manorama, R. and Rukmini, C. 1992. Crude palm oil as a source of beta-carotene. Nutr. Res. 12: 223-232.

McClements, D.J. 2005. Food emulsions: Principles, practice and techniques. 2nd Ed. CRC Press. Florida.

Muyonga, J.H., Cole, C.G.B. and Duodu, K.G. 2004. Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chem. 85: 81-89.

Nagarajan, M., Benjakul, S., Prodpran, T. and Songtipya, P. 2012. Properties of film from splendid squid (*Loligo formosana*) skin gelatin with various extraction temperatures. Int. J. Biol. Macromol. 51: 489-496.

Núñez-Flores, R., Giménez, B., Fernández-Martín, F., López-Caballero, M.E., Montero, M.P. and Gómez-Guillén, M.C. 2013. Physical and functional characterization of active fish gelatin films incorporated with lignin. Food Hydrocolloid. 30: 163-172.

Nur Hanani, Z.A., McNamara, J., Roos, Y.H. and Kerry, J.P. 2013. Effect of plasticizer content on the functional properties of extruded gelatin-based composite films. Food Hydrocolloid. 31: 264-269.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009. Characterization of porcine plasma protein-based films as affected by pretreatment and cross-linking agents. Int. J. Biol. Macromol. 44: 143-148.

Palazolo, G.G., Sobral, P.A. and Wagner, J.R. 2011. Freeze-thaw stability of oil-in-water emulsions prepared with native and thermally-denatured soybean isolates. Food Hydrocolloid. 25: 398-409.

Phan, T.T., Attaphong, C. and Sabatini, D.A. 2011. Effect of extended surfactant structure on interfacial tension and microemulsion formation with triglycerides. J. Am. Soc. 88: 1223-1228.

Pol, H., Dawson, P., Acton, J. and Ogale, A. 2002. Soy protein isolate/corn-zein laminated films: transport and mechanical properties. J. Food Sci. 67: 212-217.

Prodpran, T., Benjakul, S. and Artharn, A. 2007. Properties and microstructure of protein-based film from round scad (*Decapterus maruadsi*) muscle as affected by palm oil and chitosan incorporation. Int. J. Biol. Macromol. 41: 605-614.

Rogers, C.E. 1985. Permeation of gases and vapors in polymers. *In* Polymer Permeability. (Comyn, J., ed.). p. 11-73. Elsevier Applied Science. London, U.K.

Setiowaty, G., Che Man, Y.B., Jinap, S. and Moh, M.H. 2000. Quantitative determination of peroxide value in thermally oxidized palm olein by Fourier transform infrared spectroscopy. Phytochem. Analysis. 11: 74-78.

Shiku, Y., Hamaguchi, P.Y., Benjakul, S., Visessanguan, W. and Tanaka, M. 2004. Effect of surimi quality on properties of edible films based on Alaska pollack. Food Chem. 86: 493-499.

Sikorski, Z.E., Scott, D.N., Buisson, D.H. and Love, R.M. 1984. The role of collagen in the quality and processing of fish. Cr. Rev. Food Sci. 20: 301-343.

Sobral, P.J.A., Menegalli, F.C., Hubinger, M.D. and Roques, M.A. 2001. Mechanical, water vapor barrier and thermal properties of gelatin based edible films. Food Hydrocolloid. 15: 423-432.

Sothornvit, R. and Krochta, J.M. 2005. Plasticizers in edible films and coatings. *In* Innovations in food packaging. Vol. 403-433. (Han JH, ed.). Elsevier Academic Press. San Diego, Calif.

Staroszczyk, H., Pielichowska, J., Sztuka, K., Stangret, J. and Kołodziejska, I. 2012. Molecular and structural characteristics of cod gelatin films modified with EDC and TGase. Food Chem. 130: 335-343.

Tang, C.-H., Xiao, M.-L., Chen, Z., Yang, X.-Q. and Yin, S.-W. 2009. Properties of cast films of vicilin-rich protein isolates from *Phaseolus* legumes: Influence of heat curing. LWT - Food Sci Technol. 42: 1659-1666.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2013a. Physico-chemical properties, morphology and antioxidant activity of film from fish skin gelatin incorporated with root essential oils. J. Food Eng. 117: 350-360.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2013b. Characteristics and antioxidant activity of leaf essential oil–incorporated fish gelatin films as affected by surfactants. Int. J. Food Sci. Technol. 48: 2143–2149.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2014. Structural, morphological and thermal behaviour characterisations of fish gelatin film incorporated with basil and citronella essential oils as affected by surfactants. Food Hydrocolloid. 41: 33-43.

Valenzuela, C., Abugoch, L. and Tapia, C. 2013. Quinoa protein–chitosan–sunflower oil edible film: Mechanical, barrier and structural properties. LWT - Food Sci. Technol. 50: 531-537.

Vanin, F.M., Sobral, P.J.A., Menegalli, F.C., Carvalho, R.A. and Habitante, A.M.Q.B. 2005. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. Food Hydrocolloid. 19: 899-907.

Xie, Y.-L., Zhou, H.-M. and Qian, H.-F. 2006. Effect of addition of peach gum on physicochemical properties of gelatin-based microcapsule. J. Food Biochem. 30: 302-312.

CHAPTER 9

USE OF FISH GELATIN BASED-FILMS AS EDIBLE POUCH TO EXTEND THE SHELF LIFE OF DRIED CHICKEN POWDER AND CHICKEN OIL

9.1 Abstract

Edible pouches made form fish gelatin film incorporated without and with palm oil (PO), basil essential oil (BEO) or oil mixture (M) were prepared and used to store chicken powder and chicken skin oil in comparison with nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days. The moisture content of chicken powder packaged in pouches from fish gelatin films incorporated without and with various oils increased during 15 days of storage ($p > 0.05$). However, there was non-significant change in moisture content of sample packaged in Nylon/LDPE pouch. Samples packaged in pouches from fish gelatin films incorporated with oils had lower moisture content than those stored in pouch from gelatin film without oil added throughout the storage ($p < 0.05$). This coincided with the higher increases in darkness and yellowness for the latters. All samples packaged in pouches made from all films had the slight increase in PV, whereas a drastic increase in TBARS was observed for all samples during 15 days of storage. During 15 days of storage, chicken skin oil packaged in Nylon/LDPE pouch had higher TBARS and *p*-anisidine value than those stored in pouches made from fish gelatin, regardless of oil incorporated ($p < 0.05$). Therefore, pouches from gelatin film incorporated with oils could lower water migration and lipid oxidation in fat containing foods and oils.

9.2 Introduction

Nowadays, packaging has become important for food industry and is also related with global environment. The over-consumption of plastics is creating a global crisis of waste disposal. Plastic packaging waste disposal is one of urgent current environmental problem due to their non-biodegradability. Therefore, the developments of renewable and environmentally friendly bio-based materials have been received increasing attention. Gelatin has been used as a material for preparing

biodegradable films with several excellent properties (Jongjareonrak *et al.*, 2006; Kavoosi *et al.*, 2014; Nagarajan *et al.*, 2013). Generally, protein-based films have been widely used for coating or film production due to their relative abundance, good film-forming ability and nutritional qualities as well as excellent good barrier characteristics against gas, organic vapor and oil, compared to synthetic films (Hoque *et al.*, 2011; Jiang *et al.*, 2007; Tongnuanchan *et al.*, 2013). However, due to the hydrophilic nature of protein, water vapor barrier property of protein-based film is poorer than other bio-based materials and synthetic films. This is one of the main disadvantages of protein-based films for their application as packaging material. Therefore, the incorporation of hydrophobic substances such as essential oils (Pires *et al.*, 2011; Tongnuanchan *et al.*, 2014), oils (Ma *et al.*, 2012; Tongnuanchan *et al.*, 2015), fatty acids (Fabra *et al.*, 2009; Limpisophon *et al.*, 2010), waxs (Monedero *et al.*, 2009; Soazo *et al.*, 2013) and the use of hydrophobic plasticizers (Andreuccetti *et al.*, 2009) have been implemented to decrease water vapor permeability of protein-based films.

Heat sealing property of material is a prime factor for packing industry, particularly for manufacturing of bags, pouches or sachets, which can be used for packaging foods or ingredients or drinks. Heat sealing is the process of sealing one flexible film to another similar film via heat and pressure. Biodegradable or edible films with heat sealability have been reported for several biomaterials such as fish gelatin (Rouhi *et al.*, 2013), bovine skin gelatin (Nur Hanani *et al.*, 2014), whey protein (Hernandez-Izquierdo and Krochta, 2009; Kim and Ustunol, 2001), wheat gluten (Cho *et al.*, 2007), sago starch (Abdorreza *et al.*, 2011) and corn starch (López *et al.*, 2011). Cho *et al.* (2010) reported that edible pouch produced from corn zein layer laminated on soy protein isolate film with higher oxygen barrier property could reduce oxidative rancidity of olive oil in comparison with that packaged in Nylon/LDPE pouch. However, little information regarding the application of fish gelatin-oil emulsion film as edible pouch or bag exists. Therefore, this study aimed to investigate moisture migration and oxidative stabilities of dried chicken powder and chicken skin oil packaged in pouches from gelatin films incorporated without and with basil essential oil or palm oil or mixed oils during storage under controlled

temperature and relative humidity (RH) in comparison with those packaged in synthetic film based on pouch.

9.3 Materials and Methods

9.3.1 Chemicals

Glycerol and trichloroacetic acid were obtained from Merck (Darmstadt, Germany). Tween-20 (HLB = 16.7), ammonium thiocyanate and 1,1,3,3-tetramethoxypropane (MDA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade.

9.3.2 Fish gelatin and essential oils

Fish gelatin produced from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Palm oil was obtained from Oleen Co., Ltd. (Samutsakorn, Thailand). Basil essential oil (*Ocimum basilicum*) was purchased from *Botanicessence* (Bangkok, Thailand).

9.3.3 Preparation of film from fish gelatin incorporated with different oils

9.3.3.1 Preparation of film forming emulsion

Gelatin powder was mixed with distilled water to obtain the protein concentration of 3.5 % (w/v). The mixture was heated at 70 °C for 30 min. Glycerol at 30 % (w/w) of protein content was used as a plasticizer. The obtained solution was referred to as film-forming solution (FFS). To prepare emulsion film, palm oil, basil essential oil and their mixture (ratio 1:1) previously mixed with Tween-20 as surfactants at 25 % (w/w, based on oil) were added into FFS at levels of 50 % (w/w, based on protein content). The mixture was homogenized at 22,000 rpm for 3 min using a homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). The film-forming emulsion (FFE) was subjected to air removal by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature (28 – 30 °C).

9.3.3.2 Preparation of film

To prepare the films, FFS or FFE (4 g) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown for 12 h (27 ± 2 °C and 75 ± 10 % relative humidity (RH)). The films were further dried at 25 °C and 50 ± 5 % RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). Film from the FFS containing gelatin and glycerol (without essential oil, palm oil and surfactant) was also prepared. The resulting films were manually peeled off and used for pouch preparation. Gelatin films incorporated without and with basil essential oil, palm oil and their mixture as well as nylon/low-density polyethylene film had the thickness of 0.054 ± 0.003, 0.073 ± 0.003, 0.101 ± 0.004, 0.086 ± 0.003 and 0.066 ± 0.001 mm, respectively.

9.3.3.3 Preparation of heat-sealed pouch

The prepared films were used to prepare a heat-sealed pouch as illustrated in Figure 37 using an impulse sealer with magnet Model ME-300HIM (S.N.MARK Ltd., Park, Nonthaburi, Thailand). Fish gelatin films incorporated without and with basil essential oil, palm oil or their mixture and nylon/low-density polyethylene film were heat-sealed at 150 ± 0.5 ºC for 1.25, 1.25, 1.00, 1.25 and 1.50 sec for heating time, respectively, followed by cooling time for 1.50 s of all films.

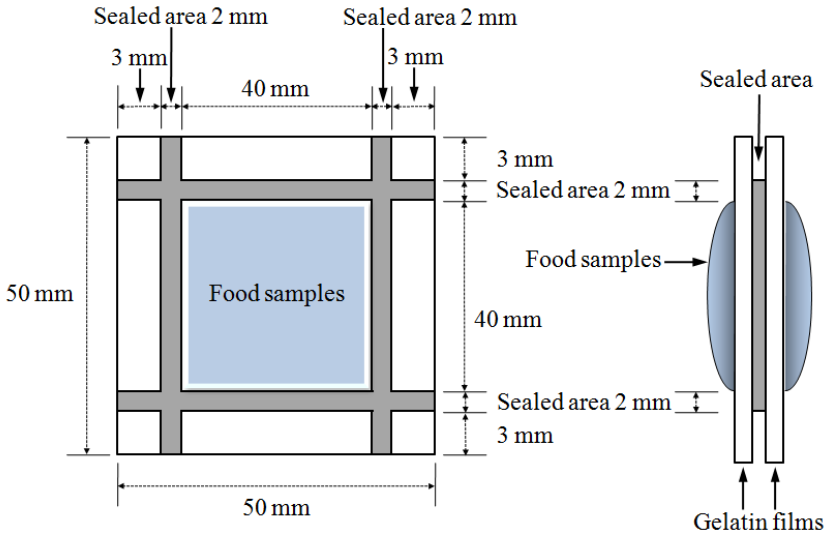


Figure 37. Simplified illustration for heat-sealed pouch.

9.3.4 Use of fish gelatin based-films as edible pouch for shelf-life extension of dried chicken powder and chicken skin oil

9.3.4.1 Preparation of chicken powder

Chicken meat was washed and cut into small pieces (3x3x3 cm^3). Prepared sample was boiled for 10 min. The sample was shredded manually, followed by drying using a hot-air oven with an air velocity of 1.5 m/s at 60 °C for 8 h. The dried sample was powderized using a blender until the uniformity was obtained.

9.3.4.2 Preparation of chicken skin oil

Chicken skin oil was prepared from chicken skin containing depot fat. Chicken skins with approximately 2 × 3 cm^2 were rendered using a hot pan at 150-180 °C. The rendering was performed for 5 min. The oil was then filtered using two layers of cheesecloth.

9.3.4.3 Shelf-life study of dried chicken powder and chicken skin oil during storage

Dried chicken powder (4 g) or chicken skin oil (4 g) was filled into a pouch from fish gelatin films incorporated without or with various oils or nylon/low-density polyethylene film. Pouches containing samples were heat-sealed. The samples were stored at 28 ± 0.5 ºC and 65 ± 5 % RH. Photographs of dried chicken powder and chicken skin oil packaged in pouch prepared from fish gelatin film are shown in Figure 38. Chicken powder samples were taken every 3 days for 15 days for analyses of moisture content (AOAC, 2000), peroxide value, TBARS and color. Chicken skin oil samples were taken every 3 days for totally 15 days and measured for peroxide value, TBARS and *p*-anisidine value.

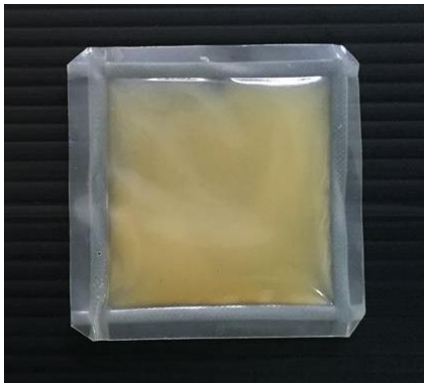
Dried chicken powder	Chicken skin oil
	

Figure 38. Photographs of dried chicken powder and chicken skin oil packaged in pouch prepared from fish gelatin film. Black background was used.

9.3.4.3.1 Peroxide value

Peroxide value (PV) was determined as per the method of Richards and Hultin (2002) with a slight modification. Sample (1 g) was homogenized at a speed of 13,500 rpm for 2 min in 11 ml of chloroform/methanol (2:1, v/v). Homogenate was then filtered using Whatman No. 1 filter paper. Two milliliters of 0.5% NaCl were then added to 7 ml of the filtrate. The mixture was vortexed at a moderate speed for 30 s and then centrifuged at 3000*g* for 3 min to separate the sample into two phases. Two milliliters of cold chloroform/methanol (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 (Shantha 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 0.5–2 ppm. PV was calculated and expressed as mg cumene/kg sample.

9.3.4.3.2 TBARS

Thiobarbituric acid-reactive substances (TBARS) were determined as described by Buege and Aust (1978). Sample (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 5000*g* 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. TBARS were expressed as mg MDA equivalents/kg of sample.

9.3.4.3.3 *p*-anisidine value

The determination of *p*-anisidine value was performed as per the method of AOCS (1998). Sample (1 g) was dissolved in 25 ml of isooctane. The solution was read at 350 nm by using UV–Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). This solution (5 ml) was mixed with 1 ml of 0.25% w/v p-anisidine in acetic acid for 10 min. The absorbance was read at 350 nm. *p*-anisidine value was calculated as follows:

$$p\text{-anisidine value} = 25 \times 1.2 \times (A_2 - A_1)/W$$

where A_1 is the absorbance before adding *p*-anisidine, A_2 is the absorbance after adding *p*-anisidine and W is the weight of sample (g).

9.3.4.3.4 Color

Color of the samples was determined using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA), using D65 (day light) and a measure cell with opening of 30 mm. The color of the sample was expressed as *L**-, *a**- and *b*-value*s.

9.3.5 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, SPSS Inc., Chicago, IL, USA).

9.4 Results and Discussion

9.4.1 Changes of dried chicken powder packaged in various pouches during storage

Moisture content of dried chicken powder packaged in pouches prepared from fish gelatin films incorporated without and with palm oil (PO), basil essential oil (BEO), oil mixture (M) in comparison with nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days at 28 ± 0.5 ºC and 65 ± 5 % RH is shown in Figure 39. In general, the moisture content of dried chicken powder in pouches from fish gelatin films incorporated without and with various oils increased within the first 3 days of storage ($p < 0.05$). On the other hand, no change in moisture content was noticeable in the sample stored in Nylon/LDPE pouch. The increasing rate was much higher for the sample packaged in gelatin pouch (without oil incorporated) than that found in samples packaged in gelatin pouches containing various oils. It was noted that the sample packaged in Nylon/LDPE pouch showed the constant moisture content throughout 15 days ($p > 0.05$). In general, slight change in moisture content was noticeable for all samples after 3 days up to the end of storage (day 15). After 15 days of storage, samples kept in pouches from fish gelatin film incorporated without and palm oil, basil essential oil, oil mixture and Nylon/LDPE film had the increase in moisture content by1.28, 1.20, 1.22, 1.23 and 1.01-fold, respectively. It was confirmed that the moisture content of dried chicken powder packaged in Nylon/LDPE pouch had the lower increase in moisture content than those placed in pouches from fish gelatin films ($p < 0.05$). In addition, the moisture contents of samples stored in pouch based on emulsion fish skin gelatin film were lower than that found in pouch for film without oil incorporation ($p < 0.05$). However, there was no difference in moisture content of samples stored in pouches based on different emulsion films ($p < 0.05$). The result indicated that the water vapor barrier property of gelatin film was lower than that of Nylon/LDPE. Also, it was indicated that all emulsion films used for pouch making had higher water vapor barrier property than gelatin film (without oils). 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 (Hamaguchi *et al.*, 2007; Hoque *et al.*, 2011; Jongjareonrak *et al.*, 2006). The increase in moisture content of dried sample packaged in pouch was due to the moisture diffusion from the surrounding atmosphere through the packaging material. Therefore, the incorporation of hydrophobic substances, such as oils from different sources, could improve the water vapor barrier property of gelatin film, as evidenced by the lower moisture content of samples after storage.

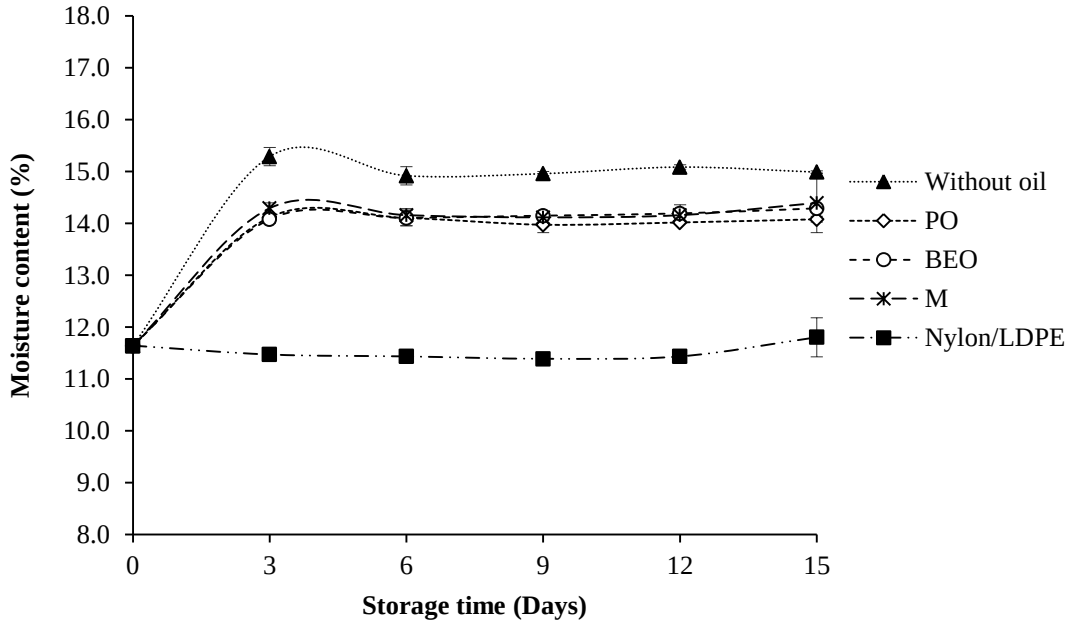


Figure 39. Changes in moisture content of dried chicken powder packaged in pouches from fish gelatin films incorporated without and with palm oil (PO), basil essential oil (BEO), oil mixture (M) and nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days at 28 ± 0.5 ºC and 65 ± 5 % RH. Bars represent the standard deviation (n=3).

Lipid oxidation of dried chicken powder packaged in pouches made from emulsion fish gelatin films and Nylon/LDPE pouch was monitored by measuring PV and TBARS values during 15 days of storage (Figure 40 and 41, respectively). PV of samples packaged in pouches based on fish gelatin films incorporated without and with various oils slightly increased during 15 days of storage, whereas the negligible change in PV value was observed in sample stored in Nylon/LDPE pouch ($p < 0.05$). During storage, the samples packaged in Nylon/LDPE pouch generally had a lower PV value than those packaged in fish gelatin pouch incorporated without and with various oils during 15 days of storage, except at day 3 of storage. Chicken meat is rich in polyunsaturated fatty acids (PUFA), which are prone to oxidation (Cortinas *et al.*, 2004). Chicken meat contained PUFA at 24.6 % (Rule *et al.*, 2002). For TBARS value, the slight increases in values were observed in all samples within the first 3 days of storage, followed by continuous increase up to 15 days. It was found that no differences in TBARS value were observed among all samples during storage. However, non-significantly lower TBARS values were obtained in sample stored in Nylon/LDPE pouch during 12 – 15 days of storage. Normally, 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 of all samples increased during 15 days of storage, lipid oxidation could be promoted, especially for the samples packaged in pouches made from fish gelatin films. Thus, packaging materials used for pouch making was a prime factor affecting water vapor and oxygen permeability, thereby influencing oxidative stability.

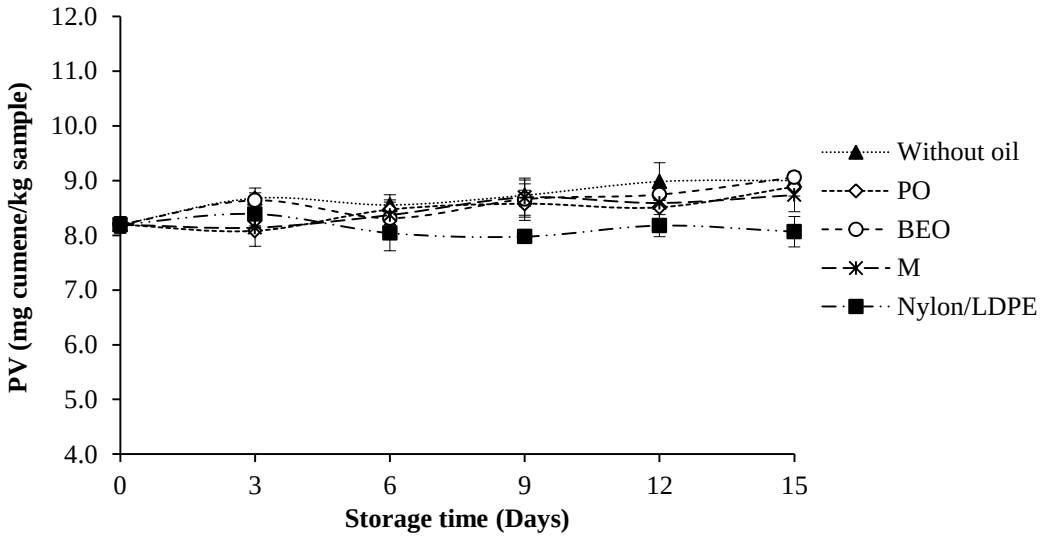


Figure 40. Changes in peroxide value of dried chicken powder packaged in pouches from fish gelatin films incorporated without and with palm oil (PO), basil essential oil (BEO), oil mixture (M) and nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days at 28 ± 0.5 ºC and 65 ± 5 % RH. Bars represent the standard deviation (n=3).

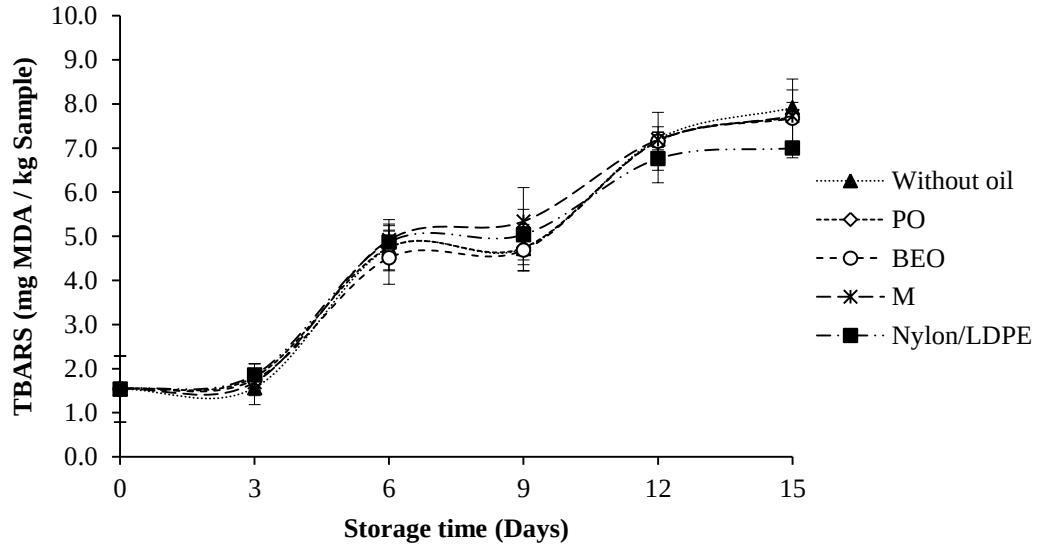


Figure 41. Changes in TBARS value of dried chicken powder packaged in pouches from fish gelatin films incorporated without and with palm oil (PO), basil essential oil (BEO), oil mixture (M) and nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days at 28 ± 0.5 ºC and 65 ± 5 % RH. Bars represent the standard deviation (n=3).

The changes in color expressed as *L**-, *a**- and *b**-values of dried chicken powder packaged in pouches made from fish gelatin films without and with various oils and Nylon/LDPE pouch during storage of 15 days are shown in Figure 42. The dried chicken powder packaged in pouches from fish gelatin films incorporated without and with various oils had the decrease in *L**-value and increases in *a**- and *b**-values within the first 3 days of storage ($p < 0.05$). Thereafter, the changing rate was much lower for all samples. A continuous increase in *a**-value was observed in all samples during 15 days storage, excepted for the sample stored in Nylon/LDPE pouch. Overall, samples packaged in fish gelatin film pouch had the highest rate of decrease in *L**- value and the increase in *a** and *b**-values, compared with those packaged in pouch made from fish gelatin films incorporated with oils. However, no differences in colors were observed between samples packaged in pouches from fish gelatin films incorporated with various oils during 15 days storage ($p > 0.05$). Generally, dried chicken powder packaged in Nylon/LDPE pouch exhibited the lowest rate of change in colors during storage of 15 days. It was noted that the color of all samples became more yellowish and darker during storage, as indicated by the increase in *b**-value and decrease in *L**-value, respectively. The result suggested that the higher *b**-value could represent the higher formation of yellowish pigment via Maillard reaction, which was plausibly enhanced by the increased moisture content of samples. The moisture content of dried chicken powder in different samples increased during 15 days of storage (Figure 39). The rate of Maillard reactions increases as the water activity increases (Labuza and Baisier, 1992). The influence of water activity on Maillard reaction kinetics can be interpreted as a consequence of mobility and diffusion of reactants (Pereyra Gonzales *et al.*, 2010). Furthermore, oxidation products, especially aldehydes, could serve as the source of carbonyl, which could undergo glycation with amino group in dried chicken powder (Kubow, 1992). This could lead to the increased Maillard reaction with increasing storage time. Artharn *et al.* (2009) reported the increase in yellowness of dried fish powder covered with round scad protein-based film during 21 days of storage at room temperature. Those changes were mediated by the enhanced lipid oxidation during the extended storage.

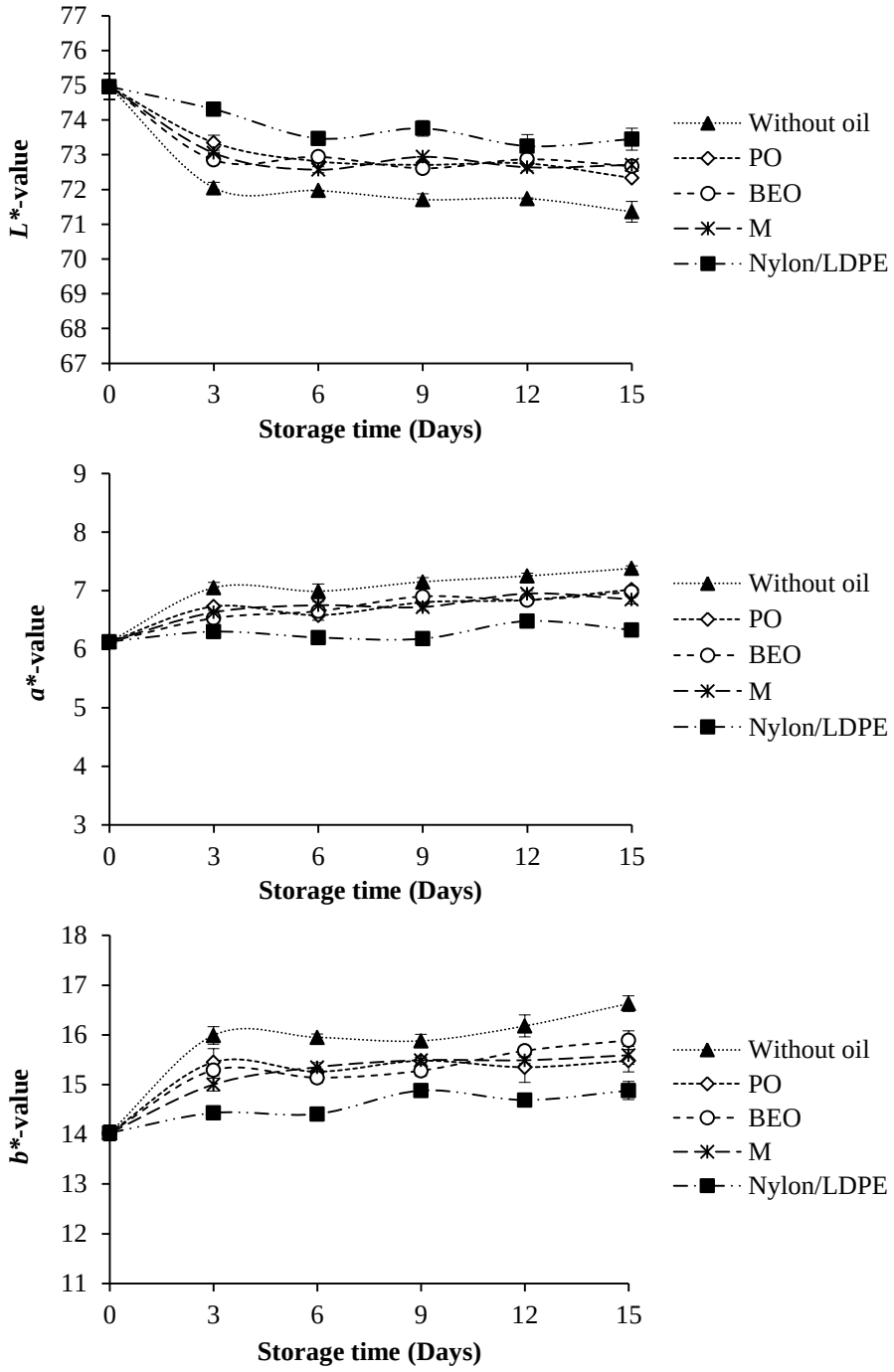


Figure 42. Changes in *L**, *a** and *b**-values of dried chicken powder packaged in pouches from fish gelatin films incorporated without and with palm oil (PO), basil essential oil (BEO), oil mixture (M) and nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days at 28 ± 0.5 ºC and 65 ± 5 % RH. Bars represent the standard deviation (n=3).

9.4.2 Changes of chicken skin oil packaged in various pouches during storage

Lipid oxidation of chicken skin oil packaged in pouches made from fish gelatin films incorporated without and with palm oil (PO), basil essential oil (BEO), oil mixture (M) films and nylon/low-density polyethylene (Nylon/LDPE) pouch was monitored using PV, TBARS and *p*-anisidine values during storage of 15 days (Figure 43, 44 and 45, respectively). The increase in PV value in all chicken skin oil samples was found within the first 3 – 6 days of storage ($p < 0.05$), regardless of types of pouch used. Thereafter, the decrease in PV value was noticeable at day 9, followed by the slight increase up to 15 days of storage. The decreases in PV of samples suggested that hydroperoxides formed were plausibly decomposed to the secondary oxidation products (Kubow, 1992). Chicken skin fat consisted of saturated (30.2 %), monounsaturated (39.0 %) and polyunsaturated fatty acids (30.6 %). Linoleic acid (ω-6) and linolenic acid (ω-3) constituted at 28.23 and 2.37 % of total fatty acids, respectively (Feddern *et al.*, 2010). At day 15, the highest PV was found in the sample packaged in Nylon/LDPE pouch, suggesting the lowest decomposition.

In general, TBARS values of samples packaged in pouch made from fish gelatin film and Nylon/LDPE pouch increased up to 6 days of storage, whereas the samples packaged in pouch made from emulsion fish gelatin films had the increase in TBARS within the first 9 days of storage, followed by slight decrease up to 12 days. Subsequently, the increase in TBARS was observed up to 15 days of storage for all samples. During 15 days of storage, the sample stored in Nylon/LDPE pouch had higher TBARS, followed by samples packaged in pouch made from fish gelatin-based films incorporated without and with various oils, respectively. The higher TBARS value in sample packaged in Nylon/LDPE pouch suggested that lipid oxidation took place at a higher extent. This was plausibly due to the poorer oxygen barrier property of Nylon/LDPE film in comparison with gelatin film. Moreover, *p*-anisidine values of all samples increased within the first 3 days of storage. Thereafter, the slight decrease in *p*-anisidine value was observed at day 6, followed by the constant value up to the end of storage (day 15). *p*-anisidine value has been used an indicator for lipid oxidation, which was measured the secondary lipid oxidation

products, particularly non-volatile aldehydes and ketones (Tompkins and Perkins, 1999).

The samples packaged in pouch from fish gelatin films incorporated without and with various oils showed the lower *p*-anisidine value than that packaged in Nylon/LDPE pouch during storage. This result indicated that oxygen barrier properties of fish gelatin films might be higher than Nylon/LDPE film. The oxygen permeability of protein-based films has been reported to be very low and comparable to that of synthetic polymers at low or intermediate RH conditions (McHugh and Krochta, 1994). Normally, oxygen initiates lipid oxidation, which decreases food quality and shortens food product shelf-life, especially for products containing high amounts of unsaturated fatty acids or oxygen sensitive vitamins (Sothornvit and Krochta, 2000). The excellent oxygen barrier property of packaging is one factor that guarantees the maximum shelf-life of the products. The results suggested that fish gelatin pouch could be potentially used as alternative packaging material for chicken skin oil or other oils.

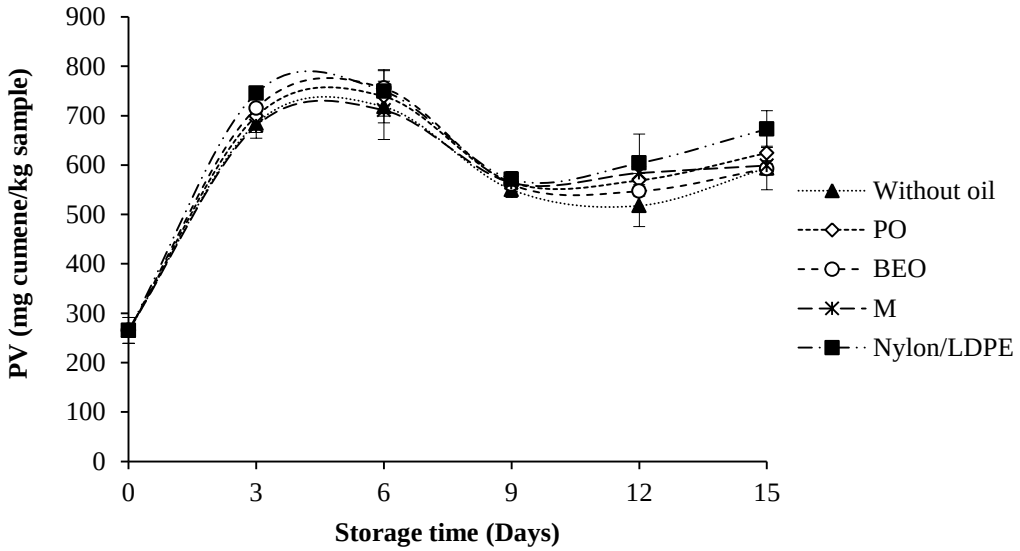


Figure 43. Changes in peroxide value of chicken skin oil packaged in pouches from fish gelatin films incorporated without and with palm oil (PO), basil essential oil (BEO), oil mixture (M) and nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days at 28 ± 0.5 ºC and 65 ± 5 % RH. Bars represent the standard deviation (n=3).

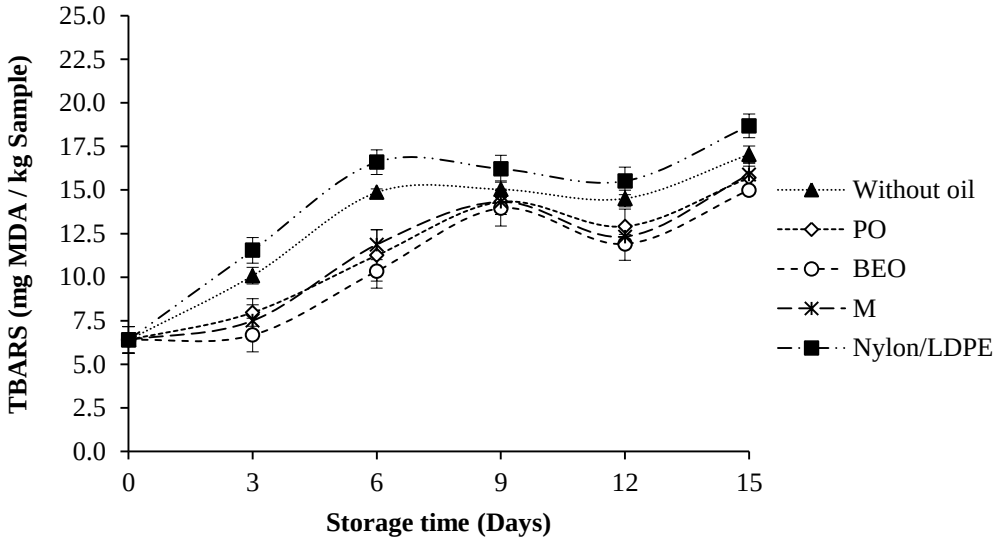


Figure 44. Changes in TBARS value of chicken skin oil packaged in pouches from fish gelatin films incorporated without and with palm oil (PO), basil essential oil (BEO), oil mixture (M) and nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days at 28 ± 0.5 ºC and 65 ± 5 % RH. Bars represent the standard deviation (n=3).

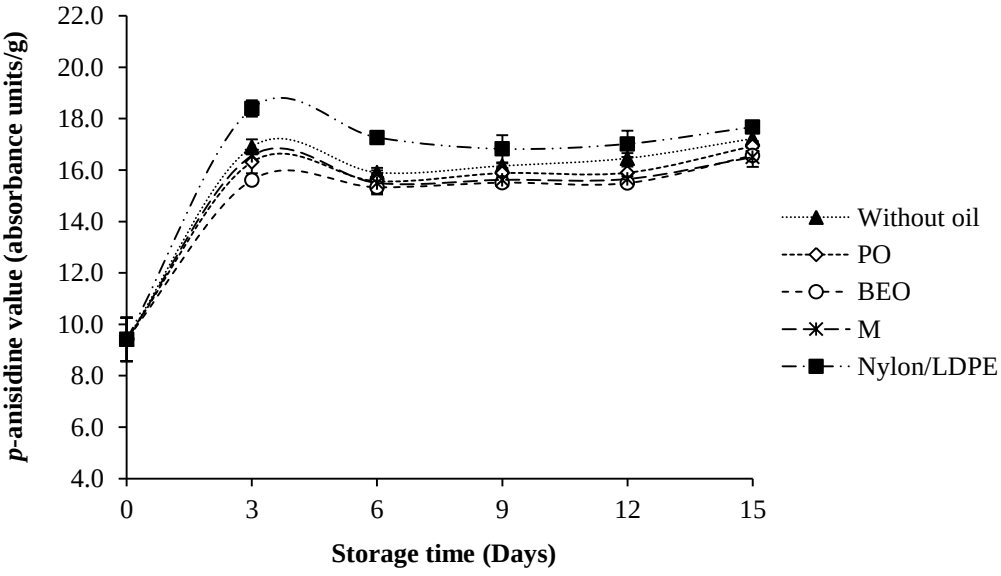


Figure 45. Changes in *p*-anisidine value of chicken skin oil packaged in pouches from fish gelatin films incorporated without and with palm oil (PO), basil essential oil (BEO), oil mixture (M) and nylon/low-density polyethylene (Nylon/LDPE) pouch during storage of 15 days at 28 ± 0.5 ºC and 65 ± 5 % RH. Bars represent the standard deviation (n=3).

9.5 Conclusion

Pouches made from gelatin films incorporated with oils could lower the increase in moisture content, darkness and yellowness of chicken powder than that packaged in pouch from fish gelatin film without oils. However, the preventive effect of pouch from gelatin film was lower than Nylon/LDPE pouch. Moreover, fish gelatin pouch incorporated with oils could prevent the oxidation of chicken skin oil more effectively than pouch from gelatin film without oil incorporated and Nylon/LDPE pouch, respectively. Therefore, fish gelatin can be used as for making an alternative edible pouch to retard lipid oxidation of food products, particularly oils.

9.6 Conclusion

Abdorreza, M.N., Cheng, L.H. and Karim, A.A. 2011. Effects of plasticizers on thermal properties and heat sealability of sago starch films. Food Hydrocolloid. 25: 56-60.

Andreuccetti, C., Carvalho, R. and Grosso, C.R.F. 2009. Effect of hydrophobic plasticizers on functional properties of gelatin-based films. Food Res. Int. 42: 1113-1121.

AOAC. 2000. Official methods of analysis. *In* 16 Ed. Association of Official Analysis Chemists Washington, DC.

AOCS. 1998. Official methods Panisidine value. *In* Official methods and recommended practices of the American Oil Chemists Society. 5 Ed. p. 18-90.

Artharn, A., Prodpran, T. and Benjakul, S. 2009. Round scad protein-based film: Storage stability and its effectiveness for shelf-life extension of dried fish powder. LWT - Food Sci Technol. 42: 1238-1244.

Buege, J.A. and Aust, S.D. 1978. Microsomal lipid peroxidation. Method. Enzymol. 52: 302-310.

Cho, S.-W., Ullsten, H., Gällstedt, M. and Hedenqvist, M.S. 2007. Heat-sealing properties of compression-molded wheat gluten films. J. Biobased Mater. Bio. 1: 56-63.

Cho, S.Y., Lee, S.Y. and Rhee, C. 2010. Edible oxygen barrier bilayer film pouches from corn zein and soy protein isolate for olive oil packaging. LWT - Food Sci. Technol. 43: 1234-1239.

Cortinas, L., Villaverde, C., Galobart, J., Baucells, M.D., Codony, R. and Barroeta, A.C. 2004. Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. Poultry Sci. 83: 1155-1164.

Fabra, M.J., Jiménez, A., Atarés, L., Talens, P. and Chiralt, A. 2009. Effect of fatty acids and beeswax addition on properties of sodium caseinate dispersions and films. Biomacromolecules. 10: 1500-1507.

Feddern, V., Kupski, L., Cipolatti, E.P., Giacobbo, G., Mendes, G.L., Badiale-Furlong, E. and de Souza-Soares, L.A. 2010. Physico-chemical composition, fractionated glycerides and fatty acid profile of chicken skin fat. Eur. J. Lipid Sci. Tech. 112: 1277-1284.

Hamaguchi, P.Y., Weng, W., Kobayashi, T., Runglertkreingkrai, J. and Tanaka, M. 2007. Effect of fish meat quality on the properties of biodegradable protein films. Food Sci. Technol. Res. 13: 200-204.

Hernandez-Izquierdo, V.M. and Krochta, J.M. 2009. Thermal transitions and heat-sealing of glycerol-plasticized whey protein films. Packag. Technol. Sci. 22: 255-260.

Hoque, M.S., Benjakul, S. and Prodpran, T. 2011. Effects of partial hydrolysis and plasticizer content on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. Food Hydrocolloid. 25: 82-90.

Jiang, Y., Tang, C.-H., Wen, Q.-B., Li, L. and Yang, X.-Q. 2007. Effect of processing parameters on the properties of transglutaminase-treated soy protein isolate films. Innov. Food Sci. Emerg. 8: 218-225.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid. 20: 492-501.

Kavoosi, G., Rahmatollahi, A., Mohammad Mahdi Dadfar, S. and Mohammadi Purfard, A. 2014. Effects of essential oil on the water binding capacity, physico-mechanical properties, antioxidant and antibacterial activity of gelatin films. LWT - Food Sci. Technol. 57: 556-561.

Kim, S.J. and Ustunol, Z. 2001. Thermal properties, heat sealability and seal attributes of whey protein isolate/ lipid emulsion edible films. J. Food Sci. 66: 985-990.

Kubow, S. 1992. Routes of formation and toxic consequences of lipid oxidation products in foods. Free Radical Bio. Med. 12: 63-81.

Labuza, T.P. and Baisier, W.M. 1992. The kinetics of nonenzymatic browning. *In* Physical chemistry of foods. (Schwartzberg, H. and Hartel, R., eds.). p. 595-649. Marcel Dekker. New York, USA.

Limpisophon, K., Tanaka, M. and Osako, K. 2010. Characterisation of gelatin-fatty acid emulsion films based on blue shark (*Prionace glauca*) skin gelatin. Food Chem. 122: 1095-1101.

López, O.V., Lecot, C.J., Zaritzky, N.E. and García, M.A. 2011. Biodegradable packages development from starch based heat sealable films. J. Food Eng. 105: 254-263.

Ma, W., Tang, C.-H., Yin, S.-W., Yang, X.-Q., Wang, Q., Liu, F. and Wei, Z.-H. 2012. Characterization of gelatin-based edible films incorporated with olive oil. Food Res. Int. 49: 572-579.

McHugh, T.H. and Krochta, J.M. 1994. Sorbital - versus glycerol - plasticized edible films: integrated oxygen permeability and tensile property evaluation. J. Agr. Food Chem. 42: 841-845.

Monedero, F.M., Fabra, M.J., Talens, P. and Chiralt, A. 2009. Effect of oleic acid-beeswax mixtures on mechanical, optical and water barrier properties of soy protein isolate based films. J. Food Eng. 91: 509-515.

Nagarajan, M., Benjakul, S., Prodpran, T., Songtipya, P. and Nuthong, P. 2013. Film forming ability of gelatins from splendid squid (*Loligo formosana*) skin bleached with hydrogen peroxide. Food Chem. 138: 1101-1108.

Nur Hanani, Z.A., O'Mahony, J.A., Roos, Y.H., Oliveira, P.M. and Kerry, J.P. 2014. Extrusion of gelatin-based composite films: Effects of processing temperature and pH of film forming solution on mechanical and barrier properties of manufactured films. Food Packag. Shelf Life. 2: 91-101.

Partanen, R., Raula, J., Seppänen, R., Buchert, J., Kauppinen, E. and Forssell, P. 2008. Effect of relative humidity on oxidation of flaxseed oil in spray dried whey protein emulsions. J. Agr. Food Chem. 56: 5717-5722.

Pereyra Gonzales, A.S., Naranjo, G.B., Leiva, G.E. and Malec, L.S. 2010. Maillard reaction kinetics in milk powder: Effect of water activity at mild temperatures. Int. Dairy J. 20: 40-45.

Pires, C., Ramos, C., Teixeira, G., Batista, I., Mendes, R., Nunes, L. and Marques, A. 2011. Characterization of biodegradable films prepared with hake proteins and thyme oil. J. Food Eng. 105: 422-428.

Richards, M.P. and Hultin, H.O. 2002. Contributions of blood and blood components to lipid oxidation in fish muscle. J. Agr. Food Chem. 50: 555-564.

Rouhi, J., Mahmud, S., Naderi, N., Ooi, C. and Mahmood, M. 2013. Physical properties of fish gelatin-based bio-nanocomposite films incorporated with ZnO nanorods. Nanoscale Res. Lett. 8: 364.

Rule, D.C., Broughton, K.S., Shellito, S.M. and Maiorano, G. 2002. Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. J. Anim. Sci. 80: 1202-1211.

Shantha, N.C. and Decker, E.A. 1994. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. J. AOAC Int. 77: 421-424.

Soazo, M., Pérez, L.M., Rubiolo, A.C. and Verdini, R.A. 2013. Effect of freezing on physical properties of whey protein emulsion films. Food Hydrocolloid. 31: 256-263.

Sothornvit, R. and Krochta, J.M. 2000. Oxygen permeability and mechanical properties of films from hydrolyzed whey protein. J. Agr. Food Chem. 48: 3913-3916.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics. McGraw-Hill Book. New York.

Tompkins, C. and Perkins, E. 1999. The evaluation of frying oils with the p-Anisidine value. J. Am. Soc. 76: 945-947.

Tongnuanchan, P., Benjakul, S., Prodpran, T. and Songtipya, P. 2013. Properties and stability of protein-based films from red tilapia (*Oreochromis niloticus*) protein isolate incorporated with antioxidant during storage. Food Bioprocess Tech. 6: 1113-1126.

Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2014. Comparative studies on properties and antioxidative activity of fish skin gelatin films incorporated with essential oils from various sources. Int. Aquat. Res. 6: 1-12.

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.

CHAPTER 10

CONCLUSION AND SUGGESTION

10.1 Conclusion

1. The incorporation of different essential oils directly affected the properties of fish skin gelatin film, but the properties varied, depending on type of essential oils.

2. Essential oils could act as plasticizer and increased water barrier property and antioxidative activity of films, especially with higher levels of essential oils.

3. Soy lecithin was the most appropriate surfactant, which yielded gelatin films with improved WVP and antioxidative activity. Moreover, soy lecithin could enhance stability of emulsion, providing homogeneous oil distribution in the matrix of gelatin-essential oil composite films.

4. Palm oil could be used to improve water vapor barrier property of film. Films incorporated with palm oil and stabilized with soy lecithin exhibited a homogeneous morphological microstructure without bilayer formed, except for those added with 100% oil.

5. Gelatin film incorporated with and without different oils were heat sealable. The seal strength and seal efficiency of film varied with film compositions. Tween-20 was the appropriate surfactant, which potentially enhanced the heat sealability of emulsion films and provided the good quality seal. Sealing time was also essential parameter determining heat sealability of emulsion films.

6. Pouches made from emulsion gelatin films could retard lipid oxidation of chicken skin oil more efficiency than pouch from LDPE/nylon film. Nevertheless, the water vapor barrier property of gelatin pouch was still poorer than that of pouch from synthetic films. Therefore, fish gelatin films could be used as alternative packaging for effective retardation of lipid oxidation of food products.

10.2 Suggestion

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

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

3. The role of biodegradable or edible surfactants from polysaccharide-based materials in emulsion films should be elucidated.

4. The application of emulsion film as edible pouch in other foods should be further investigated.

VITAE

Name Mr. Phakawat Tongnuanchan

Student ID 5411030015

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Food Science and Nutrition)	Prince of Songkla University	2008
Master of Science (Food Science and Technology)	Prince of Songkla University	2011

Scholarship Awards during Enrolment

1. Scholarship for Academic Distincetion, Prince of Songkla University.
2. National Research University Project of Thailand's Office of the Higher Education Commission.
3. Oversea Thesis Research Study Scholarship, Graduate School, Prince of Songkla University.
4. Award for Silver Metal, Water soluble pouch from fish skin gelatin, In 42nd International Exhibition of Inventions of Geneva. Geneva, Switzerland. 2-6 April 2014.

List of Publications and Proceedings

Publications

1. Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2012. Properties and antioxidant activity of fish skin gelatin film incorporated with citrus essential oils. Food Chem. 134: 1571-1579.

2. Tongnuanchan, P., Benjakul, S. and Prodpran. 2013. Physico-chemical properties, morphology and antioxidant activity of film from fish skin gelatin incorporated with root essential oils. J. Food Eng. 117: 350-360.

3. Tongnuanchan, P., Benjakul, S. and Prodpran. 2013. Characteristics and antioxidant activity of leaf essential oil-incorporated fish gelatin films as affected by surfactants. Int. J. Food Sci. Technol. 48: 2143-2149.

4. Tongnuanchan, P., Benjakul, S. and Prodpran. 2014. Structural, morphological and thermal behaviour characterisations of fish gelatin film incorporated with basil and citronella essential oils as affected by surfactants. Food Hydrocolloid. 41: 33-43.

5. Tongnuanchan, P. and Benjakul, S. 2014. Essential oils: Extraction, bioactivities and their uses for food preservation. J. Food Sci. 79: 1231-1249.

6. Tongnuanchan, P., Benjakul, S. and Prodpran. 2014. Comparative studies on properties and antioxidative activity of fish skin gelatin films incorporated with essential oils from various sources. Int. Aquat. Res. 6:1-12.

7. 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.

8. Tongnuanchan, P., Benjakul, S., Prodpran, Pisuchpen, S. and Osako, K. 2015. Heat-sealing properties and thermal behavior of fish skin gelatin film containing palm oil and basil essential oil with different surfactants. Food Hydrocolloids. Submitted.

Proceedings

1. Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2011. Properties and stability of protein-based films from red tilapia protein isolate incorporated with antioxidant during storage. In RGJ Seminar Series LXXXIII: Natural Resources and Management for Sustainable Utilization. Faculty of Science, Prince of Songkla University, Thailand. 31 August 2011. Oral presentation.

2. Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2013. Molecular and thermal properties of fish gelatin film incorporated with basil and citronella essential oils as affected by surfactants. In 13th ASEAN Food Conference (AFC2013). The MAX Atria, Singapore Expo. 9-11 September 2013. Poster presentation.

3. Tongnuanchan, P., Benjakul, S. and Prodpran, T. 2014. Properties, morphology and antioxidative activity of fish skin gelatin films incorporated with different essential Oils. In 2nd AFSA conference on food safety and food security. Dong Nai University of Technology, Bien Hoa City, Vietnam. 15-18 August 2014. Oral presentation.

4. Tongnuanchan, P., Benjakul, S., Prodpran, Pisuchpen, S. and Osako, K. 2015. Heat-sealing properties and thermal behavior of fish skin gelatin film containing palm oil and basil essential oil with different surfactants. In International Conference on Quality Improvement and Development of Food Product (QID-Food 2015), Bukittinggi, West Sumatera-Indonesia. 18 April 2015. Oral presentation.